

**Molecular, Biochemical and Morphological
Characterization of Selected *Curcuma*
Accessions**

**Thesis submitted to the
University of Calicut**

For the award of degree

of

Doctor of Philosophy
(BIOCHEMISTRY)

by

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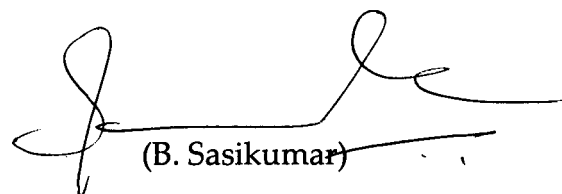
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CERTIFICATE

This is to certify that the thesis entitled "**Molecular, biochemical and morphological characterisation of selected *Curcuma* accessions**" submitted to the **University of Calicut** by Mr. **Syamkumar, S.** for the award of the degree of **Doctor of Philosophy in Biochemistry** is a bonafide record of research work carried out by him under my supervision and guidance. No part of the work has formed the basis for the award of any other degree or diploma previously.

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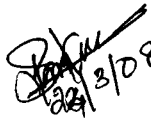


(B. Sasikumar)

DECLARATION

I hereby declare that the thesis entitled "**Molecular, biochemical and morphological characterisation of selected *Curcuma* accessions**" submitted by me for the award of the degree of **Doctor of Philosophy in Biochemistry** of the **University of Calicut** contains the results of bonafide research work done by me at Indian Institute of Spices Research, Calicut, Kerala, under the guidance of Dr. B Sasikumar, Principal Scientist, Crop Improvement and Biotechnology Division, Indian Institute of Spices Research, Calicut. This Thesis or part of it has not been submitted to any other university for the award of any other degree or diploma previously. All sources of help received by me during the course of this study have been duly acknowledged.

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22/3/08
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Abbreviation	Expansion
A	Adenine
A _{260/280}	Absorbance at 260/absorbance at 280
AE HPLC	Anion exchange high performance liquid chromatography
AR	Aroma of rhizome
ARMS	Amplification refracton mutation system
ASTA	American spice trade association
C	Cytosine
CLS	Colour of leaf sheath
Cm	Centimeter
CTAB	Cetyl trimethyl ammonium bromide
CW	Clump weight
D	Dextro
DEAE	Diethyl amino ethyl cellulose
DNA	Deoxy ribonucleic acid
dNTP	Deoxy Nucleotide triphosphate
ERK	Extracellular signal regulated kinase
EV	Electron volt
Fig.	Figure
F/T	Flavour/Taste of rhizome
g	Gravitational force
G	Guanine
GC	Gas chromatography
GC-MS	Gas chromatography-Mass spectroscopy
g l ⁻¹	Gram per liter
HIV	Human immuno deficiency virus
HP	Height of pseudostem
HPLC	High performance liquid chromatography
ICC	Inner core colour
I.D	Internal diameter
INL	Internode length
IR	Infra red
ISSR	Inter simple sequence repeats
ITS	Internal transcribed region
kb	Kilo bases
kDa	Kilo dalton
L	Leavo
LB	Leaf breadth
LC-MS	Liquid Chromatography -Mass spectroscopy
LDL	Low density lipoprotein
LL	Leaf length
LMC	Leaf midrib colour
LT	Leaf texture
m	Meter
mg%	Milligram percentage
min	Minute
ml	Millilitre
ml ⁻¹	Per millilitre
mm	Millimeter
mM	Millimolar
mM µl ⁻¹	Millimolar per microliter
MME 9	Matrix metalloproteinases 9
MS	Mass spectroscopy
NCBI	National Centre for Biotechnology Information
ng	Nanogram

ng μl^{-1}	Nanogram per microlitre
NLC	Number of leaves per clump
NLM	Number of leaves in main tiller
nm	Nanometer
NM	Number of mother rhizome
NMR	Nuclear magnetic resonance
NP	Number of primary rhizome
NS	Number of secondary rhizome
NTC	Number of tillers per clump
NTSYS	Numerical taxonomic system
PCR	Polymerase chain reaction
PG	Pseudostem girth
ppm	Parts per million
RAPD	Random amplified polymorphic DNA
RI	Retention index
ROS	Reactive oxygen species
rpm	Rotation per minute
rRNA	Ribosomal ribonucleic acid
RT	Retention time
S	Sedimentation coefficient
SCAR	Sequence characterized amplified region
SNP	Single nucleotide polymorphism
spp.	Species
syn.	Synonym
T	Thymine
TAE	Tris Acetic acid EDTA
TE	Tris EDTA
TLC	Thin layer chromatography
U	Unit
U μl^{-1}	Unit per microlitre
UPGMA	Unweighted pair group arithmetic mean analysis
UV	Ultra violet
WHO	World health organization
WM	Weight of mother rhizome
WP	Weight of primary rhizome
WS	Weight of secondary rhizome
Wt	Weight

Introduction

Chapter 1

INTRODUCTION

Curcuma is gaining importance globally as a mighty cure to combat a variety of ailments as the genus is credited with molecules having anti-inflammatory, hypocholesteremic, choleric, antimicrobial, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic, anticancerous properties and insect repellent activity besides of course its known use as a spice. A US Patent (US Patent No. 20030185907) is now there on a method of treatment of inflammation and pain in mammals including human beings using curcuminoids along with other plant derived molecules. Turmeric oil is also now used in aromatherapy and perfume industry. Correct taxonomic identification of the specimen is very important for meaningful bioprospecting as well as for effective conservation.

Genus *Curcuma* belonging to the family *Zingiberaceae*, originated in the Indo- Malayan Region (Purseglove, 1968) has a widespread occurrence in the tropics of Asia to Africa and Australia. Out of the one hundred odd species reported in the genus about forty are of Indian origin (Velayudhan *et al.*, 1999).

The genus is taxonomically quite confusing. A few studies on morphological and anatomical characterization of *Curcuma* species and turmeric varieties have been attempted, but no much studies are reported on molecular characterization except a few studies on isozyme polymorphism and identification of species based

on 18S rRNA and *trn K* genes. Cytology of twelve species of *Curcuma* is also reported. Taxonomic revision of the genus is now under progress in India and the number of Indian species may be narrowed down to thirty or so from the reported forty species as some of the species are now being treated as synonyms. (Sasikumar, 2005). Morphological characterization of the genus has its limitation as some of the key taxonomic traits remain controversial (Santapau, 1945). Currently molecular characterization is being used as an adjunct to the traditional methods of germplasm characterization in many crop plants (Semagn *et al.*, 2006).

Though *C. longa* syn. *Curcuma domestica* is the most commonly utilized species, other species such as *C. aromatica*, *C. amada*, *C. kwangsiensis*, *C. zedoaria*, *C. caesia*, *C. malabarica*, *C. angustifolia*, *C. montana*, *C. decipiens*, *C. alismatifolia*, *C. thorelii*, *C. comosa* and *C. xanthorrhiza* are also economically important .

Turmeric (*C. longa* L.), the most important species of the genus is widely known as a spice, medicine and dye plant all over the world. The name turmeric originated from the Latin name '*terramerita*', which became '*terre merite*' of French meaning meritorious earth. The antiquity of turmeric dates back to '*Atharva Veda*' of 1000 - 1500 BC, a holy treatise of the Hindus, as 'Haldi' or 'Haridrar' (AV/1/22/4) (Shah, 1997). '*Ayurveda*' (Science of Life), the offshoot of '*Atharva Veda*', mentions approximately 5000 years of antiquity to the medicinal uses of turmeric (Duke, 2003).

Curcumin, essential oil and oleoresin are the three most important biochemical constituents of therapeutic and/or spice value found in *Curcuma* species besides the biologically active peptides and immunologically active polysaccharides (Srinivas *et al.*, 1992; Gonda *et al.*, 1990).

Curcuma longa, the cultivated turmeric, is now grown in countries like India, China, Pakistan, Bangladesh, Vietnam, Thailand, Philippines, Japan, Korea, Sri Lanka, Nepal, South Pacific Islands, East and West Africa, Malaysia, Caribbean Islands and Central America.

India harbours a rich diversity of *Curcuma*, especially species and cultivar diversity (Sasikumar *et al.*, 1996). About forty *Curcuma* species and more than fifty cultivars besides twenty five improved *C. longa* varieties are available in India. India is the leading producer and exporter of turmeric in the world. Indian turmeric is exported to countries like UK, USA, Iran, Japan, UAE, Saudi Arabia, The Netherlands, South Africa and Singapore in different forms such as whole dry rhizome, turmeric powder, turmeric oleoresin, curcumin, essential oil and curry powder.

As India harbours a rich diversity of *Curcuma*, this country stands to gain from its bioprospecting. These facts make it ideal to initiate a study on characterizing the *Curcuma* species/turmeric of India.

The present study is an attempt to characterize selected *Curcuma* accessions from India using morphological, molecular and biochemical markers with the following objectives:

- 1) Morphological characterization of selected *Curcuma* species, thirty six cultivars/varieties of turmeric (*C. longa*) besides twelve open pollinated progenies of turmeric based on aerial morphological features and underground rhizome characters.
- 2) Molecular characterization of selected *Curcuma* species, thirty six *C. longa* varieties/cultivars and twelve open pollinated progenies of *C. longa* using RAPD and ISSR markers.
- 3) Development of a viable PCR protocol for varietal characterization of turmeric based on fresh rhizome DNA.
- 4) Characterization of *Curcuma* species based on 18S rRNA sequence analysis.
- 5) Chemotyping of *Curcuma* by GC MS analysis of volatile oil and identification of specific chemotypes.
- 6) Extraction of curcumin and separation of curcuminoids from *C. longa* accessions and selected *Curcuma* species to study the distribution pattern of curcuminoids.

- 7) Rhizome protein studies and peptide mapping of selected *Curcuma* species and accessions using HPLC.
- 8) Studying the levels of the antioxidant peptide, turmerin, in different *Curcuma* species and selected turmeric varieties/cultivars

Review of Literature

Chapter 2

REVIEW OF LITERATURE

2.1. General

The genus *Curcuma* belongs to the family Zingiberaceae within the sub family Zingiberoideae and tribe Zingiberae (Kress *et al.*, 2002) and the genus include one hundred and twenty species distributed in tropical and subtropical Asia (Skornickova *et al.*, 2007). The generic epithet is derived from the Arabic word *Karkum*, meaning yellow, and *Curcuma* is the Latinised version. The genus was first established by Linnaeus in his '*Species Plantarum*' in 1753 and is an important natural resource that provides many useful products such as spice, medicine, dyes, food, perfume and aesthetics to people. The first classification of the genus was done by Baker (1890 - 1892) and he reported twenty-nine species from India. The first published report of the genus *Curcuma* in India appeared in *Hortus Malabaricus* by Rheede (1678-1693). He identified and recorded two *Curcuma* species 'Kua' - *Curcuma zedoaria* Christam Rosc. and 'Manjella Kua' - *Curcuma longa* Linn. in their present binomial identity. This was latter established by Burt (1977) as the lectotypes of *Curcuma* and reinstated the name *Curcuma longa* L. and *Curcuma domestica* as its synonym. Baker (1898) further confirmed twenty seven species of *Curcuma* in the 'Flora of British India' and subdivided the genus into three sections, *Exantha*, *Mesantha* and *Hitcheniopsis*. The section *Exantha* comprises fourteen species including the turmeric (*C. longa* L.) and other economically important species such as *C. angustifolia* Roxb., *C. aromatica* Salisb., *C. zedoaria* Rosc. etc. (Velayudhan *et al.*, 1996).

A comprehensive attempt to study the taxonomy of genus was done by Valeton (1918) and classified the genus into two sub genera namely *Paracurcuma* and *Eucurcuma* based on the presence/absence of anther spur. In the genus *Paracurcuma*, Valeton has included two species viz, *C. ecalcarata* and *C. aurantiaca* either lacking anther spur or possessing a very short spur. The sub genera *Eucurcuma* was further divided into three main sections namely *tuberosa*, *nontuberosa* and *stolonifera* based on the presence or absence of tubers and stolons. Each section was again divided into subsections based on aerial characters and underground features. Velayudhan *et al.*, (1996) accommodated the Indian *Curcuma* species into the two subgenera of Valeton's based on the presence or absence of anther spurs.

Though Velayudhan *et al.*, (1996) reported forty *Curcuma* species from India (Table 1.) it is now believed that there may not be forty true species and some of them may be duplicates and are now being recognized as synonyms such as *C. zedoaria* syn. *C. xanthorrhiza* (Sasikumar, 2005; Skornickova and Sabu, 2005). The existence of duplicate entities in the *Curcuma* species of China is also reported. *C. albicoma* and *C. chuanyujin* are synonyms of *C. sichuanensis* and *C. kwangsiensis*, respectively. *Curcuma wenyujin* is considered as synonym of *C. aromatica*; *C. sichuanensis* as synonym of *C. albicoma*. As per the latest studies *C. sichuanensis* and *C. chuanyujin* are treated as varieties of *C. longa* (Liu and Wu, 1999). *C. phaeocaulis* has been misidentified in the past as *C. zedoaria*, *C. caesia* and *C. aeruginosa* in China (Nian and Wu, 1999).

Correct taxonomic identity of the *Curcuma* species is important for bioprospecting as well as for conservation.

2.1.1. Origin, distribution and diversity

The genus is considered to have originated in the Indo-Malayan region (Purseglove, 1968) and is widely distributed in the tropics of Asia to Africa and Australia. It has a wide spread occurrence from sea level to an altitude as high as two thousand meters in the Western Ghats and Himalayas. *Curcumas* are found to occur in tropical and subtropical forests, margins of forests, open grass lands,

secondary forests, plantations, plains in coconut and arecanut groves. In India *Curcuma* species diversity is concentrated in North Eastern India, South India and Andaman and Nicobar Islands. The other countries rich in *Curcuma* species diversity are China, Thailand, Malaysia, Philippines, Vietnam and Indonesia (Sabu, 1991).

Table 1. *Curcuma* species occurring in India

Sl. No	Species	Sl. No	Species
1	<i>C. aeruginosa</i> Roxb.	21.	<i>C. leucorrhiza</i> Roxb.
2.	<i>C. albiflora</i> Thw.	22.	<i>C. longa</i> L.
3.	<i>C. amada</i> Roxb.	23.	<i>C. lutea</i> Ansari & Nair
4.	<i>C. amarissima</i> Rosc.	24.	<i>C. malabarica</i> Vel. et al.,
5.	<i>C. angustifoila</i> Dalz & Cibs.	25.	<i>C. montana</i> Roxb.
6.	<i>C. aromatica</i> Salisb.	26.	<i>C. neilgherrensis</i> Sp. nov. Vel. et al.,
7.	<i>C. aurantiaca</i> Van Zijp.	27.	<i>C. oligantha</i> Trim.
8.	<i>C. brog</i> Val.	28.	<i>C. petiolata</i> Roxb.
9.	<i>C. caesia</i> Roxb.	29.	<i>C. pseudomontana</i> F.
10.	<i>C. kannanorensis</i> Ansari & Nair	30.	<i>C. purpurea</i> Blatter
11.	<i>C. caulina</i> F.	31.	<i>C. raktakanta</i> Mangaly and Sabu
12.	<i>C. comosa</i> Roxb.	32.	<i>C. reclinata</i> Roxb.
13.	<i>C. coriacea</i> Mangaly & Sabu	33.	<i>C. rubescens</i> Roxb.
14.	<i>C. decipiens</i> Dalz.	34.	<i>C. soloensis</i> Val.
15.	<i>C. ecalcarata</i> Sivarajan and Indu Balachandran	35.	<i>C. sulcata</i> Haines
16.	<i>C. ferrugenia</i> Roxb.	36.	<i>C. sylvatica</i> Val.
17.	<i>C. haritha</i> Mangaly and Sabu	37.	<i>C. thalakaveriensis</i> Vel. et al.,
18.	<i>C. inodora</i> Blat.	38.	<i>C. vamana</i> Mangaly & Sabu
19.	<i>C. karnatakensis</i> Amalraj et al.,	39.	<i>C. xanthorrhiza</i> Roxb.
20.	<i>C. kudagensis</i> Vel. et al.,	40.	<i>C. zeodaria</i> Rosc.

Source: Velayudhan et al., 1996.

2.1.2. Morphology and taxonomy of genus *Curcuma*

The genus *Curcuma* is morphologically highly variable for the different conventional taxonomic traits. *Curcumas* are perennial rhizomatous herbs, 50 – 200 cm tall, and the leafy shoot dying back during the dry period of tropical areas. Rhizomes are ovoid without branches, or branched, fleshy and aromatic. Rhizomes are usually light brown externally, but they can be of different shades of yellow, white, light to deep orange, bluish to deep blue, yellow with greenish borders internally. Roots are many, tubers are present in all *Curcuma* species placed on ends of roots containing mostly starch. A pseudostem formed by closely embracing leaf

sheaths is present in most of the species. It can be pure green or with a deep red or pink tinge low to the ground or rather high up depending on the size and habit of a particular species. The leaves are petiolate or sessile, lamina is usually lanceolate, oblong or ovate, rarely linear, deep green to bright green above and pale green below. Some species have purple or violet streak on the upper side of the lamina, which in some species penetrates to the lower surface.

The inflorescence is either lateral or terminal. Lateral inflorescence usually appears in April – May and central inflorescence in July – September. Flowers are arranged in a cylindrical spike inflorescence formed by bracts. The bracts in the lower portion of the inflorescence are fertile, less coloured often green. The upper bracts usually called coma bracts are larger, longer and brightly coloured. In some species all bracts are of the same colour, their spikes are fertile almost to the top, which are usually smaller. Flowers are arranged in the axil of the bract in a cincinni, bracteoles are thin, elliptic, with the sides inflexed. Calyx short unequally toothed and split nearly half way down one side. Corolla tube plus staminal tube tubular at base, the upper half cup shaped, the corolla lobes inserted on the edges of the cup, the lip, staminodes and stamen just above them. Corolla lobes thin, translucent, white or pink to purplish, staminodes elliptic – oblong, labellum ovate consisting of a thickened yellow middle band. Stamen filaments short and broad constricted at the top, anther versatile, pollen sacs parallel with a curved spur at the base. Ovary trilocular, fruit ellipsoid, thin walled, dehiscent, seeds ellipsoid with a lacerate aril (Holttum, 1950; Sabu, 1991; Ravindran *et al.*, 2007).

Linnaeus (1753) described ten species under five genera of the present day Zingiberaceae including two of the *Curcuma* species namely *C. rotunda* and *C. longa*. Later Roxburgh (1820-1824) in his *Flora Indica* described sixty five species of Zingiberaceae under the class Monandria monogynia distributed over eight genera. Bentham and Hooker (1862-1883) proposed a new classification and included tribe Zingiberæ (zingiberaceae) under the order (Family) Scitamineae. Under Scitamineae they recognized four tribes, Cannaceae, Maranteae, Museae and Zingiberæ and included twentyone genera under the Zingiberæ. Later the authors recognised these tribes as separate families and gave ordinal status to Scitamineae. Petersen (1889) further divided Zingiberæ into three tribes- Hedychieae, Zingiberæ

and Globbeae. Schumann (1904) divided Zingiberaceae into two subfamilies viz, Zingiberoideae and Costoideae. Burt and Smith (1972) modified Schumann's classification and proposed a new classification of the family Zingiberaceae. The family Zingiberaceae is divided into four tribes namely Globbeae, Zingibereae, Hedychieae and Alpineae and the genus *Curcuma* is included in the tribe Hedychieae.

Sirirugsa (1997) studied the Zingiberaceae in Thailand in order to complete the taxonomic revision of the family. More than fifty species of genus *Curcuma* have been found to occur in Thailand with only twenty-six of them being formally identified. The main representatives of the Thai *Curcuma* species include *C. longa* L., *C. parviflora* Wall., *C. xanthorrhiza* Roxb., *C. zedoaria* Rosc., *C. alismatifolia* Gagnep., *C. aurantiaca* Van Zijp., *C. petiolata* Wall., and *C. roscoeana* Wall.

Fourteen different types of *Curcuma* species from Thailand were studied by Paisooksantivatana *et al.* (2001a) based on morphological, palynological and cytological evidences. The results obtained were used to confirm the identity of different *Curcuma* species. The locally called *Curcuma* 'Waan – torahod' and 'Waan chak modluk' were identified as *C. comosa* and *C. bua cham* and *C. bulai prachin* were identified as *C. petiolata* and *C. gracillima*, respectively. *C. bulai lao* which is superficially similar to 'bulai prachin' was identified as a distinct species and the study also supports the classification of genus *Curcuma* into *Eucurcuma* and *Paracurcuma* by Valeton (1918). The study also states that *C. zedoaria* recorded as 'Khamin oi' in Thai literature has a close relationship with *C. longa* and was clustered in the subgenus *Eucurcuma*.

The phylogeny of the tribe Zingibereae based on nuclear ribosomal DNA and chloroplast DNA was performed by Ngambriabsakul *et al.* (2004). The study indicated that the Zingibereae is monophyletic with *Curcuma* and *Hedychium* clade and the genus *Curcuma* is paraphyletic in which infrageneric relationships are more complicated.

A taxonomic and phylogenetic analysis of Zingiberaceae from South India comprising the state of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and union

territories of Mahe and Pondicheri was attempted by Sabu (1991). A total of fifty five taxa of Zingiberaceae belonging to eleven genera are collected from South India including both subfamilies- Costoideae and Zingiberoideae and all tribes of Zingiberoideae viz, Globbeae, Zingibereae, Hedychieae and Alpineae. Among these, the genus *paracautleya* and twelve species including *C. coriaceae*, *C. ecalcarata*, *C. haritha*, *C. kudagensis*, *C. neilgherrensis*, *C. raktakanta*, *C. vamana* and one variety of *C. oligantha* var. *lutea* are reported as endemic to South India. He also reported that five more species are endemic to peninsular India extending to the state of Maharashtra including three *Curcuma* species namely *C. decipiens*, *C. inodora* and *C. pseudomontana*. The work included the identification of eight new taxa from south India of which four *Curcuma* species are new reports namely *C. coriaceae* Mangalay & Sabu, *C. haritha* Mangalay & Sabu, *C. raktakanta* Mangalay & Sabu and *C. vamana* Sabu & Mangalay and described new species *C. aeruginosa* Roxb. to South Indian flora.

Velayudhan *et al.* (1996) proposed a new conspectus of the genus in India. These authors found that the Indian species can be accommodated in the two subgenera of Valeton (1918) viz. *Eucurcuma* and *Paracurcuma*. *Eucurcuma* contains three main sections namely *tuberosa*, *nontuberosa* and *stolonifera* based on the presence or absence of tubers and stolons, each section again encompassing one or more subsections based on floral, vegetative and rhizome characters. Under the subgenus *Paracurcuma*, only the anther spurs lacking species viz. *C. aurantiaca* and *C. ecalcarata* are included.

The more recent studies on the genus are those of Mangaly and Sabu (1993), Velayudhan *et al.* (1999) and molecular taxonomy by Kress *et al.* (2002).

The taxonomic hierarchy of the genus *Curcuma* is presented below (Kress *et al.*, 2002)

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Subfamily	Zingiberoideae
Tribe	Zingibereae
Genus	<i>Curcuma</i>

2.1.3. Intraspecific diversity

Turmeric (*C. longa* L.) is the most economically important species in the genus. It is grown in India, Pakistan, Malaysia, Myanmar, Vietnam, Thailand, Philippines, Japan, China, Korea, Sri Lanka, Nepal, South Pacific Island, East and West Africa, Malagasi, Carribean Islands and Central America. India is the major producer and exporter of turmeric. Turmeric is propagated vegetatively by means of rhizomes. However, unlike in ginger, viable sexual reproduction and true turmeric seedling propagation are there in this species, though *C. longa* is triploid ($2n=63$) (Nambiar *et al.*, 1982; Ramachandran, 1961).

Turmeric is a perennial herb cultivated as annual. The rhizome of turmeric consists of two parts, the central, pear shaped mother rhizome and its lateral, axillary branches known as the fingers. The planting unit (seed rhizome) consists of either a mother rhizome or a complete finger. Usually there is only one main axis which develops into the aerial leafy shoot. The base of the main axis enlarges and becomes the first formed unit of the rhizome, the mother rhizome or the bulb. The bulb undergoes branching, due to the development of axillary buds from the lower nodes of the main axis and give rise to the first order branches, often called the primary fingers. The number of primary fingers usually varies from two to five. The primary

branches grow to some length and then either develop into an aerial shoot or stop growing further. The primary fingers undergo further branching producing secondary and tertiary branches, and these branches do not produce aerial shoots. Roots emerge from the bulbs and sometimes from the primary fingers, but not from the secondary or tertiary fingers. The roots enlarge and become fleshy due to storage of food material. They serve the functions of absorption, anchorage and storage. In most of the species roots terminate in bulbous tubers (Shah and Raju, 1975; Ravindran *et al.*, 2007).

Cultivar diversity of turmeric is highest in India. Most of the cultivars grown in India are popular by their place of cultivation/collection/origin. In India, Southern India, Orissa and North Eastern states are rich in cultivar diversity (Sabu, 1991). A good number of varieties also exist in Bangladesh, Pakistan, Vietnam, Nepal, Myanmar, South Pacific Islands and Malagasi (Purseglove, 1981; Islam, 2004). Velayudhan *et al.* (1999) proposed the existence of twentyone distinct morphotypes in *C. longa*. These morphotypes are grouped into six taxonomic varieties namely *C. longa* var. *typica*, *C. longa* var. *atypica*, *C. longa* var. *camphora*, *C. longa* var. *spiralifolia*, *C. longa* var. *musacifolia*, *C. longa* var. *paltifolia*. Most of the Indian morphotypes belong to *C. longa* var. *atypica*. Cultivar diversity of turmeric reported from India is given in Table 2.

Table 2. Cultivar/variety diversity of turmeric

Sl. No.	Cultivar/variety	Sl. No.	Cultivar/variety
1	'Alleppey'	37	'Lokhande'
2	'Alleppey' Supreme	38	'Megha turmeric'
3	'Amalapuram'	39	'Mundage'
4	'Amrithapani'	40	'Mydukkur'
5	'Armoor'	41	'Nandyal'
6	'Avanigadda'	42	'Pakistan'
7	'Balaga'	43	'Pant Peetabh'
8	'Bilaspur'	44	'Panamalur'
9	'BSR-1'	45	'Pattani'
10	'BSR-2'	46	'Perumnadan'
11	'Bullapura'	47	'Prabha'
12	'Ca-12'	48	'Prathibha'
13	'Ca-72 Udayagiri'	49	'Rajapuri'
14	'Chayapaspu'	50	'Rajendra Sonia'
15	'Chinnanadan'	51	'Ranga'
16	'CLL- 324'	52	'Rasmi'
17	'CLL- 328'	53	'Renuka'
18	'Co-1'	54	'Roma'
19	'Deshi'	55	'Shimla'
20	'Duggirala'	56	'Sobha'
21	'Dughi'	57	'Sona'
22	'Dindigam'	58	'Soni'
23	'Erode Local'	59	'Sudarsana'
24	'Ethamukkala'	60	'Sugandham'
25	'Gorakpur'	61	'Suguna'
26	'GL Puram'	62	'Suranjana'
27	'Guntur'	63	'Suroma'
28	'Jabedi' (G-67)	64	'Suvarna'
29	'Kanthi'	65	'Thalachira'
30	'Kasturi'	66	'Thekkurpetta'
31	'Kasturi Tanaka'	67	'TSundar'
32	'Katpadi Local'	68	'Varna'
33	'Kedaram'	69	'Vombinitta'
34	'Kothapetta'	70	'Wynadan'
35	'Krishna'	71	'Yelachage'
36	'Lekadong'		

2.1.4. Economically important *Curcuma* Species

Curcuma is one of the few genus having diverse uses in fields such as religion, traditional/folk/modern medicines, aromatherapy, cosmetics, dye, floriculture and food industry. Several economically important species are reported from the genus *Curcuma*. *C. longa* L. syn *C. domestica* Val. is the most utilized species. Table 3. gives the list of some economically important *Curcuma* species.

Table 3. Economically important *Curcuma* Spp.

Species	Use
<i>C. longa</i> L. syn <i>C. domestica</i> Val.	Spice, medicine, dye, religious local delicacies, insect repellent, aroma therapy and perfume.
<i>C. amada</i> Roxb and <i>C. mangga</i> Val.& Zijp.	Spice, medicine, pickles and salads
<i>C. zedoaria</i> Roxb.	Folk medicine, arrow root industry
<i>C. ochrorrhiza</i> Val & Van Zijp	Malayan traditional medicine
<i>C. pierreana</i> Gagnep.	Vietnamese traditional medicine
<i>C. aromatica</i> Salisb.	Medicine, toiletry articles, insect repellent
<i>C. kwangsiensis</i> S. G. Lec & C.F.Liang.	Chinese traditional medicine
syn	
<i>C. chuanyujin</i> , and <i>C. phaeocaulis</i> Val.	Chinese traditional medicine
<i>C. caesia</i> Roxb.	Spice and medicine
<i>C. comosa</i> Roxb.	Traditional medicine of Thailand
<i>C. angustifolia</i> Roxb., <i>C. zedoaria</i> Roxb..	
<i>C. caulina</i> F. Grah, <i>C. pseudomontana</i> F. Grah.	Arrow root industry
<i>C. montana</i> Roxb., <i>C. rubescens</i> Roxb.	
<i>C. leucorrhiza</i> , <i>C. xanthorrhiza</i> Roxb.	
<i>C. decipiens</i> Dalz. <i>C. malabarica</i> vel. <i>et al.</i> ,	
<i>C. raktakanta</i> Mangaly & Sabu, <i>C. haritha</i>	
Mangaly& Sabu and <i>C. aeruginosa</i> Roxb.	
<i>C. alismatifolia</i> Gagnep., <i>C. cordata</i> Wal., <i>C. petiolata</i> Roxb., <i>C. gracillima</i> Gagnep., <i>C. thorelii</i> Gagnep., <i>C. parviflora</i> Wall.,	
<i>C. roscoeana</i> Wall., <i>C. pseudomontana</i> F. Grah., <i>C. aurantiaca</i> van Zijp, <i>C. Australasica</i> Hook.f. and <i>C. bicolor</i> Mood & K.Larsen	Ornamental (Cut flower)

Source: (Sasikumar 2005; Kuehny *et al.*, 2002; Skormickova *et al.*, 2007).

2.2. Uses

2.2.1. Religious and cosmetics

The earliest description about turmeric can be seen in *Atharvaveda* and a reference to turmeric has also been made in the *Yajnavalkyasamhitha* at the time of the epic Ramayana (Ravindran *et al.*, 2007). Ethanobotanical evidences indicate that the use of turmeric has been in India since very ancient days connected with the

religious worship and tantric or magic rites of Pre Aryan Indians. This would have made Sopher (1964) to remark that turmeric was rather a material of magic property and colouring source in the beginning and later on it became a medicine and spice. Turmeric assumes ambrosias in India as it is associated with the worship of the Hindu deities such as 'Kali' (Mother Goddess) and 'Naga' (Serpent deity). In Kerala, India, the dried turmeric powder, rice powder and other few plant derived powders are used as colouring materials in making and decorating the huge 'Kalams' of various deities connected with 'Sakthi' worship (Mother Goddess), a folklore tradition even today. 'Kumkum' (mixture of turmeric & lime) a sort of natural vermilion distributed to the devotees from temples, is applied on the forehead, as it is believed to be auspicious by the Hindus. In North India, a red dye locally known as 'pithyan' or 'roli' prepared from turmeric is smeared on the body during religious occasions or ceremonies. Turmeric powder mixed with mustard oil is smeared on the body of the bride and bridegroom in some parts of India. In southern India 'Mangalyasutra' (cotton thread carrying the 'thali' - a heart shaped golden locket - worn around the neck at the time of marriage by Hindu women) is coloured with turmeric. Turmeric is also used in sorcery. It is believed that keeping a piece of turmeric saves a person from evil eyes (Sasikumar, 2005; Ravindran *et al.*, 2007).

The essential oil of the dry leaf of *C. longa* is used as potential oil for application in the perfumery, cosmetics and soap industry (Ramachandraiah *et al.*, 1998). *C. zedoaria* is also used in perfumery in Thailand (Sirirugsa, 1997).

2.2.2. Traditional food and delicacies

Curcuma longa is extensively used in Indian and South East Asian traditional dishes. In Asian countries whole fresh, dry or turmeric powder with other spices is used for making vegetable and meat preparation and soups (Sasikumar, 2001). A traditional delicacy 'Kadbu' (a sweet made from steaming rice paste wrapped in fresh turmeric leaves) is prepared during the Hindu festival of 'Ganesha Chaturthi' in Karnataka, India. Turmeric chutney, turmeric pickle, salad from immature turmeric rhizome, 'tumbli' (coconut-curd based appetizer made with turmeric rhizome) are some of the other turmeric based delicacies in rural India

(Sasikumar, 2005). It is an essential component in curry, curry powders and also used in colouring butter, cheese and vanaspathi (Kapur *et al.*, 1960). Fresh or dried turmeric is used to colour the sacred dish 'nasi kuning' (yellow rice) in the Eastern Islands of Indonesia. Fresh turmeric leaves are used as flavouring agent in some parts of Indonesia. A popular yellow curry paste in Thailand is prepared from the fresh rhizomes of turmeric. Turmeric has also found its application in baked products, dairy products, yogurts, yellow cakes, biscuits, popcorn colour, sweets, cake icings, cereals, sauces, gelatins and direct compression tablets (Balakrishnan, 2007). Turmeric oleoresin is mainly used in brine pickles (Eiserle, 1966; Cripps, 1967) and to relish formulations in non alcoholic beverages such as orangeades and lemonades, in breading of frozen fish sticks, in potato croquettes and in ice creams mainly as a coloring material (Perotti, 1975). *C. zedoaria* is cultivated in countries like Indonesia, Thailand, China and Vietnam primarily for the 'Shoti starch' of commerce by the arrowroot industry. In Thailand the young rhizomes is eaten as a vegetable, pickles and salads, in soups and are used to relieve stomachache. The leaves are used for flavouring fish and other food (Sirirugsa, 1997). *C. aeruginosa* rhizomes are used in south India for the extraction of East Indian arrowroot or Travancore starch (Skornickova *et al.*, 2007). *C. pseudomontana* tubers are boiled and eaten in times of scarcity (Graham, 1839) and *C. amada* rhizomes used as condiment and vegetable (Watt, 1889).

2.2.3. Traditional medicine

In Indian system of medicine, *C. longa* is used as a remedy for conjunctivitis, joint inflammations, as antidiabetes, stomachache, against chronic constipation, irregular menstruation, ring worm infections, eczema, for scars of chicken pox, cough, menorrhagia, piles with inflammation, filariasis, prurigo, chronic otorrhoea, dysentery, and asthma. A paste of turmeric is smeared topically on the head in vertigo, on body sprains, swellings, cuts, wounds, injuries, skin infection, pimples and foul ulcers, common cold, bronchitis and internal fevers, flatulence, indigestion and diarrhea, biliary and hepatic disorders, anorexia and diabetic wounds. Dried turmeric rhizome fume is inhaled for sinusitis, catarrh, coryza and fits. After delivery, women are given a body massage with turmeric powder mixed with mustard oil to relieve inflammatory affections of joints (Shah,

1997). Turmeric powder or paste is smeared on the severed umbilical chord of the newborn babies by the Indian rural folk, as an antiseptic practice. Poultice of turmeric, wheat flour, castor oil and salt is an effective remedy for easing out plant thorns stuck on a toe (Sasikumar, 2005; Ravindran *et al.*, 2007). In tribal medicine, *C. longa* is used for snake bite, burns, concussion and swelling, blood formation, asthma, spider/rat/ poisonous insect/scorpion bites, improving complexion and body smell, ulcers in mouth and intestine, dental pain, diabetes, fracture, headache, ringworm infections, sprain, fever and skin diseases (Remadevi *et al.*, 2007).

C. zedoaria is used as spice and medicine, the fresh root arrests all inflammation of the intestines, purges the kidneys, cures gonorrhea and purges the blood, and the juice of the leaves is a moderate laxative (Burt, 1977). In Bangladesh, a decoction of *C. zedoaria* is used to cure painful bowel movements and is known as a diuretic, a stimulant and to have carminative and antihelmintic properties. It is also applied to bruises and pains (Dymock *et al.*, 1893). *C. zedoaria* is also used as antipyretic, carminative, demulcent, expectorant, stomachic, stimulant and tonic. Fresh rhizomes have diuretic properties and are used in checking leucorrhoea and gonorrheal discharge and for purifying blood, fevers, and cough (Duke, 2003).

C. aeruginosa is used as medicine for stomach disorders and as an ingredient in various cuisines in India. In Malaysia, it is used as a medicine for asthma, cough, and a paste with coconut oil for dandruff and in Indonesia as purgative during childbirth (Skornickova *et al.*, 2007).

C. amada tubers are regarded as cooling and as useful in prurigo. *C. amada* paste with spirit and egg white are applied for chronic rheumatisms and bruises. Roots of *C. amada* are expectorant and astringent used for diarrhea and gleet. (Watt, 1889). *C. amada* rhizome extract and essence showed hypercholesteromic effects in rabbits, strong antibiotic activity against *Aspergillus niger* and *Trichophyton rubrum*, respectively (Pachauri and Mukherjee, 1970; Gupta and Banerjee, 1972). In Ayurveda, *C. amada* is used as an appetizer, alexteric, antipyretic, aphrodisiac and

laxative. It is also used in biliousness, itching, skin diseases, bronchitis, asthma, hiccups and inflammation due to injuries (Remadevi *et al.*, 2007).

C. aromatica rhizomes are used as medicine, as tonic and carminative. Externally used for scabies and eruptions of small pox. The rhizome paste with benzoin is used for headache. It gives a peculiar lively tinge and a delicious fragrance to the naturally dark complexion when applied externally.

C. caesia is used in rheumatic pain and a paste made from rhizome is used to cure blood dysentery. In North East India, it is used in local medicines and as a talisman to keep the evil spirits away (Yusuf *et al.*, 2002).

In Chinese medicine *Curcuma* drugs are mainly used for treating various syndromes due to obstruction of blood circulation and retention of blood stasis such as arthralgia, psychataxia, and dysmenorrhea. In China, “Jianghuang” from the rhizome of *C. longa*, “Pianjianghuang” from the sliced rhizome of *C. wenyujin*, “Ezhu” from the rhizomes of *C. phaeocaulis*, *C. wenyujin* or *C. kwangsiensis*, and “Yujin” from the tubers of *C. wenyujin*, *C. longa*, *C. aromatica*, *C. kwangsiensis* or *C. phaeocaulis* are in use. “Jianghuang,” “Ezhu” and “Yujin” have been used discriminatingly for invigorating blood circulation, promoting digestion to alleviate pain, and as a cholagogic in traditional Chinese medicine, respectively. In Japan, “Gajutsu” from the rhizome of *C. zedoaria*, “Ukon” from the rhizome of *C. longa*, and “Haruukon” from the rhizome of *C. aromatica* are commonly used. “Gajutsu,” “Ukon” and “Haruukon” have been used as a stomachic, a cholagogic, and a health food, respectively. Recent pharmacological studies have demonstrated that the rhizomes of *C. longa* and *C. zedoaria* from China expressed antiallergic and hepatoprotective activities, respectively (Cao *et al.*, 2001; Sasaki *et al.*, 2002; Xia *et al.*, 2005).

2.2.4. Veterinary medicine

Turmeric (*C. longa* L.) plays an important role in traditional veterinary medicine. Fresh turmeric and garlic ground together and mixed in water are given to newly hatched chickens by the rural poultry farmers in India. Turmeric poultice is

applied to broken legs of chickens, ducks and other domestic birds. Young puppies are given a massage with coconut oil impregnated with turmeric powder and sulphur to prevent hair fall. Turmeric ground with neem leaf is applied in case of scabies in cattle and to heal cuts and wounds in cattle, dogs and other animals. Turmeric ointment prepared in ghee and white petroleum jelly is used in the treatment of abscesses and wounds of cattle (Mandal and Chauhan, 2000). Fresh rhizome juice of turmeric is applied to recent wounds, bruises and leech bites in domestic animals. A paste of turmeric and neem leaf is used in ringworm infection, itching, eczema and other parasitic diseases of cattle. A boiled mixture of turmeric, clarified butter, milk and sugar mixed with black pepper, ginger and cinnamon is administered in the case of prurigo, boils, urticaria and chronic skin eruptions of domestic animals (Sharma and Joshi, 2004), in treating skin diseases of camel and buffalo (Chhabra *et al.*, 1994) and subclinical mastitis in cattle (Burgohain and Dutta, 1998). Turmeric is used for controlling endoparasites, external and internal injuries, and pregnancy related problems of ruminants in Trinidad and Tobago (Lans and Brown, 1998). Effective mastitis treatment in cows using gel (Mastelep AV/AMP/4) containing turmeric is reported from India by Buragohain and Dutta (1998). Dietary supplement of turmeric to broiler chicken results in liver parenchymal and portal infiltration of mononuclear cells and hyperaemia of portal vessels, dilatation of bile ducts, mild proliferation of biliary epithelium and periportal hepatocyte degeneration (Sultan and Gameel, 2004). Use of turmeric as feed additive enhances overall performance of broiler chickens by higher body weight gain, better feed conversion, lower fat percentage, higher bursa, thymus and spleen weight, increased count of erythrocytes leukocytes count (Sultan, 2003). Anti-inflammatory activity of the extracts of *C. aromatica* in cattle is also reported (Jangde *et al.*, 1998).

2.2.5. Pharmacognosy

A variety of compounds from *Curcuma* are considered as a mighty cure for a number of ailments. The pharmacological properties of *Curcuma* are mainly attributed to curcumin and essential oil components. In addition to this, several under exploited biologically active compounds from *Curcuma* are also reported. Srinivas *et al.* (1992) isolated a heat stable, water soluble, 5-kDa noncyclic peptide containing fourty amino acid residues, with a blocked N-terminal and leucine at the

C-terminal from *C. longa* L. with protective effect against reactive oxygen species (ROS) induced, lipid peroxide-mediated membrane and DNA damage, mutagenicity and antivenomous properties. Turmerin forms 0.1% of the dry weight of turmeric and insensitive to trypsin and pepsin, heat, and UV radiation. The attenuation of arterial endothelial injury by local application and oral administration of turmerin and inhibition of ATPase (Ca^{2+} , Na/K^{+}) level by turmerin on Concanavalin A stimulated human blood mononuclear T cell are also reported (Cohly *et al.*, 2002 & 2003).

Amadannulen ($\text{C}_{24}\text{H}_{40}\text{O}_3$), a novel compound from mango ginger (*C. amada* Roxb.) rhizome was isolated by Policegoudra *et al.*, 2007. Amadannulen showed antibacterial activity against both Gram-positive and Gram-negative bacteria and bactericidal activity against *M. luteus*, *B. cereus* and *B. subtilis* in addition to the DPPH radical scavenging activity, super oxide radical scavenging activity, lipid peroxidation inhibitory activity and metal chelating activity. Abas *et al.* (2005) isolated a new labdane diterpene glucoside, curcumanggoside from the rhizomes of *C. mangga* with cytotoxic activity against human tumor cell lines.

Three acidic polysaccharides viz., ukonanan A (composed of L arabinose, D xylose, D galactose, D glucose, L rhamnose and D galacturonic acid (12:4:12:1:4:10) with a backbone chain of beta -1,3-linked D-galactose, beta -1,4-linked D-xylose and alpha -1,2-linked L-rhamnose residues) with phagocytosis-activating property, ukonan B (composed of L arabinose, D xylose, D galactose, D glucose, L rhamnose and D galacturonic acid in the ratio 12:4:12:1:2:4) and Ukonan C (composed of beta-1,3-linked D-galactose and beta-1,4-linked D-xylose with or without terminal alpha-L-arabinose units at position three), both having reticuloendothelial system potentiating and alkaline phosphatase inducing activity was isolated from the rhizome of *C. longa* L., by Gonda *et al.* (1990, 1992 & 1993). A neutral polysaccharide, named ukonan D, was isolated from the rhizome of *C. longa* L by Gonda *et al.* (1992). It showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test. ukonan D is composed of L-arabinose: D-galactose: D- glucose: D-mannose in the molar ratio of 1:1:12:0.2, in

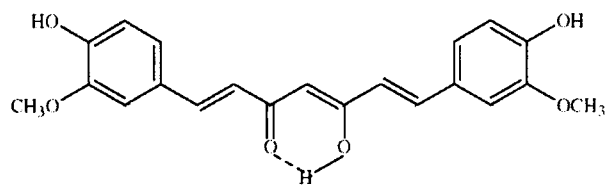
addition to small amounts of peptide moiety. Biological activity of turmeric and its compounds are summarized in Table 4. Fig. 1 gives the structure of some important bioactive components isolated from *Curcuma*.

Table 4. Biological activity of turmeric and its compounds

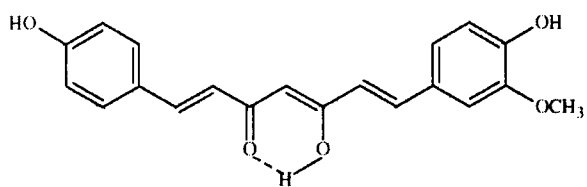
Compounds/ extract	Biological activity	Reference
Turmeric powder	Wound healing	Gujral <i>et al.</i> (1983)
	Antiinflammatory	Yeghnananarayan <i>et al.</i> (1976)
	Hypolipemic	Dixit <i>et al.</i> (1988)
	Antitumour	Kuttan <i>et al.</i> (1985)
	Antiprotozoan	Dhar <i>et al.</i> (1968)
		Rasmussen <i>et al.</i> (2000)
Petroleum extract	Antiinflammatory	Yeghnananarayan <i>et al.</i> (1976)
	Antifertility	Garg (1974)
Alcohol extract	Antibacterial	Bhavani Shankar and Srinivasa Murthy (1979)
Crude ether extract	Antifungal	Misra and Sahu (1977)
Chloroform extract	Antifungal	Misra and Sahu (1977)
Aqueous extract	Antifertility	Garg (1974)
	Antifungal	Kapoor (1998)
Volatile oil	Insect repellency	Tripathi <i>et al.</i> (2001); Pitasawat <i>et al.</i> (2003)
	Antiinflammatory	Leun Mau <i>et al.</i> (2003)
	Antibacterial	Chandra and Gupta (1972)
	Aphid vector control	Bhavani Shankar and Srinivasa Murthy (1979) ; Khanna (1999)
Curcumin	Antifungal	Banerjee and Nigam (1978); Kapoor (1998)
	Antibacterial	Lutomski <i>et al.</i> (1974)
	Antiprotozoan	Bhavani Shankar and Srinivasa Murthy (1979)
	Antitoxicity	Rajakrishnan and Menon (2002)
	Antiviral	Mazumdar <i>et al.</i> (1995),
	Pollution control	Varma <i>et al.</i> (1998)
	Antiinflammatory	Rao <i>et al.</i> (1970); Ramsewak <i>et al.</i> (2000);
	Antiarthritic	Deodar <i>et al.</i> (1980)
	Antithrombotic	Chandra and Gupta (1972)
	Antimutagenic	Srivastava <i>et al.</i> (1985)
		Nagabhushan and Bhide (1987); Polasa <i>et al.</i> (1990)
	Hypolipemic	Rao <i>et al.</i> (1970)
	Hypoglycaemic	Blasiak <i>et al.</i> (1999)
	Anticoagulant	Srivastava <i>et al.</i> (1985)
	Antioxidant	Sharma (1976); Srinivas <i>et al.</i> (1992);
		Ramsewak <i>et al.</i> (2000)
	Estimation of boron in solutions and biological sample	Winner and Goldbatch (1999)
	Antilipid peroxidase	
	Antitumourous	Sharma <i>et al.</i> (1972)
	Anticarcinogenic	Deeb <i>et al.</i> (2003)

	Protects against	Chen <i>et al.</i> (1996)
	a) Pancreatitis	
	b) Endotoxin shock	Gukovsky <i>et al.</i> (2003)
	c) Stress	Madan and Ghosh (2003)
	d) Inflammatory bowel disease	Balaogun <i>et al.</i> (2003)
	e) Alcohol induced liver injury	Salh <i>et al.</i> (2003); Ukil <i>et al.</i> (2003)
	f) Drug induced myocardial toxicity	Nanji <i>et al.</i> (2003)
	g) Cataract formation	Venkatesan <i>et al.</i> (1998)
	h) Drug induced lung injury	Awasthi <i>et al.</i> (1996)
		Venkatesan <i>et al.</i> (1997); Punithavathi <i>et al.</i> (2000)
	i) Scarring	Phan <i>et al.</i> (2003)
	Blocks the replication of HIV	Sui <i>et al.</i> (1993); Mazumder <i>et al.</i> (1997); Barthelemy <i>et al.</i> (1998)
		Lim <i>et al.</i> (2001)
	Prevents Alzheimer's disease	Arun <i>et al.</i> (2002); Babu <i>et al.</i> (1997)
	Anti diabetic	
	Inhibits	Dikshit <i>et al.</i> (1995); Nirmala <i>et al.</i> (1996)
	a) Myocardial infarction	Mehta <i>et al.</i> (1997); Pivocka <i>et al.</i> (2002)
	b) Multidrug resistance	
	c) LDL oxidation	Ramirez-Tortosa <i>et al.</i> (1999); Asai <i>et al.</i> (2001)
	d) Platelet aggregation	Srivastava <i>et al.</i> (1986)
	Hypocholesterolemic	Rao <i>et al.</i> (1970); Patil <i>et al.</i> (1971)
	Stimulates muscle regeneration	Thaloor <i>et al.</i> (1999)
	Modulates multiple sclerosis	Natarajan and Bright (2002)
Turmerin	Antioxidant	Srinivas <i>et al.</i> (1992)
Ar-turmerone	Antivenomous	Ferreira <i>et al.</i> (1992)
Methyl curcumin	Antiprotozoan	Gomes Dde <i>et al.</i> (2002)
Demethoxycurcumin	Antioxidant	Unnikrishnan and Rao (1995)
Bisdimethoxy curcumin	Antioxidant	Unnikrishnan and Rao (1995)
Sodium curcumin	Antiinflammatory and antibacterial	Ramprasad and Sirsi (1956)

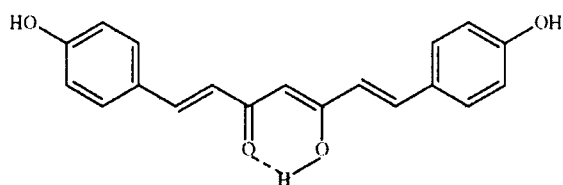
Source: Sasikumar 2005; Compiled from Aggarwal *et al.*, 2007.



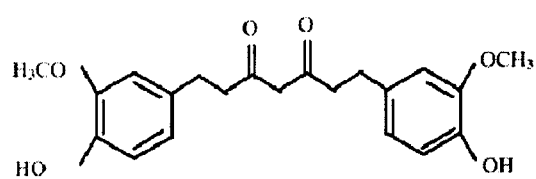
curcumin



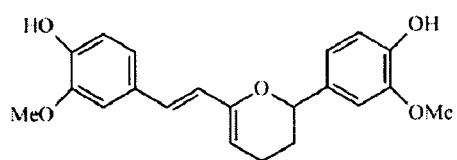
demethoxycurcumin



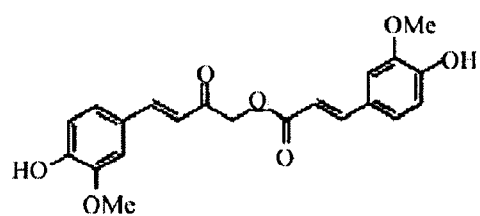
bisdemethoxycurcumin



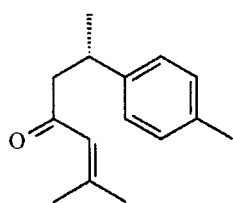
tetrahydrocurcumin



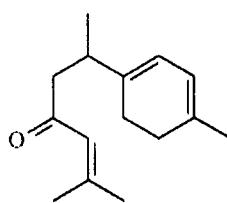
cyclocurcumin



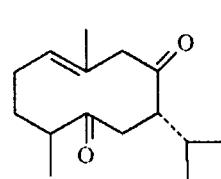
calebin



ar – turmerone



turmerone



curdione

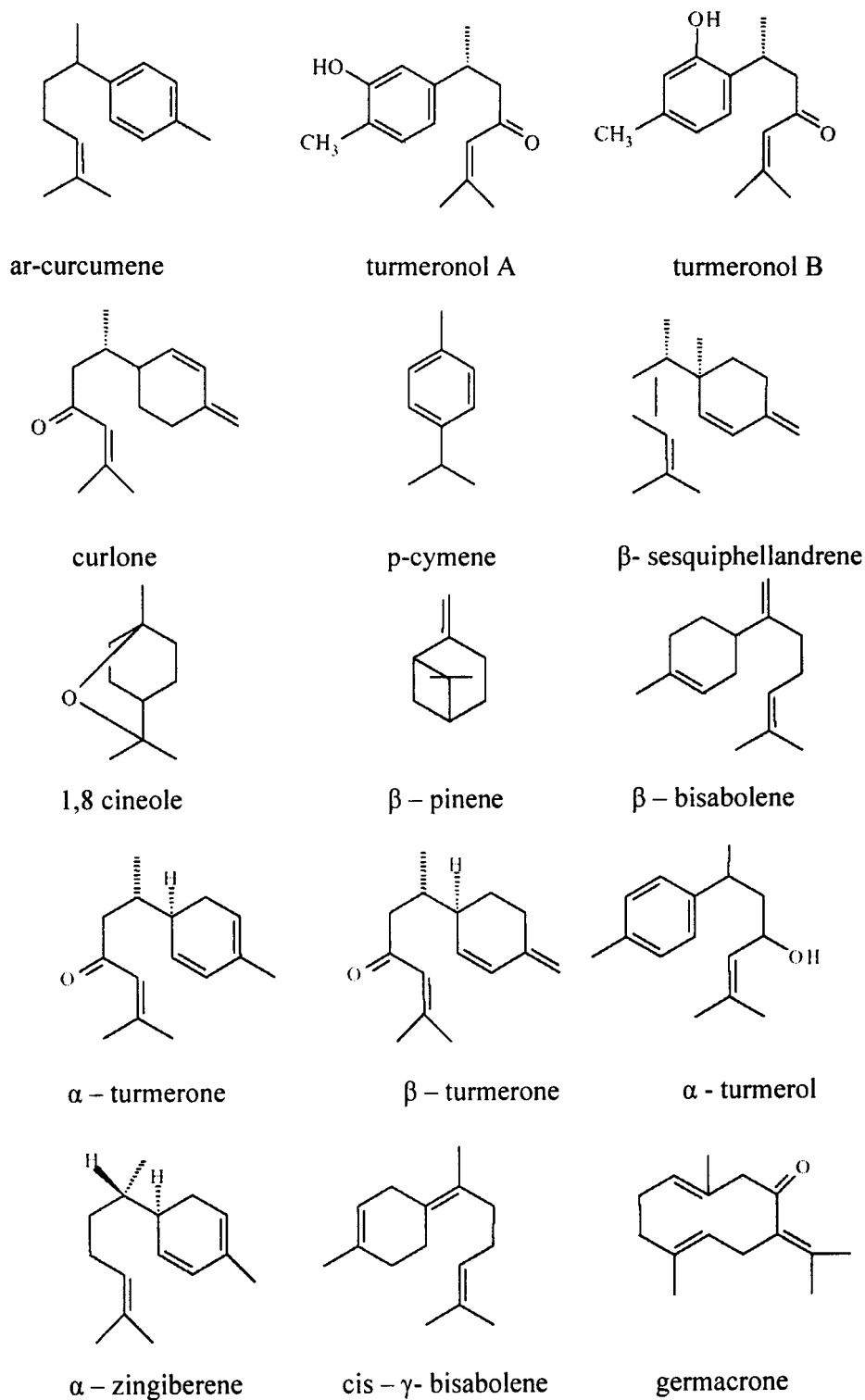


Fig.1. Structure of important biologically active compounds isolated from *Curcuma*.

Hypotriglyceridaemic activity of the active principles of the essential oil and hexane-soluble fractions prepared from *C. xanthorrhiza* was investigated in rats by Yasni *et al.* (1994). Rats fed the essential oil and hexane soluble fraction had lower hepatic fatty acid synthase activity. The fraction containing α -curcumene, prepared from the hexane soluble fraction by silica gel column chromatography, suppressed the synthesis of fatty acids from [14 C] acetate in primary cultured rat hepatocytes. Thus, α -curcumene is one of the active principles exerting triglyceride-lowering activity in *C. xanthorrhiza*.

Mau *et al.* (2003) reported that the essential oil of *C. zedoaria* was moderate to good in antioxidant activities. The study showed that the oil possesses good reducing power and excellent in scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl radical. The antioxidant properties of the oil might be due to the presence of 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone.

2.2.6. Antimicrobial and insecticidal activity

The antimicrobial activity of extracts of *Curcuma zedoaria* and *Curcuma malabarica* tubers was reported by Wilson *et al.* (2005) against six bacterial and two fungal strains. Petroleum ether, hexane, chloroform, acetone and ethanol extracts exhibited antibacterial as well as antifungal activity.

The antimicrobial efficacy of essential oil from *C. caesia* was reported by Garg and Jain (1998). *C. caesia* oil exhibited strong to moderate inhibitory action against *Bacillus subtilis*, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Shigella*, *Aspergillus niger*, *A. fumigatus*, *Curvularia lunata*, *Fusarium psidi* and *Rhizopus oryzae*.

Lai *et al.* (2004) studied the antimicrobial activity and cytotoxicity of the essential oil of *C. zedoaria*. The results suggests that the essential oil has the antimicrobial activity against Gram positive and negative pathogenic microorganisms and the components of the extract lead to the apoptosis of human cancer cell line and thus could inhibit the proliferation of human promyelocytic leukemia HL-60 cells.

Babu *et al.* (2007) studied the antifungal activity of *C. longa* leaf essential oil against fungal pathogens viz. *Fusarium oxysporum* f. sp. *dianthi*, *Alternaria dianthi*, *Fusarium oxysporum* f. sp. *gladioli* and *Curvularia trifolii* f. sp. *gladioli* and showed a significant antifungal activity of the essential oil by inhibiting the mycelial growth of the four pathogens studied.

Fungicidal activity of *C. longa* rhizome derived materials against *Botrytis cineria*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia oryzae*, and *Rhizoctonia solani* was tested by Kim *et al.* (2003) using a whole plant method *in vivo*. It was compared with synthetic fungicides and four commercially available compounds derived from *C. longa*. At 1000 mgL⁻¹, the hexane extract of *C. longa* showed fungicidal activities against *E. graminis*, *P. infestans*, and *R. solani*, and the ethyl acetate extract of *C. longa* showed fungicidal activities against *B. cineria*, *P. infestans*, *Pu. recondita*, and *R. solani*. Curcumin was isolated from the ethyl acetate fraction using chromatographic techniques and also showed fungicidal activities against *P. infestans*, *Pu. recondita*, and *R. solani*.

Antifungal activity of essential oil from *C. longa* (rhizome), *C. zedoaria* (rhizome), *C. aromatica* (leaf) and *C. amada* (rhizome) was reported by Singh *et al.* (2002). Bioassay of these oils indicated *C. longa* as a good antifungal material and cause the complete inhibition of mycelial growth of *Colletotrichum falcatum*, *Fusarium moniliforme* at 1000 ppm and *Curvularia pallescens*, *Aspergillus niger*, *Fusarium oxysporum* at 2000 ppm concentrations. *C. zedoaria* and *C. aromatica* also caused complete inhibition of mycelial growth of *Colletotrichum falcatum* at 2000 ppm and 3000 ppm concentrations, respectively. *C. aromatica* and *C. amada* oils also have good insecticidal action against the sugarcane pest *Odontotermes obesus* Rhamb.

Singh *et al.* (2002) reported the the antifungal activity of rhizome volatile oil *C. zedoaria* against *Colletotrichum falcatum* and also having high insecticidal action against *Odontotermes obesus* Rhamb.

Antifungal activity of leaf essential oil of *C. longa* against human pathogens viz. *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Sporothrix schenckii* and *Microsporum gypseum* was reported by Rath *et al.* (1999).

Larvicidal action of petroleum ether extract of *C. raktakanta* against four mosquito species viz, *Culex quinquefasciatus*, *Culex sitiens*, *Aedes aegypti* and *Anopheles stephensi* was reported by Latha and Ammini, (2000).

Curcuma essential oil is gaining importance for its components with pharmacological importance. The mosquito repellent activity of 95% ethanol extract of *C. aromatica* against *Ae. togoi* on human volunteers was reported by Pitasawat *et al.* (2003). The ethanolic extract of *C. aromatica* was therefore chosen for further insect repellent activity under field conditions, where it had a protective effect against *Armigeres subalbatus*, *Culex quinquefasciatus*, and *Cx. tritaeniorhynchus* and can be applied as an effective personal protection measure against mosquito bites.

Essential oil extracted from the leaves of turmeric was investigated for contact and fumigant toxicity and its effect on progeny production in three stored product beetles, *Rhyzopertha dominica* F., *Sitophilus oryzae* L., and *Tribolium castaneum* Herbst. by Tripathi *et al.* (2001). Oviposition-deterrent and ovicidal actions of *C. longa* leaf oil were also evaluated against *T. castaneum*. *C. longa* oil reduced oviposition and egg hatching and totally suppressed progeny production of all the three test insects.

Larvicidal efficacy of zedoary oil against *Aedes aegypti* mosquitoes were investigated by Champakaew *et al.* (2007) and it was found potential against the fourth instar larvae of *A. aegypti*.

2.3. Characterization of *Curcuma*

2.3.1 Morphological characterization of *Curcuma*

Morphological characterization of thirtyone *Curcuma* species from India was studied by Velayudhan *et al.* (1999) using numerical taxonomy tools. The studies were carried out on sixteen sessile tuberising species and fifteen non sessile tuber bearing species. These authors collected wide data on distribution, habitat, flowering time, floral characters, and quantitative characters of the floral parts, qualitative and quantitative features of aerial and below ground characters of the thirtyone *Curcuma* species. The thirtyone species studied were clustered into nine groups in the

dendrogram and the sessile tuberising species were distinct from species without sessile tubers.

Maciel and Criley (2003) studied morphology, growth and flowering behaviour of *C. zedoaria*. It is observed that from March to April, axillary buds of the corm and epical buds of the third order rhizomes emerge above ground as inflorescence and the flower spike will grow about 30 cm tall and appears just before the foliage. The nodes closest to the flower spike develop vegetative buds and by autumn the above ground foliage dies back. From November to December storage roots are formed with a high content of carbohydrates.

Many authors have reported good variability for yield, yield attributes and quality traits in *C. longa* (Sasikumar *et al.*, 2005; Thomar *et al.*, 2005; Srivastava *et al.*, 2003; Panja *et al.*, 2002; Shanmughasundaram *et al.*, 2001; Chandra *et al.*, 1999; Sasikumar *et al.*, 1996; Sinkar *et al.*, 2005; Singh *et al.*, 2000; Hazra *et al.*, 2000; Hegde *et al.*, 1997; Pujari *et al.*, 1987; Radhakrishnan *et al.*, 1995; Ratnambal, 1986; Philip and Nair, 1983; Philip and Nair, 1986; Rao *et al.*, 2005; Jalgaonkar *et al.*, 1990, Singh *et al.*, 2003; Indiresk *et al.*, 1992).

2.3.2 Molecular characterization of *Curcuma*

Molecular markers are proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. Besides the isozymes markers (Hamrick and Godt, 1990; Loveless and Hamrick, 1984), RAPD technique developed over the past two decades (Williams *et al.*, 1990; Welsh and McCellard, 1990) has been the most employed technique because of its cost effectiveness, simplicity in operation and non requirement of the prior knowledge of the DNA sequences (Schnell *et al.*, 1995). RAPD markers are widely used for the germplasm characterization, genetic diversity analysis population genetic studies, construction of linkage maps, taxonomic reevaluation, sex determination, and development of sequence characterized amplified region (SCAR) (Dongre *et al.*, 2004; Sharma *et al.*, 2002; Due *et al.*, 1999; Yang *et al.*, 2005; Zheng *et al.*, 2003).

Inter Simple Sequence Repeat markers utilize primers that anneal to microsatellites during PCR. ISSR primers are constructed to anneal to microsatellites with one extra nucleotide (anchors) in the 3' end which permits amplification only if the primer binds to a 5' end of the microsatellite with a suitable nucleotide in the flanking sequence (Zietkiewicz *et al.*, 1994). ISSR markers provide more sophisticated analysis of genetic diversity, cultivar identification, population genetic structure and other events of evolutionary biological processes (Gupta *et al.*, 1994; Albertson *et al.*, 1999; Gerber *et al.*, 2000; Hardy, 2003; Hill and Weir, 2004; Jacquemyn *et al.*, 2004; Bornet and Branchard, 2001; Kantety *et al.*, 1995; Pejic *et al.*, 1998; Prevost and Wilkinson, 1999; Fang *et al.*, 1997; Nagaoka and Ogihara, 1997; Metais *et al.*, 2000; Fernandez *et al.*, 2002; Tanyolac, 2003) and are considered superior to RAPD (Quian *et al.*, 2001) and extensively used to characterize genebank accessions, cultivar identification, assessing genetic variation among different taxa at DNA level (Ang Li and Song Ge, 2001; Bornet *et al.*, 2002; Ghariani *et al.*, 2003; Yang *et al.*, 2005; Pomper *et al.*, 2003; Nan *et al.*, 2003; Fang *et al.*, 1997; Blair *et al.*, 1999; Archak *et al.*, 2003).

Molecular marker studies in *Curcuma* are limited. Shamina *et al.* (1998) studied the variability in fifteen *C. longa* accessions collected from different geographical locations in India with respect to six isozymes viz. acid phosphates, superoxide dismutase, esterase, polyphenol oxidase, peroxidase and catalase. Acid phosphatase produced maximum number of bands among the accessions, while polyphenol oxidase and peroxidase were more consistent and reproducible. A high degree of polymorphism (63.8- 96%) was observed in the population studied for these isozymes. Accessions collected from the same geographical area showed highest similarity (above 90%) and two true turmeric seedlings included in the study stood out distinctly from the rest in the dendrogram.

Isozyme markers were used to resolve the taxonomic confusion prevailing in the genus *Curcuma* in Thailand by Apavatjirut *et al.* (1999). Out of the twentyone isozymes tested, eight isozymes namely phosphoglucosyltransferase, glutamic oxaloacetate transaminase, diaphorase, aconitase, esterase, shikimate dehydrogenase, leucine amino peptidase and isocitrate dehydrogenase which showed reliable polymorphism were used to discriminate the *Curcuma* species such as *C. zedoaria*, *C.*

xanthorrhiza, *C. rubescens*, *C. elata*, *C. aeruginosa* along with two unidentified *Curcuma* species. The analysis based on the isozyme data showed that the species *C. rubescens* is distinct from the other species which was in agreement with the morphological observation.

Paisooksantivatana *et al.* (2001b) used allozyme polymorphism at seven loci to study the level of genetic diversity in the natural and domesticated population of *C. alismatifolia* of Thailand and Japan and observed that natural populations are more diverse than the cultivated ones.

Random Amplified Polymorphic DNA (RAPD) based estimations of genetic diversity in *Curcuma zedoaria* from Bangladesh was reported by Islam *et al.* (2007). The study revealed that hilly populations maintain higher genetic diversity, which was also found to be distinct from plain land and plateau land populations.

Hybrid detection and characterization of *Curcuma* species from Thailand using sequence characterized amplified (SCAR) DNA markers was done by Anuntalabhochai *et al.* (2007). The tropical cut flower, *C. alismatifolia* variety 'Patumma' analysed using eleven RAPD primers produced polymorphic bands of size ranged from 100 – 2500 bp. A robust sequence characterized amplified region with 600 bp in length was present in all 'Patumma' varieties and hybrids, which did not amplify in an additional series of 24 distinct *Curcuma* species used as an independent test. The marker produced was useful for cost effective morphologically independent characterization of *Curcuma* hybrids.

Nayak *et al.* (2006) carried out 4 C nuclear DNA content and RAPD analysis of seventeen promising cultivars of turmeric (*C. longa*) from India. RAPD analysis clearly showed the genetic variation among the seventeen cultivars using twenty random decamer primers. The intercultivar polymorphism ranged from 35.6 % to 98.6% among the seventeen cultivars studied and the amplification fragments per primer ranged from four to seventeen with fragment size ranging from 0.4 kb to 3 kb.

Genetic diversity analysis of twenty accessions of *C. longa* from different parts of Brazil using RAPD markers produced fortyfive polymorphic loci and the dendrogram produced by UPGMA grouping using Jaccard's Index of similarity formed two groups. Among the groups, 44. 4% genetic variability was observed and most part of the variation was found within the groups (Pinheiro *et al.*, 2003).

Five species of *Curcuma* namely *C. longa*, *C. zedoaria*, *C. aromatica*, *C. amada* and *C. caesia* were characterized based on the RAPD profiles of DNA isolated from fresh rhizomes by Sreeja (2002). Eleven polymorphic bands were produced in the five species studied using three RAPD markers.

Kress *et al.* (2001) proposed a new phylogenetic analysis of Zingiberaceae based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid *mat K* regions. The results suggest that *Curcuma* is paraphyletic with *Hitchenia*, *Stahlianthus* and *Smithatris*.

Genetic fidelity analysis of eight leaf base callus regenerated plants of turmeric (*C. longa*) using fourteen RAPD primers was done by Salvi *et al.* (2001). The result indicated that lack of genetic fidelity and variation at DNA level has occurred during *in vitro* culture.

Praveen (2005) studied the genetic fidelity analysis of micropropagated plants of *C. longa* using RAPD markers. Plants regenerated from vegetative buds showed high genetic fidelity while the callus regenerated plants showed lack of genetic fidelity.

Genetic stability of twelve month old *in vitro* conserved *C. longa* cv. 'Prathibha' plants was assessed using twentyfive random amplified polymorphic DNA (RAPD) primers by Tyagi *et al.* (2007). No significant variation was observed in RAPD profiles of mother plants and *in vitro* conserved plantlets.

A polymerase chain reaction based method for detection of adulteration of marketed *C. longa* (turmeric) powder with that of a wild species, *C. zedoaria* was developed by Sasikumar *et al.* (2004). These authors postulated the scope for developing species specific SCAR markers for identifying the *Curcuma* species.

DNA and RNA markers are extensively used in Chinese traditional medicine for the identification of particular species in the medicinal preparations. RAPD was used to detect the DNA polymorphism within and among *C. wenyujin*, *C. sichuanensis* and *C. aromatica* from China by Chen *et al.* (1999). It was hard to differentiate between *C. wenyujin* and *C. sichuanensis* at the DNA level. The relationship between *C. wenyujin* and *C. aromatica* was also analyzed. Based on the morphological and chemical data, it was suggested that these two species should be combined into one and that classification based on peduncle central or peduncle lateral may not be right.

Xia *et al.* (2005) used 5S rRNA spacer domain specific primers to ensure the quality of *Rhizoma Curcumae* derived from three species of *Curcuma* namely *C. phaeocalis*, *C. wenyujin* and *C. kwangensis* from their common adulterants. *C. longa* and *C. chanyujin* are identified as the common adulterants used in the *Rhizoma Curcumae*. The phylogenetic tree generated by comparing the sequence identity among the five *Curcuma* species showed that *C. phaeocalis*, *C. kwangensis* and *C. wenyujin* formed a single group with closest homology between *C. phaeocalis* and *C. wenyujin*, *C. longa* and *C. chanyujin* showed only 50 – 55% DNA similarity between them. This study also reports the taxonomic confusion regarding the position of *C. wenyujin* and *C. chuanyujin*, the later growing in the Sichuan province of China is also known as *C. sichuanensis*.

Application of single nucleotide polymorphism analysis based on species specific nucleotide sequence was developed by Sasaki *et al.* (2004) to identify the plants and drugs derived from *C. longa*, *C. phaeocalis*, *C. zedoaria* and *C. aromatica*. Based on the difference in the nucleotide positions at 177, 645, 724 and a 4 base indel on the *trnK* gene obtained using three different lengths of (26 mer, 30 mer & 34 mer) reverse primers helped to identify the four *Curcuma* species studied. The SNP analysis method developed become a useful method for the identification of botanical origins of *Curcuma* drugs such as ‘Ezhu’ used in Chinese medicine, which is difficult to identify morphologically and phytochemically.

An original method for the determination of the botanical origins of Chinese and Japanese *Curcuma* drugs was developed by Sasaki *et al.* (2002). The

Amplification Refraction Mutation System (ARMS) analysis of the 18S rRNA gene using two types of forward primers based on the nucleotide difference at the 234 positions of 18S rRNA gene and *trnK* gene using a mixture of four types of specific reverse primers designed on the basis of base substitution and indels among the six species could clearly identify *Curcuma* plants and the authentication of *Curcuma* drugs. The ARMS method developed using 18S rRNA gene could be applied only to the identification of plants while ARMS method for *trnK* could be applied to identify the botanical origin of *Curcuma* plants and drugs used. Based on the 18S rRNA and *trnK* ARMS twenty five drugs were identified in China.

Cao *et al.* (2001) studied the 18S rRNA gene sequences of six medicinally used *Curcuma* species from China and Japan for authenticating the *Curcuma* drugs. The 18S rRNA gene sequence of *Curcuma* species having 1810 base pair length and is a conserved region among the six *Curcuma* species. Only one base transversion substitution from cytosine to thymine was observed at nucleotide positions 234 in *C. kwangsiensis* when compared to the common sequence of *C. longa*, *C. phaeocaulis*, *C. wenyujin* and *C. aromatica*, while the two *C. zedoaria* populations from China and Japan formed separate group based on the base substitution at position 234. The study also indicated that the length of the *trnK* gene varied from 2698 – 2705 bp and the gene is highly conserved among the six *Curcuma* species particularly in the *matK* region. Based on the *trnK* and 18S rRNA gene studies, the authors confirmed that the Chinese population of *C. zedoaria* will be the same species as *C. phaeocaulis* and the identity of the Japanese population of *C. zedoaria* was doubted.

Chloroplast *trnK* nucleotide sequencing was used to develop a rapid and simple method for identification six medicinal *Curcuma* species viz. *C. longa*, *C. phaeocaulis*, *C. sichuanensis*, *C. chuanyujin*, *C. chuanhuangjiang*, and *C. chuanezhu* from Sichuan Province of China by Komatsu and Cao (2003). They sequenced entire chloroplast *trnK* gene region spanned 2699-2705 bp. The *matK* gene (an intron embodied in *trnK* gene) sequence and the intron spacer region of the *trnK* gene have great diversity within these six medicinal *Curcuma* species. There were six single base substitutions between the *trnK* coding region and the *matK* region, the 9-bp deletion and 4-bp or 14-bp insertion repeat at some sites of *matK*

region in each taxon. These relatively variable sequences were potentially informative in the identification for these six *Curcuma* species at the DNA level.

2.3.3. Biochemical characterization of *Curcuma*

Curcumin, essential oil and oleoresin are the most important biochemical constituents besides the antioxidant peptide turmerin, phagocytosis activating polysaccharide ukonan and amadannulen found in *Curcuma*.

Only about twenty *Curcuma* species have been studied phytochemically (Nahar and Sarker, 2007). The compounds isolated from *Curcuma* species belongs to three major classes, diphenyl alkaloids, phenylpropene derivatives of cinnamic acid type and terpenoids. There are two types of diphenyl alkaloids present in *Curcuma* ie. diphenyl heptanoids and diphenylpentanoids and the former with about twenty two numbers of identified compounds including curcumin and its derivatives constitutes the major class and only two diphenyl pentanoids reported sofar. Monomers and dimmers of phenyl propane derivatives of cinnamic acid types are seen in *Curcuma* but monomeric phenyl propanes are common. Seven monomeric phenyl propanes namely caffeic acid, cinnamic acid, p coumaric acid, p methoxycinnamic acid, cinnamaldehyde, calebin A (ester of ferulic acid and phenyl butane), ethyl p methoxycinnamic acid and three dimeric phenyl propane derivatives such as 1- p coumaroyl cinnamic acid, 1 – feruloyl 2 methoxy cinnamic acid and 1 feruloyl cinnamic acid are present in *Curcuma* (Nahar and Sarker, 2007).

Curcuma longa contains 2–8 % volatile oil, composed mainly of sesquiterpenes such as *a*- and *b*-turmerone, *ar*-turmerone, *a*-curcumene, and zingiberene, and minor amounts of monoterpenes such as cineol (Kapoor, 1990; Leung and Foster, 1996; Sasikumar, 2001), 3–5% curcuminoids (dicinnomoyl derivatives) mainly curcumin (Budavari, 1996; Iwu, 1993), demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin (But *et al.*, 1996; Kiuchi *et al.*, 1993) 3.5% minerals, carotene, vitamin C (Kapoor, 1990; Leung and Foster, 1996), a water soluble peptide (e.g., 0.1% 5–kDa peptide–turmerin) (Srinivas *et al.*, 1992) and 45–55% gelatinized starch, composed of polysaccharides such as the immunologically active arabinogalactans (Bruneton, 1996), particularly the phagocytosis–activating polysaccharides ukonan A and C (Gonda *et al.*, 1992; Gonda *et al.*, 1993). A new

labdane diterpene glucoside, curcumanggoside was isolated from the rhizomes of *C. mangga* by Abas *et al.* (2005). A novel compound amadannulen was isolated from *C. amada* having multisystem antioxidant and antibacterial properties (Policegoudra *et al.*, 2007).

Many reports are available on the extraction, quantification and profiling of essential oil, oleoresin and curcumin from different *Curcuma* species. Oleoresins are generally mixtures of compounds, volatiles and other active ingredients, non volatile fatty and resinous materials obtained by the solvent extraction. Acetone, dichloromethane, 1,2-dichloroethane, methanol, ethanol, isopropanol and light petroleum (hexanes) are the commonly used solvents for the extraction of oleoresins (Adamson, 1971). Turmeric oleoresins are deep brownish orange viscous oily fluids, pasty semisolids or hard amorphous solids containing 37-55% curcuminoids and up to 25% volatile oil. It is mainly used as a colour and flavouring agent. Turmeric oleoresin is insoluble in water and soluble in ethanol will produce pure yellow colour and light green fluorescence.

Krishnamurthy *et al.* (1976) reported that acetone will be the choice of solvent for the extraction of good quality turmeric oleoresin than alcohol and ethylene dichloride. The soxhlet extraction of turmeric powder with acetone for 4 – 5 hrs yielded 5% oleoresin containing 42% of curcuminoids. The prolonged extraction upto 24m hrs yielded a marginal difference in yield of oleoresin (5.7 – 5.9) and curcuminoids (42.2 – 46%). It was also found that the yield of oleoresin and efficiency of extraction of curcumin are higher with the finer ground powder than the coarse ground in soxhlet and cold percolation extraction methods.

Curcumin (diferuloy methane) is a low molecular weight polyphenol that is regarded as the most active constituent and comprises 2-8 % of turmeric (Milobedzka *et al.*, 1910; Heath *et al.*, 2004). Curcumin is a bis- α , β - unsaturated β -diketone. As such, curcumin exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane (Wang *et al.*, 1997). Curcumin has a molecular weight of 368.37 and a melting point of 183°C (Sharma *et al.*, 2005). Three curcuminoids are isolated from turmeric viz., curcumin, demethoxycurcumin and bisdemethoxycurcumin. All three

impart the hallmark yellow pigmentation to the *C. longa* plant and particularly to its rhizomes (Jayaprakasha *et al.*, 2005). Curcuminoids isolated from *C. longa* exhibits strong absorption between 420-430 nm in organic solvents. The methods for assaying curcumin in *Curcuma* products as well as food colour additives are based upon direct spectrophotometric absorption measurements (British Standard Methods of Test for Spices and Condiments, 1983; WHO Food Additive Series, 1976).

Various methods are reported for the extraction of curcumin and curcuminoids from turmeric. The evaluation of total amount of curcuminoids in a sample by use of direct absorption measurements is valid only if the calculations are based on reference value obtained from pure standards. The presence of other compounds absorbing in the region of 420-430 nm influences the results strongly (Jayaprakasha *et al.*, 2005). Direct fluorimetric method for the assay of curcumin in food products was reported by Karasz *et al.* (1973). The difficulties in obtaining reproducible results by spectrophotometric methods are due to the difference in fluorescence intensity of curcumin and the 2-demethoxy compounds in organic solvents at fixed excitation and emission wave lengths (420-470 nm). The relative fluorescence intensities of curcumin, demethoxycurcumin and bisdemethoxycurcumin in ethanol are 1:2.2:10.4 at equimolar concentrations (Tonnessen and Karlsen, 1983). Commercial curcumin/ turmeric products contain mixtures of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Absan *et al.*, 1999). Spectroscopic methods (IR, NMR, MS) are widely used for identification and characterization of curcuminoids. However, it is not possible to quantify the individual curcuminoids with spectrophotometric methods. A pre separation of the curcuminoids can be accomplished by thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) (Tonnesen and Karlsen, 1983).

Separation of the curcuminoids is strongly dependant on the chromatographic conditions. Quantitative analysis of curcumin and related compounds by TLC or HPLC is difficult to carry out unless the chromatographic support is properly deactivated. HPLC systems based on C₁₈ stationary phases did not completely resolve the three curcuminoids (Asakawa *et al.*, 1981; Smith and Witowska, 1984). A reproducible separation of the coloured compounds was achieved by the use of an amino-bonded stationary phase, provided that the water

content of the system is kept below 10%. HPLC system based on an amino-bonded stationary phase, however, seems to have a catalytic effect upon curcumin degradation. To obtain reproducible results the experimental conditions must be carefully controlled (Khurana and Ho, 1988).

Different solvent systems and techniques are used for the extraction of curcumin/curcuminoids. Methanol, acetonitrile and tetrahydrofuran (THF) are the commonly used reverse phase solvents for the extraction of curcuminoids of this methanol didn't provide the necessary resolution. Using THF instead of acetonitrile as the organic modifier reverses the elution order of the curcuminoids (Rouseff, 1988; Smith and Witowska, 1984). Separation of three curcuminoids, using a non silica polymeric column and aqueous 55% acetonitrile as the mobile phase was reported by Taylor and McDowell, (1992). Marsin *et al.* (1993) reported the HPLC analysis of curcuminoids extracted with supercritical CO₂ using acetonitrile-acetate buffer as the mobile phase.

Hiserodt *et al.* (1996) reported the LC-MS and GC-MS methods for the separation of curcuminoids in an octadecyl stationary phase using a mobile phase consisting of ammonium acetate with 5% AcOH and acetonitrile. An online HPLC–UV diode array and electrospray mass spectrometry to analyze curcuminoids and sesquiterpenoids in a fresh turmeric extract was reported He *et al.* (1998). A rapid and simple high performance thin layer chromatographic method has been developed for the simultaneous quantitation of pharmacologically important diaryl heptanoids curcumin, demethoxy curcumin, and bisdemethoxy curcumin in *C. longa* and *C. amada*. The assay combines the isolation and separation of curcuminoids on silica gel 60F₂₅₄ high performance thin layer chromatographic plates, followed by scanning of the spots at 366 nm using a UV detection mode.

Inoue *et al.* (2001) reported a method for evaluation of curcuminoids in turmeric based on a flow injection analysis system with online UV and fluorometric detection. Jayaprakasha *et al.* (2002a&b) reported an HPLC method for the separation and quantification of curcumin, demethoxycurcumin and bisdemethoxycurcumin using a ternary mobile phase, methanol: 2% acetic acid: acetonitrile. The advantage of this method was that individual curcuminoids could

be estimated from the varieties of turmeric rhizomes. LC-MS equipped with electrospray ionization interface is used for the determination of major curcuminoids obtained by solid phase extraction from foods by Inoue *et al.* (2003).

An HPLC method with photodiode array detection was developed to analyse the curcuminoids in rhizomes of *C. mangga*, *C. heyneana*, *C. aeruginosa* and *C. soloensis* from Indonesia by Bos *et al.* (2007). The curcuminoid content was 0.18–0.47% in *C. mangga*, 0.98–3.21% in *C. heyneana*, 0.02–0.03% in *C. aeruginosa* and 0.40% in *C. soloensis*.

The essential oil profile of the different *Curcuma* species are studied by various workers and among them *C. longa* is the most extensively studied species. The essential oil components of *Curcuma* composed mainly of monoterpenes and sesquiterpenes. A great variety of monoterpenes and sesquiterpenes are reported from *Curcuma* but no tri or tetraterpenes. 8, (7), 12 labda diene¹⁵, 16 dial is the only diterpene found in *Curcuma*. The monoterpenes give fragrance to oils and are important in perfumery and flavour industries. About 30 monoterpenes are reported from genus *Curcuma*. *Curcuma* monoterpenes are categorized into cineole derivatives (1,8 - cineole & β - 3 - carene), 2,6 dimethyloctane derivatives (citronellol, linalool, myrcene, nerol, (E) β - ocimene & (Z) β - ocimene, menthane type (P - Cymene, P - Cymene – 8 – ol, carvacrol, limonene, α - phellandrene, β - phellandrene, D - sabinene, α - terpinene, γ - terpinene, terpinolele, terpinen – 4 – ol & α - terpineol and pinene type monoterpenes (α - pinene, β - pinene, borneol, bornyl acetate, isoborneol, isoborneol acetate, camphor and camphene (Nahar and Sarker, 2007).

The sesquiterpenes are fifteen carbon compounds formed from a common precursor farnesyl pyrophosphate by crystallization and skeletal rearrangement. About one hundred and forty sesquiterpenes are isolated from *Curcuma* species and are classified into ten different structural types. But most of them come under the bisabolene type, germacrene type and guaiane type sesquiterpenes. Table 5. give the list of sesquiterpenes and their profile isolated from *Curcuma*, so far.

Table. 5. Sesquiterpenes and their profile in *Curcuma* species

Sl. No.	Sesquiterpene type	Compounds identified
1	Bisabolene type sesquiterpenes	α - atlantone, β - atlantone, turmerone, α - turmerone, β - turmerone, γ - turmerone, α - curcumene, β - curcumene, γ - curcumene, turmeronol A, turmeronol B, xanthorrhizol, zingiberene, β - bisabolene, bisacurone, bisacurone A, bisacurone B, bisacurone C, bisacurool, bisacumol, bisacurone epoxide, 1, 10 bisaboladiene 3,4 - diol, 2,10 bisaboladiene 1, 4 - diol, 1,3,5,10 bisabolapentaen - 9 - ol, 1,3,5,10 bisabolatetraene, 1,3,5,11 bisabolatetraene, bisabola 3,10 diene - 2 - one, 2,5 dihydroxy bisabola 3,10 diene, 4,5 dihydroxybisabola 2,10 - diene, 4 hydroxy 3 methoxy 2,10 bisaboladien - 9 - one, 3 hydroxy 1,10 bisaboladien - 9 - one, 4 hydroxy bisabola 2,10 dien - 9 - one, 4 methoxy 5 hydroxy bisabola 2,10 diene - 9 - one.
2	Cadalene type sesquiterpenes	cadalene quinine, α calacorene, 8 hydroxy cadalene.
3	Cadinane and furanocadinane type sesquiterpenes:	α - cadinol, curzeone and pyrocurzerenone
4	Carabranne type sesquiterpenes	curcumenone, 4 S dihydrocurcumenone, curcurabranol A, curcurabranol B, curcumalactone A, curcumalactone B and curcumalactone C.
5	Curcumane type sesquiterpenes	curmadione, curcumadione, isocurcumadione and curcumenone
6	Elemene type sesquiterpenes	curzerene, curzerenone, 5 epicurzerenone, β - elemene, γ - elemene, elemol and β - elemenone.
7	Eudesmane and furanoeudesmane type sesquiterpenes	β - dictyopetrol, β - eudesmol, α - silenene (α - eudesmene), β - selinene (eudesmane type sesquiterpenes) and curcolonol, curcolone, 1,4 dihydroxyfurano eremophilan - 6 - one are the different eudesmane and furanoeudesmane
8	Germacrane type sesquiterpenes	curdione, acetoxycurdione, dehydrocurdione, 13 hydroxy dehydrocurdione, 13 acetoxycurdione, 3, 4 epoxy 6, 9 germacrane dione, 1R, 10R epoxy- 1,10dihydrocurdione, furanogermenone, furanodiene, furanodienone, germacrone, germacrone 13 al, germacrene B, germacrone 4, 5 epoxide, glechomanolide, 1S, 10S, 4S,5S, germacrone 1, (10) 4 epoxide, 4,5 epoxy 12 hydroxy 1 (10) 7 (11) germacradien - 8 - one, 4,5 epoxy 12 acetoxyc 1 (10) 7 (11) germacradien - 8 - one, 4,5 epoxy 12 acetoxyc 7 α , 11 α dihydrogermacradien - 8 - one, 13 hydroxy germacrone, isofuranodienone, neocurdione, wenjine, zederone, 1 α , 4 β , 5 α , 10 β , 1,10,4,5 diepoxy 7 (11) germacren - 8 - one.
9	Guaiane type sesquiterpenes	isocurcumenol, aerugidiol, alismoxide, curcumol, curcumenol, epicurcumenol, 4 epicurcumenol, oxycurcumenol, neocurcumenol, procumadiol, curcumadiol, procumumenol, isoprocumumenol, 9 oxo neoprocumumenol, neoprocumumenol, gweicurculactone, spathulenol, isospathulenol, 4 hydroxy 7 (11) 10 (14) guaiaien 8 one, 7 α , 11 α epoxy 5 β hydroxyl 9 guaiaen 8 one, zedoarondiol, isozedoarondiol, zedoalactone A, zedoalactone B, 1 β , 4 β , 5 β , 10 β zedoarondiol, methyl zedoarondiol, zedoarol, zedoarolide A, and zedoarolide B.
10	Sesquiterpene dimers	parviflorene A, parviflorene B, parviflorene C, parviflorene D, and parviflorene F.

Source: Compiled from Phytochemistry of the genus *Curcuma*. Nahar and Sarker, 2007).

Other sesquiterpenes found in *Curcuma* are calarene, α - copaene, β - farnesene, farnesol, curcumalactone A, curcumalactone B, curcumalactone C, α - caryophyllene (α - humulene), β - caryophyllene (β - humulene) , gajutsulactone A, gajutsulactone B, β - himachalene, curcumanolide A and curcumanolide B. Eugenol, myrciaphenone A, Phloracetaphenone, Vanillic acid, Syringic acid, malvidin 3 rutinoside, 1,4 methylphenyl ethanol, 3,7 dimethyl 5 indane carboxylic acid are the other pharmacologically important compounds isolated from *Curcuma* (Nahar and Sarker, 2007).

The volatile oil and total curcumin content in dried powder of *C. longa* collected from thirteen locations from North, Northeast, Central and South Thailand was analysed by Pothitirat *et al.* (2006). The study observed that both oil and curcumin content in turmeric varies with location and highest oil content was observed in plants growing in cool climate while highest curcumin content was obtained from South where it rains all year. Lowest curcumin content was found in the Northern samples.

Rhizomes of thirtyeight types of *Curcuma* species were analysed for essential oil and curcumin content by Mathai *et al.* (1976) and a wide variability was observed in the above constituents.

Essential oil was extracted from turmeric (*Curcuma longa*) with supercritical carbon dioxide in a semi continuous -flow extractor by Gopalam *et al.*, 2000. The major components (~60%) of the extracted oil were identified as turmerone and *ar*-turmerone by GC MS.

The essential oil profile in the *Curcuma* species are observed to vary with the explant, species, methodology and locations. The GC MS profile of essential oil from some of the important *Curcuma* species are given in Table. 6.

Table 6. GC MS profiles of essential oil from different *Curcuma* species

Sl. No.	Species	Major compounds identified	Reference
1	<i>C. longa</i>	α -turmerone (44.1%), β -turmerone (18.5%) and ar-turmerone (5.4%)	Raina <i>et al.</i> (2005)
		α -pinene (0.512), β -pinene (0.77), linalool (1.35), caryophyllene (1.02), borneol (1.48), turmerone (11.33), ar-turmerone (30.54) and cis- β -ocimene (9.76).	Behura <i>et al.</i> (2002)
		α -pinene (0.2), myrcene (0.3), α -phellandrene (1.0), δ -3-carene (0.3), α -terpinene (1.4), p-cymene (0.6), 1,8-cineole (2.0), p-cymenene (0.4), terpinolene (15.8), terpinen-4-ol (0.2), cis- α -bergamotene (0.3), β -caryophyllene (5.7), α -humulene (1.4), (E)- β -farnesene (0.6), ar-curcumene (4.5), zingiberene (11.8), β -bisabolene (1.9), β -sesquiphellandrene (8.8), (E)- γ -bisabolene (0.7), (E)-nerolidol (0.2), ar-turmerol (0.3), ar-dihydro turmerone (0.6), ar-turmerone (7.7), α -turmerone (21.4), β -turmerone (7.1) and curcuphenol (0.2)	Chane-Ming <i>et al.</i> (2002)
		ar-turmerone (31.1), curlone (10.6), turmerone (10.0) and ar - curcumene (6.3)	Leela <i>et al.</i> (2002)
		ar-turmerone (51.8%) and ar-turmerol (11.9%)	Singh <i>et al.</i> (2002)
		β -turmerone (30–32), ar-turmerone (17–26) and β -turmerone (15–18)	Sharma <i>et al.</i> (1997)
		ar-turmerone (24.78), turmerone (29.58), turmerol (20.0) and α -atlantone (2.4)	Zwaving and Bos, (1992)
		Root ar-turmerone (46.8), ar-curcumene (7.0) and dehydrocurcumene(4.3)	Leela <i>et al.</i> (2002)
		Flower p-cymen - 8 -ol (26.0), terpinolene (7.4) and 1,8-cineole (4.1)	Leela <i>et al.</i> (2002)
		Leaf α -phellandrene (53.4%), terpinolene (11.5%) and 1,8-cineole (10.5%).	Raina <i>et al.</i>

				(2005)
			α -pinene (2.8), β -pinene (4.0), myrcene (48.8), β -ocimene (E and Z) (2.0), limonene (2.5), 1,8-cineole (6.4), p-cymene (4.3), terpinolene (10.1), methyl eugenol (1.3), eugenol (1.8) and tamesol (1.8)	Behura and Srivastava, (2004)
			myrcene (5.3), limonene (3.6), 1,8-cineole (3.4) and terpinolene (87.8)	Behura and Srivastava, (2004)
			α -pinene (1.45), β -pinene (2.29), 1,8-cineole (10.74), camphor (1.4), linalool (11.95), β -phellandrene (12.61), geraniol (6.72) and cis- β -ocimene (13.76)	Behura et al. (2002)
			α -pinene (2.88), β -pinene (2.36), sabinene (0.4), myrcene (1.17), α -phellandrene (38.24), 1,8-cineole (8.64), p-cymene (6.05), C ₈ -aldehyde (20.58), linalool (0.58), caryophyllene (0.70), geraniol (1.77) and methyl heptanone (0.05)	Behura et al. (2002)
			tricyclene (0.1), α -pinene (0.7), α -fenchene (0.1), sabinene (0.1), β -pinene (0.7), myrcene (1.4), δ -2-carene (0.1), α -phellandrene (2.8), δ -3-carene (1.2), α terpinene (3.7), p-cymene (0.3), 1,8-cineole (4.6), (Z)- β -cineole (0.4), (E)- β -cineloe (0.7), γ -terpinene (0.4), terpinolene (76.8), linalool (0.7), p-mentha-1,3,8-triene (0.2), p-cymene-8-ol (0.2), α -terpineol (0.3), β -caryophyllene (0.1), (E)- β -farnesene (0.1), ar-curcumene (0.1), zingiberene (1.0), β -bisabolene (0.1), β -sesquiphellandrene (0.4), (E) nerolidol (0.1), (Z)- γ -atlantone (0.1) and germacrone (0.1)	Chane-Ming et al. (2002)
			α -phellandrene (32.6), terpinolene (26.0), 1,8-cineole (6.5) and p-cymene (5.9)	Leela et al. (2002)
			α -phellandrene (18.28), α -pinene (14.65), p-cymene (13.35)	Sharma et al. (1997).
2	<i>C. amada</i>	Rhizome	(Z)- β -farnesene (21.9%), guaia-6,9-diene (19.8%), α -guaiene (14.5%), α -longipinene (14.8%), camphor (5.5%) and thymol (ca 4.9%).	Mustafa et al. (2005)

3	<i>C. caesia</i>	Rhizome	α -pinene (0.7), β -pinene (4.64), myrcene (80.54), β -ocimene (E and Z) (2.1), perillene (1.47), limonene (0.13), camphor (trace), curzerenone (0.14), 1,8-cineole (0.06) and camphene (0.18)	Singh <i>et al.</i> (2003)
			α -pinene (0.49), β -pinene (0.72), camphor (0.33), linalool (1.3), β -phellandrene (6.85), turmerone (10.8), ar-turmerone (29.12) and cis- β -ocimene (18.79).	Behura <i>et al.</i> (2000)
			myrcene (80.54%) and β - pinene (4.64%),	Singh <i>et al.</i> (2002)
			α -pinene (0.4), β -pinene (0.6), myrcene (0.2), limonene (0.4), camphor (11.2), β -elemene (2.8), isoborneol (4.5), β -curcumene (11.2), curzerenone (7.1), ar-curcumene (28.1), α -pinene (6.0), linalool (0.4), zingiberene (1.4) and camphene (0.18)	Srivastava <i>et al.</i> (2001)
			α -pinene (0.9), β -pinene (4.9), myrcene (0.2), β -ocimene (E and Z) (0.4), perillene (0.4), limonene (0.1) and α -pinene (0.1)	Choudhury <i>et al.</i> (1996)
			α -pinene (18.0), β -ocimene (E and Z) (47.2), linalool (11.2), linalyl acetate (9.1) and safrole (9.3)	Dutt and Tayal, (1941)
		Rhizome	camphor (28.3%), ar-turmerone (12.3%), (Z)- β -ocimene (8.2%), ar-curcumene (6.8%), 1,8-cineole (5.3%), β -elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and γ -curcumene (2.82%)	Pandey & Chowdhury, (2003)
			α -pinene (0.40), β -pinene (0.60), β -ocimene (E and Z) (2.1), camphor (7.73), linalool (0.99), caryophyllene (3.15), borneol (4.3), camphene (1.67), anethole (1.79) and cis- β -ocimene (14.54)	Behura <i>et al.</i> (2002)
		Leaf	α -pinene (1.5), β -pinene (6.3), myrcene (0.5), limonene (2.1), 1,8-cineole (27.0), camphor (1.68), linalool (2.8), β -elemene (2.4), borneol (8.7), α -terpenol (5.2) and eugenol (2.0)	Behura and Srivastava, (2004)
			camphor (32.3), curzerenone (11.0), α -turmerone (6.7), ar-turmerone (6.3) and 1,8-cineole (5.5)	Bordoloi <i>et al.</i> (1999)

			curdione, germacrone, 1,8-cineole, germacrone- 4,5-epoxide, β - elemene and linalool 1,8-cineole (9.3), camphor (25.6), germacrone (10.6), isoborneol (8.2), camphene (7.4) and curzerenone (10.9) ar-curcumene (18.6), β -curcumene (25.5) and xanthorrhizol (25.7) α -pinene (4.8), α -camphene (2.7), β -pinene (5.8), azulene (0.5), l-zingiberene (10.7), 1-ar-curcumene (14.6), 1- β -curcumene (33.8), camphor (3.2), α -terpineol (7.8), cuminyl alcohol (5.5), de-borneol (5.3) and zingiberol (3.5) α -pinene (4.77), β -pinene (3.7), myrcene (0.39), 1,8-cineole (28.01), p-cymene (1.45), caryophyllene (2.01), α -phellandrene (1.40), C₈-aldehyde (2.62) and geraniol (1.28) α -Pinene (4.77), β -pinene (3.7), sabinene (0.68) myrcene (0.39), α -phellandrene (1.4), 1,8-cineole (28.01), p-cymene (1.45), C₈-aldehyde (2.62), linalool (7.67), caryophyllene (2.01) and geraniol (1.28) p-cymene (25.2%) and 1,8-cineole (24.8%)	Kojima <i>et al.</i> (1998) Choudhury <i>et al.</i> (1996) Zwaving and Bos, (1992) Rao and Nigam, (1974) Singh <i>et al.</i> (2003) Behura <i>et al.</i> (2002) Singh <i>et al.</i> (2002)
5	<i>C. haritha</i>	Rhizome	camphor (25.58), ar-turmenene (13.2), curzerenone (6.2), 1,8-cineole (6.0) and α -turmerone (2.5) 1,8-cineole (20), camphor (18), germacrone (11.8), isoborneol (6.4), camphene (9.4) and limonene (8.6) α -pinene (1.75), β -pinene (6.67), p-cymol (2.89), camphor (21.24), camphene (14.59), borneol (1.37), terpinyl acetate (2.43), pentadecane (1.84), turmerone (5.39), ar-turmerone (9.63) and ethyl p-methoxycinnamate (3.53)	Bordoloi <i>et al.</i> (1999) Choudhury <i>et al.</i> (1996) Dan <i>et al.</i> (2002)
6	<i>C. raktakanta</i>	Rhizome	α - pinene (5.25), β - pinene (13.42), camphor (12.78), terpinyl acetate (3.43), bornyl acetate (2.82), ethyl cinnamate (4.23), caryophyllene (11.25), tridecane (3.87), turmerone (5.31) and ethyl p-methoxycinnamate	Dan <i>et al.</i> (2002)

			(16.57)	
7	<i>C. aeruginosa</i>	Rhizome	curzerenone (24.68), 1,8-cineole (11.6), camphor (16.6), zedoarol (6.3), isocurcumenol (5.8), curcumenol (5.6) and furanogermanone (5.5) (total 26 compounds)	Sirat <i>et al.</i> (1998)
			isocurcumenol (8.5), β - eudesmol (6.5), curdione (3.6), curcumenol (9.9), curcumenolides A, B (11.4), dehydrocurdione (9.4) and curcumenone (1.9)	Zwaving and Bos, (1992)
		Leaf	1,8-Cineole (17.7), curzerenone (10.5), furanogermenone (7.0), camphor (7.5), (2)-3-hexanol (5.08), furanodienone (5.1), curcumenol (4.5), isocurcumenol (3.7) and β -elemene (3.3)	Jirovetz <i>et al.</i> (2000)
			curzerenone (16.2), germacrone (3.6), 1,8-cineole (13.5) and camphor (5.7) (total 25 compounds)	Nguyen <i>et al.</i> (1995a)
8	<i>C. xanthorrhiza</i>	Rhizome	ar-curcumene (41.4) and xanthorrhizol (21.5)	Zwaving and Bos, (1992)
9	<i>C. pierreana</i>	Rhizome	isoborneol (22.9) and isobornyl acetate (18.8)	Nguyen <i>et al.</i> (1995b)
		Stem	isoborneol (12.4), isobronyl acetate (14.4), β -caryophyllene (10.4) and Z- β -farnesene (10.8)	Nguyen <i>et al.</i> (1995b)
		Leaf	camphor (13.0), and isoborneol (12.8)	Nguyen <i>et al.</i> (1995b)
10	<i>C. trichosanthes</i>	Rhizome	curdione (47.48), curcumol (7.0) and germacrone (6.1)	Pham <i>et al.</i> (1994)
11	<i>C. mangga</i>	Rhizome	myrcene (78.68), (E)- β -ocimene (5.1), β -pinene (3.7) and pinene (2.9)	Wong <i>et al.</i> (1999)
12	<i>C. ochrorhiza</i>	Rhizome	furanogermenone (53.24), germacrone (9.62), β -elemene (8.84), camphor (6.31) and α -isofuranodiene (5.6)	Sirat <i>et al.</i> (1997)
13	<i>C. harmandii</i>	Mother rhizome	1, 8-cineole, (4.5), germacrone (20.5) β -pinene (1.2), β -elemene (6.5) and isocurcumenol (13.4)	Nguyen <i>et al.</i> (1997)

		Finger rhizome	1, 8-cineole, (12.5), germacrone (9.0) and curdione (22.6), β -elemene (11.3) and α -isocurcumenol (3.7)	Nguyen <i>et al.</i> (1997)
		Stem	1, 8-cineole, (21.8), germacrone (15.5) and curdione (25.3)	Nguyen <i>et al.</i> (1997)
		Leaf	1, 8-cineole, (13.5), germacrone (11.5) and curdione (36.8)	Nguyen <i>et al.</i> (1997)
14	<i>C. heyneana</i>	Rhizome	1,8-cineole/limonene (14.2), isocurcumenol (7.4), β - eudesmol (4.78), curcumanolides A, B (13.18), dehydrocurdione (10.2) and curcumenone (2.3)	Zwaving and Bos, (1992)
15	<i>C. zedoaria</i>	Rhizome	β -tumerone (19.88%), 1,8-cineole (8.93%), and 7-zingiberene (7.84%) curzerenone (22.3%), 1,8-cineole (15.9%) and germacrone (9.0%). 1,8-cineole (15.8%) and β -eudesmol (10.6%) epicurzerenone (46.6) and curdione (13.7) epicurzerenone(24.1) and curzerene (10.4%). 1,8-cineole (18.5%), o- and p-cymene (18.42%), α -phellandrene (14.93%), terpinolene (4.11%), α -pinene (3.28%), β -turmerone (3.1%), β -pinene (2.93%) and β -phellandrene (2.0%). 1,8 cineol (18.5%), cymene (18.42%) and α -phellandrene (14.9%)	Champakaew <i>et al.</i> (2007) Purkayastha <i>et al.</i> (2006) Yonzon <i>et al.</i> (2005) Lai <i>et al.</i> (2004) Mau <i>et al.</i> (2003) Singh <i>et al.</i> (2003) Singh <i>et al.</i> (2002)
		Leaf	α -terpinyl acetate (8.4%), isoborneol (7%), dehydrocurdione (9%) and selina-4(15), 7(11)-dien-8-one (9.4%)	Garg <i>et al.</i> (2005)

Materials and Methods

Chapter 3

MATERIALS AND METHODS

3. 1. Plant material

The present study was conducted at the Indian Institute of Spices Research, Kozhikode and its experimental farm at Peruvannamuzhi, Kozhikode, Kerala from 2003 through 2006. Fifteen *Curcuma* species (Fig. 2- 3), thirty six turmeric (*Curcuma longa* L.) cultivars/varieties and twelve open pollinated progenies of turmeric available in the turmeric germplasm conservatory were used in the study. The list of *Curcuma* accessions used in the study is given in Table (7 – 9).

Table 7. *Curcuma* species used in the study

Sl. No	Species	Sl. No	Species
1	<i>Curcuma amada</i> Roxb.	9	<i>Curcuma haritha</i> Mangalay & Sabu
2	* <i>Curcuma aromatica</i> ^a Salisb.	10	<i>Curcuma longa</i> L.
3	* <i>Curcuma aromatica</i> ^b Salisb.	11	<i>Curcuma montana</i> Wall.
4	<i>Curcuma aeruginosa</i> Roxb.	12	<i>Curcuma malabarica</i> Vel et al.
5	<i>Curcuma caesia</i> Roxb.	13	<i>Curcuma pseudomontana</i> Grah.
6	<i>Curcuma comosa</i> Roxb.	14	<i>Curcuma raktakanta</i> Mangalay & Sabu
7	<i>Curcuma decipiens</i> Dalz.	15	<i>Curcuma amada</i> Roxb.
8	<i>Curcuma</i> species	16	<i>Curcuma zedoaria</i> Rosc.

* *C. aromatica*^a collected from NBPGR, Trissur and *C. aromatica*^b collected from 'Wynad' are maintained as separate entities in the IISR field genebank, Peruvannamuzhi, Calicut, Kerala.

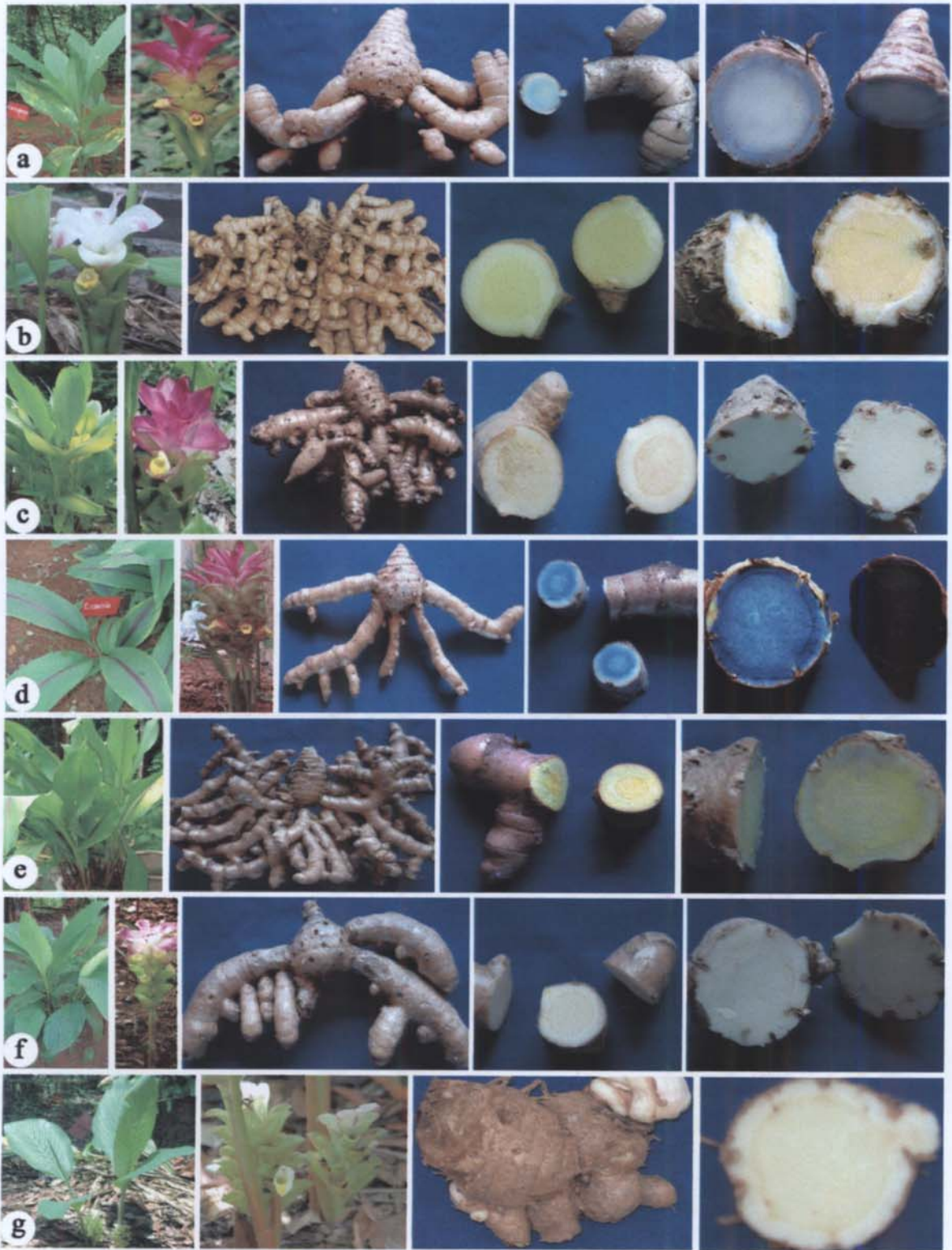


Fig. 2. Representative photographs of different *Curcuma* species studied. a - g are *C. aeruginosa*, *C. amada*, *C. aromatica*, *C. caesia*, *C. comosa*, *C. haritha* and *Curcuma* species, respectively.



Fig. 3. Representative photographs of different *Curcuma* species studied. a - g are *C. longa*, *C. malabarica*, *C. montana*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

Table 8. Turmeric cultivars/varieties used in the study

Sl. No	Acc. No.	Cultivar name	Sl. No	Acc. No.	Cultivar name
1	01	'Alleppey'	19	34	'Nandyal type'
2	02	'Amalapuram'	20	35	'Rajapuri'
3	06	'Amrithapani'	21	37	'Sugandham'
4	08	'Armoor'	22	40	'Vondimita'
5	10	'Jabedi'	23	42	'Wynad'
6	11	'Dhagi'	24	44	'Aizwal'
7	12	'Katergia'	25	52	'Ayur'
8	14	'Chayapasupu'	26	54	'Basar Along'
9	17	'Avanigadda'	27	71	'Gaspani'
10	19	'Dindigam'	28	72	'Hajo'
11	21	'Ethamkula'	29	75	'Jorhat'
12	23	'GL Puram'	30	98	'Suvarna'
13	27	Karhadi	31	119	'Sudarsana'
14	28	'Kasturi'	32	126	'Kedaram'
15	30	'Kodur'	33	143	'Suguna'
16	31	'Kuchipudi'	34	360	'Prathibha'
17	32	'Mamomthody'	35	361	'Prabha'
18	33	'Muvatupuzha'	36	585	'Alleppey Supreme'

Table 9. Open pollinated progenies of turmeric used in the study

Sl. No.	Acc. No.	Progeny No./Mother clone
1	363	Seedling progeny 'Muvatupuzha'
2	367	Seedling progeny 'Alleppey'
3	375	Seedling progeny 3/ 'Amrithapani'
4	383	Seedling progeny 'Katergia'
5	384	Seedling progeny 'Dindigam'
6	389	Seedling progeny 'Kasturi'
7	405	Seedling progeny 23/ 'Ayur'
8	408	Seedling progeny 'Sugandham'
9	428	Seedling progeny 'Aizwal'
10	429	Seedling progeny 'Amalapuram'
11	446	Seedling progeny 2/ 'Jabedi'
12	450	Seedling progeny 'Avanigadda'

The various aspects studied in fifteen *Curcuma* species, thirty six turmeric cultivars/varieties and twelve open pollinated progenies of turmeric are listed in Table 10.

Table 10. Various aspects studied and material used

Sl. No.	Material	Aspects
1	<i>Curcuma</i> species	Morphological characterization of fifteen <i>Curcuma</i> species using aerial/underground morphological characters. Molecular characterization using RAPD, ISSR markers and 18S rRNA sequence analysis Analysis of fresh rhizome/leaf oil content from twelve <i>Curcuma</i> species GC MS analysis of fresh rhizome oil from eleven <i>Curcuma</i> species Total curcumin and curcuminoids levels in six <i>Curcuma</i> species Extraction and quantification of turmerin from fifteen <i>Curcuma</i> species. Peptide mapping of rhizome proteins from thirteen <i>Curcuma</i> species using HPLC.
2	Turmeric varieties/ cultivars	Morphological characterization of thirtysix turmeric cultivar/varieties using aerial/underground morphological characters. Molecular characterization of cultivar/varieties using RAPD and ISSR markers Molecular characterization of selected turmeric varieties/cultivars (twelve No.) using fresh rhizome DNA Assessment of fresh leaf oil, dried rhizome oil, total curcumin and oleoresin percentage in thirtysix turmeric cultivar/varieties GC MS analysis of fresh leaf oil from ten selected cultivars/varieties Curcuminoid profiling of 36 turmeric cultivar/varieties using HPLC Peptide mapping of rhizome proteins from ten selected cultivars/varieties using HPLC. Extraction and quantification of turmerin from fifteen selected cultivars/varieties of turmeric.
3	Open pollinated progenies of turmeric	Morphological characterization of twelve open pollinated progenies of turmeric using aerial/underground morphological characters. Molecular characterization of twelve open pollinated progenies of turmeric using RAPD and ISSR markers

3.2. Characterization of *Curcuma*

3.2.1. Morphological characterization

Morphological characterization was carried out on fully grown plants established in the germplasm fields of Indian Institute of Spices Research,

Experimental farm, Peruvannamuzhi, Kozhikode. The plants were grown in cement tubs (45 x 45 x 45 cm) as per the standard package of practices. Details of the qualitative and quantitative characters used for the study are given in Table 11.

Table 11. List of morphological characters studied

Sl. No	Character	Unit	Abbreviation
1	Number of tillers per clump	number	NTC
2	Number of leaves in main tiller	number	NLM
3	Number of leaves per clump	number	NLC
4	Height of pseudostem	cm	HP
5	Leaf length	cm	LL
6	Leaf breadth	cm	LB
7	Pseudostem girth	cm	PG
8	Colour of leaf sheath	colour	CLS
9	Leaf midrib colour	colour	LMC
10	Leaf texture	texture	LT
11	Inner core colour	colour	ICC
12	Internode length	cm	INL
13	Clump weight	g	CW
14	Number of mother rhizome	number	NM
15	Weight of mother rhizome	g	WM
16	Number of primary rhizome	number	NP
17	Weight of primary rhizome	g	WP
18	Number of secondary rhizome	number	NS
19	Weight of secondary rhizome	g	WS
20	Aroma of rhizome	odour	AR
21	Flavour/Taste of rhizome	taste	F/T
22	Dry recovery	g	Dry

3.2.1.1. Aerial morphological characters

Aerial morphological data was recorded at full maturity (200 - 210 days after planting). The characters studied from three random plants per accession were recorded.

3.2.1.1.1. Height of pseudostem (Plant height) (HP)

Height of the main tiller was recorded as plant height. The height of the pseudostem from the ground level to the crown was measured.

3.2.1.1.2. Pseudostem girth (PG)

Girth of the three main tillers was recorded as pseudostem girth and the mean values computed.

3.2.1.1.3. Number of tillers (NTC)

Total number of tillers per clump was recorded and the mean worked out.

3.2.1.1.4. Number of leaves per tiller (NLM)

The total number of leaves present in the main tiller was recorded and the average of three was computed.

3.2.1.1.5. Number of leaves per clump (NLC)

Total number leaves present in the clump were recorded and the mean was calculated.

3.2.1.1.6. Leaf length (LL)

Length of the 3rd and 4th leaf (from the top) in the main tiller was recorded and the average was calculated.

3.2.1.1.7. Leaf breadth (LB)

Breadth of the middle portion of the leaf (3rd and 4th leaf) was recorded as leaf breadth.

3.2.1.1.8. Colour of leaf sheath (CLS)

Colour of the leaf sheath was recorded by visual observation.

3.2.1.1.9. Leaf midrib colour (LMC)

The colour of the leaf mid rib was recorded by visual observation.

3.2.1.1.10. Leaf texture (LT)

The texture of the leaf lamina was recorded as pubescent or glabrous based on visual observation or physical feeling.

3.2.1.2 Rhizome characters

The rhizome characters were recorded after harvest. The characters studied from three random plants per accession were recorded.

3.2.1.2.1. Clump weight /Weight of rhizome (CW)

The whole weight of fresh rhizome was recorded as clump weight

3.2.1.2.2. Number of mother rhizome (NM)

Number of mother rhizomes per clump was recorded and the mean worked out.

3.2.1.2.3. Weight of mother rhizome (WM)

Weight of mother rhizomes per clump was recorded and its mean calculated.

3.2.1.2.4. Number of primary rhizome (NP)

Number of primary rhizomes per clump was recorded.

3.2.1.2.5. Weight of primary rhizome (WP)

Total weight of primary rhizomes per clump was recorded as weight of primary rhizomes.

3.2.1.2.6. Number of secondary rhizome (NS)

Number of secondary rhizomes per clump was recorded.

3.2.1.2.7. Weight of secondary rhizome (WS)

Total weight of secondary rhizomes per clump was recorded as weight of secondary rhizome.

3.2.1.2.8. Inter node length (INL)

Internodal length of the primary rhizomes was recorded and the average of ten observations was computed.

3.2.1.2.9. Inner core colour (ICC)

Inner core colour of the primary rhizomes was recorded visually.

3.2.1.2.10. Aroma of rhizome (AR)

Aroma of the rhizome was recorded based on olfactory evaluation.

3.2.1.2.11. Flavour/Taste of rhizome (F/T)

The flavour/taste of the fresh matured rhizomes was recorded by organoleptic analysis.

3.2.1.3. Floral characters

3.2.1.3.1. Spike position

The position of the spike at emergence was recorded either lateral or terminal, visually.

3.2.1.3.2. Colour of the fertile bract

Colour of the fertile bract was recorded by visual observation.

3.2.1.3.3. Colour of the coma bract

Colour of the coma bract was recorded by visual observation.

3.2.1.3.4. Colour of the corolla

Colour of the corolla was recorded by visual observation.

3.2.1.3.5. Colour of the labellum

Colour of the labellum was recorded by visual observation.

3.2.1.3.6. Anther spur

Presence or absence of anther spur in the flowers was recorded by visual observation.

3.2.2. Molecular characterization

3.2.2.1. Isolation of DNA

High quality genomic DNA was isolated from both fresh leaves and matured fresh rhizomes.

3.2.2.1.1. Isolation of DNA from young leaves

Fresh and young leaves of all the genotypes were selected for DNA isolation. The leaves were washed thoroughly with distilled water and wiped with tissue paper. Two grams of the leaves were weighed and used for DNA isolation. Genomic DNA from different *Curcuma* species, turmeric varieties/cultivars and open pollinated progenies of turmeric were isolated using the modified CTAB method of Doyle & Doyle, (1987). The procedure is as follows:

- (1) Grind two grams of cleaned leaf tissue in liquid nitrogen to a fine powder using pre-chilled mortar and pestle.
- (2) Transfer the powder to 50 ml Oakridge tube containing 8 ml preheated CTAB extraction buffer.
- (3) Incubate the sample at 65° C for 30-60 minutes with occasional mixing by gentle swirling.
- (4) Add equal volume of Chloroform: Isoamyl alcohol (24:1) and mix by gentle swirling. Spin at 12,000 g for ten minutes at 4°C.
- (5) Transfer the aqueous phase to fresh tubes with cut tips.
- (6) Add 2/3 volume of Isopropanol and mix by gentle inversions and keep at room temperature for 30 min.
- (7) Scoop out the DNA strands into eppendorf tubes containing 1 ml of 70 % ethanol. Then, spin at 8000 g for 2 - 5min, discard the supernatant and vacuum dry for 20 min and dissolve in 500 µl of TE buffer/nuclease free water.

3.2.2.1.1.1. Purification of genomic DNA

The isolated DNA was purified as below:

- Add 10 µg ml⁻¹ of RNAase A and incubated at 37°C for 30 minutes in a waterbath.

- Add equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1), mix well and centrifuge at 14,000 g for 15 minutes.
- Take the aqueous phase and extract with equal volume chloroform: isoamyl alcohol (24:1) and centrifuge at 14,000 rpm for 15 minutes.
- To the aqueous phase add equal volume of chilled 100 % ethanol and incubate at 4°C for 1 hour and centrifuge at 8,000 rpm for 5 minutes.
- Decant the supernatant carefully, wash pellet with 70% ethanol.
- Air dry the pellet and dissolve in nuclease free water.

3.2.2.1.2. Isolation of DNA from fresh matured rhizomes

Fresh matured rhizomes were washed in sterile distilled water, scraped, and sliced into thin pieces. Genomic DNA from fresh mature rhizomes was isolated using the modified CTAB method of Syamkumar *et al.* (2003) as described below:

- (1) Grind the sliced rhizome samples (4 g fresh tissue) in liquid nitrogen.
- (2) Transfer to a 50ml polypropylene tube. Add 3 ml of freshly prepared preheated extraction buffer per gram of tissue.
- (3) Incubate at 60°C in a water bath for 45-60 min, with occasional mixing.
- (4) Add an equal volume of chloroform-isoamylalcohol (24:1). Mix by inversion for 15 min.
- (5) Spin at 8410 g at 25-30°C for 10 min. Carefully transfer the clear upper aqueous layer to another 50ml polypropylene tube. Add 0.6 volume of ice cold ethanol.
- (6) Incubate at -20°C for 2 h. Careful mixing will produce floating fibrous DNA, which can be separated by centrifugation at 13,141 g at 25-30°C for 10 min.
- (7) Discard the supernatant and wash the pellet with 80% ethanol.
- (8) Dry the pellet in a vacuum for 15 min. Dissolve in sterile double distilled water.
- (9) Add 10 µg l⁻¹ of RNase A and incubate at 37°C for 30 min.
- (10) Extract with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1) and mix by inversion for 15 min. Centrifuge at 18,922 g at 25-30°C for 15 min.

- (11) Transfer the aqueous layer. Add an equal volume of chloroform-isoamylalcohol (24:1) and mix by inversion for 15 min. Centrifuge at 18,922 g at 25-30°C for 15 min.
- (12) Transfer the aqueous layer to a fresh 2ml Eppendorf tube. Add an equal volume of ice cold 100% ethanol to precipitate the DNA.
- (13) Spin at 8410 g at 25-30°C for 5 min.
- (14) Discard the supernatant and wash the pellet with 80% ethanol.
- (15) Dry the pellet in a vacuum and dissolve in nuclease free water.

3.2.2.2. Quantification of DNA

3.2.2.2.1. Agarose gel analysis

Quantity of the DNA was estimated by comparing the intensity of the DNA band of the samples with that of the standard λ DNA marker (Genei, Bangalore, India). Agarose gel electrophoresis was carried out in a maxi submarine electrophoresis system (Genei, India) using a programmable electrophoresis power supply PS 9009 TX (Apelex, Germany) as below:

- Fix the tapes to ends of gel tray. Keep the combs and place the gel tray horizontally.
- Prepare 0.8 % agarose in 1 X TAE (0.8 g agarose in 100 ml 1 X TAE). Boil the solution in a microwave oven until all the agarose particles are completely dissolved. Allow to cool (60°C).
- Add 2 μ l of ethidium bromide (10 mg ml⁻¹) and pour to gel tray.
- After 20 minutes remove the tape and comb, place the gel in to the tank and pour 1 X TAE until the gel is fully immersed.
- Load the DNA samples with loading dye (6 X) in to the wells with a standard marker DNA.
- Run gel at 50 – 60 volts.
- Visualize the DNA bands on a UV transilluminator and document gel using a Gel Documentation system (Alpha Imager 2200).

3.2.2.2.2. Spectrophotometric analysis

Spectrophotometer based DNA quantification was done by measuring the absorbance of the DNA at 260 nm and 280 nm using a Biophotometer (Eppendorf, Germany).

3.2.2.3. Quality analysis of DNA

Quality of the DNA was estimated using three different methods - agarose gel electrophoresis, spectrophotometric analysis and restriction digestion.

3.2.2.3.1. Gel based analysis

The quality of DNA extracted was visualized on 0.8% agarose gel.

3.2.2.3.2. Spectrophotometer based analysis

The quality of the isolated DNA was detected by estimating the $A_{260/280}$ ratio using a spectrophotometer.

3.2.2.3.3. Restriction digestion

The quality of the isolated DNA was assayed by digesting the DNA with suitable restriction enzymes. 2.0 μ g of DNA was digested with *Eco*R I and *Hind* III according to the manufacturer's instructions. The fragments were visualized on 1% agarose gel along with standard DNA ladder (1 kb DNA ladder, Biogene, USA).

3.2.2.4. DNA amplification

The isolated DNA was amplified using RAPD, ISSR and 18S rRNA primers.

3.2.2.4.1. RAPD analysis

RAPD reaction was performed as per the method suggested by Williams *et al.*, (1990) with minor modifications.

3.2.2.4.1.1. Optimization of PCR components for RAPD analysis

By keeping the assay buffer concentration constant (1 X), concentrations of all other PCR components for RAPD reactions such as template DNA, dNTP's, *Taq* DNA polymerase, primer and $MgCl_2$ were tested in different concentrations in 25 μ l total reaction volume.

- Template DNA concentration – A concentration of 10 - 50 ng DNA was used.
- *Taq* DNA polymerase – the enzyme concentration taken was 0.5 U – 2 U (Bangalore Genei, India).
- dNTPs – final concentrations of 0.1 mM – 0.3 mM were used (Bangalore Genei, India).
- $MgCl_2$ – a final concentration of 1.5 – 2.5 mM $MgCl_2$ per reaction. (Bangalore Genei, India).

- Primers –Primer concentration of 5 - 20 picomoles per reaction (Operon Technologies, USA).

3.2.2.4.1.2. *PCR protocol for RAPD analysis*

To a 0.2 ml thin walled PCR tube add the following in order.

- Nuclease free water - 16.92 μ l
- 10X reaction buffer - 2.5 μ l
- $MgCl_2$ (50 mM μ l⁻¹) - 0.25 μ l
- dNTP mix (10 mM μ l⁻¹) - 0.5 μ l
- Primer (5 pico moles μ l⁻¹) - 2.0 μ l
- *Taq* DNA polymerase (3 U μ l⁻¹) - 0.33 μ l
- Template DNA (10 ng μ l⁻¹) - 2.5 μ l

3.2.2.4.1.3. *Optimization of PCR programming*

Different temperature profiles and cycle repeats were tested for optimizing the PCR. For RAPD-PCR amplification, the following temperature profile was found to be optimum.

- Denaturation at 94°C for 3 minutes.
 - Denaturation at 94°C for 1 minute.
 - Annealing at 37°C for 1 minute.
 - Extension at 72°C for 1 minute.
 - Final extension at 72°C for 10 minutes.
 - Refrigerate at 4°C.
- } 34 cycles

3.2.2.4.1.4. *Screening of primers for RAPD analysis*

After optimizing the PCR conditions and variables, one hundred random decamer primers (Operon Technologies, USA) were screened using ‘Alleppey’ and ‘Prabha’ DNA as templates. Primers from OPA, OPB, OPC, OPD and OPJ series were used for the screening and 68 primers were selected for the subsequent amplification in fifteen *Curcuma* species, thirtysix turmeric varieties/cultivars and twelve open pollinated progenies (Table 12).

Table 12. Operon primers used for RAPD analysis

Sl. No.	Primer	Sequence (5' – 3')	GC content (%)	Sl. No.	Primer	Sequence (5' – 3')	GC content (%)
1	OPA 02	TGCCGAGCTG	70	35	OPC 09	CTCACCGTCC	70
2	OPA 03	AGTCAGCCAC	60	36	OPC 10	TGTCTGGGTG	60
3	OPA 04	AATCGGGCTG	60	37	OPC 11	AAAGCTGCGG	60
4	OPA 05	AGGGGTCTTG	60	38	OPC 12	TGTCATCCCC	60
5	OPA 07	GAAACGGGTG	60	39	OPC 13	AAGCCTCGTC	60
6	OPA 08	GTGACGTAGG	60	40	OPC 14	TGCGTGCTTG	60
7	OPA 09	GGGTAACGCC	70	41	OPC 16	CACACTCCAG	60
8	OPA 10	GTGATCGCAG	60	42	OPC 18	TGAGTGGGTG	60
9	OPA 11	CAATCGCCGT	60	43	OPC 20	ACTTCGCCAC	60
10	OPA 12	TCGGCGATAG	60	44	OPD 01	ACCGCGAAGG	70
11	OPA 13	CAGCACCCAC	70	45	OPD 02	GGACCCAACC	70
12	OPA 14	TCTGTGCTGG	60	46	OPD 03	GTCGCCGTCA	70
13	OPA 15	TTCCGAACCC	60	47	OPD 04	TCTGGTGAGG	60
14	OPA 17	GACCGTTGT	60	48	OPD 05	TGAGCGGACA	60
15	OPA 18	AGGTGACCGT	60	49	OPD 06	ACCTGAACGG	60
16	OPA 19	CAAACGTCGG	60	50	OPD 07	TTGGCACGGG	70
17	OPA 20	GTTGCGATCC	60	51	OPD 08	GTGTGCCCCA	70
18	OPB 01	GTTTCGCTCC	60	52	OPD 09	CTCTGGAGAC	60
19	OPB 02	TGATCCCTGG	60	53	OPD 11	AGCGCCATTG	60
20	OPB 07	GGTGACGCAG	70	54	OPD 12	CACCGTATCC	60
21	OPB 08	GTCCACACGG	70	55	OPD 13	GGGGTGACGA	70
22	OPB 09	TGGGGGACTC	70	56	OPD 14	CTTCCCCAAG	60
23	OPB 10	CTGCTGGGAC	70	57	OPD 15	CATCCGTGCT	60
24	OPB 11	GTAGACCCGT	60	58	OPD 16	AGGGCGTAAG	60
25	OPB 12	CCTTGACGCA	60	59	OPD 18	GAGAGCCAAC	60
26	OPB 14	TCCGCTCTGG	70	60	OPJ 05	CTCCATGGGG	70
27	OPB 16	TTTGCCCGGA	60	61	OPJ 07	CCTCTCGACA	60
28	OPB 18	CCACAGCAGT	60	62	OPJ 09	TGAGCCTCAC	60
29	OPB 19	ACCCCCGAAG	70	63	OPJ 10	AAGCCCGAGG	70
30	OPC 03	GGGGGTCTTT	60	64	OPJ 11	ACTCCTGCGA	60
31	OPC 05	GATGACCGCC	70	65	OPJ 13	CCACACTACC	60
32	OPC 06	GAACGGACTC	60	66	OPJ 16	CTGCTTAGGG	60
33	OPC 07	GTCCCGACGA	70	67	OPJ 17	ACGCCAGTTC	60
34	OPC 08	TGGACCGGTG	70	68	OPJ 18	TGGTCGCAGA	60

3.2.2.4.2 . ISSR analysis

ISSR reaction was performed as per the method suggested by Zeitzkiewicz *et al.*, (1994) with minor modifications.

3.2.2.4.2.1. Optimization of PCR components for ISSR analysis

Concentrations of all PCR components for ISSR reactions such as template DNA, dNTP's, *Taq* DNA polymerase, primer and MgCl₂ were tested in different concentrations keeping the assay buffer concentration constant (1X) in 25µl reaction volume.

- Template DNA concentration – A concentration of 10 - 80 ng DNA was used.

- *Taq* DNA polymerase – the enzyme concentration taken was 0.5 U-2 U (Bangalore Genei, India).
- dNTPs – final concentrations of 0.1 - 0.3 mM were used (Bangalore Genei, India).
- $MgCl_2$ – a final concentration of 1.5 - 2.5 mM $MgCl_2$ per reaction. (Bangalore Genei, India).
- Primers –Primer concentration of 10- 80 picomoles per reaction (Integrated DNA Technologies, USA).

3.2.2.4.2.2. *PCR protocol for ISSR analysis*

To a 0.2 ml thin walled PCR tube add the following in order.

- | | |
|--|-----------------|
| • Nuclease free water | - 14.42 μ l |
| • 10 X reaction buffer | - 2.50 μ l |
| • $MgCl_2$ (50 mM μ l ⁻¹) | - 0.25 μ l |
| • d NTP mix (10 mM μ l ⁻¹) | - 0.50 μ l |
| • Primer (10 pico moles μ l ⁻¹) | - 3.00 μ l |
| • <i>Taq</i> DNA polymerase (3 U μ l ⁻¹) | - 0.33 μ l |
| • Template DNA (10 ng μ l ⁻¹) | - 4.00 μ l |

3.2.2.4.2.3 . *Optimization of PCR programming*

Different temperature profiles and cycle repeats were tested for optimizing the PCR. For ISSR PCR amplification the following temperature profile was found to be optimum.

- | | |
|---|-------------|
| • Denaturation at 94°C for 3 minutes. | } 31 cycles |
| • Denaturation at 94°C for 1 minute. | |
| • Annealing at 50°C for 1 minute. | |
| • Extension at 72°C for 1 minute. | |
| • Final extension at 72°C for 10 minutes. | |
| • Refrigerate at 4°C. | |

3.2.2.4.2.4. *Screening of primers for ISSR analysis*

Thirty two ISSR primers synthesized from Integrated Technologies USA were screened using “Alleppey” and “Prabha” DNA as templates. Eighteen selected primers were used for subsequent amplification (Table 13).

Table 13. Primers used for ISSR reaction

Sl. No.	Primer	Sequence (5' – 3')	GC content (%)
1	ISSR 1	(CT) ₈ TG	50.0
2	ISSR 2	(CT) ₈ AC	50.0
3	ISSR 3	(TCC) ₅ AG	64.7
4	ISSR 4	(AGC) ₄ GT	64.3
5	ISSR 5	(CAC) ₃ GC	63.6
6	ISSR 6	(CTC) ₃ GC	63.6
7	ISSR 7	(GACA) ₃	50.0
8	ISSR 8	(GACA) ₃ GC	50.0
9	ISSR9	(TC) ₇ G	53.3
10	ISSR10	(CT) ₇ TG	50.0
11	ISSR11	(AGTG) ₃ TT	42.9
12	ISSR12	(CCCT) ₄	81.3
13	ISSR13	(AGTG) ₃	50.0
14	ISSR14	(AG) ₈ T	47.1
15	ISSR15	(TC) ₈ C	52.9
16	ISSR16	(AC) ₇ T	47.1
17	ISSR17	(GA) ₇ A	47.1
18	ISSR18	(GA) ₈ T	47.1

3.2.2.4.3. 18S rRNA amplification

Amplification of 18S rRNA gene was performed according to Cao *et al.*, (2001).

3.2.2.4.3.1. Optimization of PCR components for 18S rRNA amplification

All the PCR components for the 18S rRNA gene amplification such as template DNA, dNTP's, *Taq* DNA polymerase, primer and MgCl₂ except the assay buffer concentration (1X) were tested in different concentrations in 50 µl total reaction volume.

- Template DNA concentration – A concentration of 20 - 150 ng DNA was used.
- *Taq* DNA polymerase – the enzyme concentration taken was 0.5 U - 2 U (Bangalore Genei, India).
- dNTPs – final concentrations of 0.1 mM-0.5 mM were used (Bangalore Genei, India).
- MgCl₂ – a final concentration of 1.5 - 2.5 mM MgCl₂ per reaction. (Bangalore Genei, India).
- Primers – Primer concentration of 20-100 picomoles per reaction were used. (Integrated DNA Technologies, USA).

3.2.2.4.3.2. PCR protocol for 18S rRNA amplification

To a 0.2 ml thin walled PCR tube add the following in order.

- Nuclease free water - 31.17 μ l
- 10 X reaction buffer - 5.00 μ l
- dNTP mix (10 mM μ l⁻¹) - 0.50 μ l
- Primer (10 pico moles μ l⁻¹) - 4.00 μ l each of forward & reverse primer
- *Taq* DNA polymerase (3 U μ l⁻¹) - 0.33 μ l
- Template DNA (10 ng μ l⁻¹) - 5.00 μ l

3.2.2.4.3.3. PCR programming

For 18S RNA gene amplification the following temperature profile was used.

- Denaturation at 94°C for 4 minutes.
- Denaturation at 94°C for 40 seconds
- Annealing & Extension at 65°C for 8 minutes. } 30 cycles
- Final extension at 72°C for 30 minutes.
- Refrigerate at 4°C.

3.2.2.4.4. 18S rRNA restriction analysis

10 μ l of the amplified product was digested with different restriction enzymes according to the manufacturer's instruction. The restricted products were loaded on a 2 % agarose gel and visualized by ethidium bromide staining under the UV light. List of restriction enzymes used for the analysis are given in Table 14.

Table 14. Restriction enzymes used for 18S rRNA restriction analysis

Sl. No.	Enzyme	Restriction site
1	<i>Bam</i> H I	G↓GATCC CCTAG↑G
2	<i>Hae</i> III	GG↓CC CC↑GG
3	<i>Msp</i> I	C↓CGG GGC↑C
4	<i>Rsa</i> I	GT↓AC CA↑TG

3.2.2.4.5. Sequence retrieval and primer designing

Partial nucleotide sequences of 18S rRNA gene from twenty species of *Curcuma* were retrieved from the NCBI GenBank. Multiple sequence alignments

were made using Clustal X (version 1.81) (Thomson *et al.*, 1997). Details of the nucleotide sequences retrived from the GenBank alongwith their accession number and sizes are listed in Table 15.

Table 15. Details of the nucleotide sequences of 18s rRNA gene retrived from the GenBank

Sl. No.	Accession No.	Species	Size
1	AB047724	<i>Curcuma xanthorrhiza</i>	1810 bp
2	AB047727	<i>Curcuma zedoaria</i> (Japan)	1810 bp
3	AB047717	<i>Curcuma kwangsiensis</i>	1810 bp
4	AB047725	<i>Curcuma yunnanensis</i>	1810 bp
5	AB047722	<i>Curcuma sichuanensis</i>	1810 bp
6	AB047721	<i>Curcuma roscoeana</i>	1810 bp
7	AB047715	<i>Curcuma exigua</i>	1810 bp
8	AB047714	<i>Curcuma elata</i>	1810 bp
9	AB047713	<i>Curcuma chuanyujin</i>	1810 bp
10	AB047712	<i>Curcuma chuanhuangjiang</i>	1810 bp
11	AB047711	<i>Curcuma chuanhezhu</i>	1810 bp
12	AB047708	<i>Curcuma aromatica</i>	1810 bp
13	AB047707	<i>Curcuma sichuanensis</i>	1810 bp
14	AB047726	<i>Curcuma zedoaria</i> (China)	1810 bp
15	AB047723	<i>Curcuma wenyujin</i>	1810 bp
16	AB047720	<i>Curcuma phaeocaulis</i>	1810 bp
17	AB047718	<i>Curcuma longa</i>	1810 bp
18	AB047710	<i>Curcuma aromatica</i>	1810 bp
19	AB261849	<i>Curcuma aeruginosa</i>	1758bp
20	AB261848	<i>Curcuma zedoaria</i>	1758bp

Three pairs of compatible internal primers (forward & reverse) were designed to amplify 18S rRNA fragment into three overlapping fragments from selected *Curcuma* species manually and with the aid of Primer 3 (Rozen and Skaletsky, 2000). The specificity of the designed primers was assessed by performing a BLASTn (Altschul *et al.*, 1990). Sequence of the internal primers used to amplify *Curcuma* 18S rRNA genes are listed in Table16.

Table 16. Sequence of the internal primers used to amplify *Curcuma* 18S rRNA gene

Sl. No.	Primer	Sequence (5' - 3')	GC content (%)
1	18S F1	CAACCTGGTTGATCCTGCCAGT	55
2	18S F2	GGTGCCAGCAGCCGCGGTAATTC	65
3	18S F3	GGGCACCACCAGGAGTGGAGC	71
4	18S R1	GACCGATCGACTCAACCCAAGG	59
5	18S R2	GTCTGGACCTGGTAAGTTTCCCCGTG	58
6	18S R3	CTGATCCTTCTGCAGGTTCACTAC	50

3.2.2.4.6. PCR amplification

PCR amplification of the internal sequence of 18S rRNA gene was done as follows.

3.2.2.4.6.1. PCR components

Nuclease free water	- 30.67 μ l
10 X reaction buffer	- 5.00 μ l
dNTP mix (10 mM μ l ⁻¹)	- 1.0 μ l
Primer (10 pico moles μ l ⁻¹)	- 4.00 μ l each of forward & reverse primer
Taq DNA polymerase (3 U μ l ⁻¹)	- 0.33 μ l
Template DNA (10 ng μ l ⁻¹)	- 5.00 μ l

3.2.2.4.6.2. PCR Conditions

Denaturation at 94°C for 4 minutes.

Denaturation at 94°C for 1 minute	} 30 cycles
Annealing at 65°C for 1 minute	
Extension at 72°C for 1 minute	

Final extension at 72°C for 15 minutes.

Refrigerate at 4°C.

3.2.2.4.7. Elution of DNA fragments from gel

The desired DNA fragments from agarosed gel was excised and purified using the Perfect Prep Gel Cleanup kit (Eppendorf), Germany as follows.

3.2.2.4.7.1. Protocol

1. Excise the desired ethidium bromide stained DNA fragment from agarose gel.
2. Weigh gel slice in a microcentrifuge tube, add 3 volumes of binding buffer for every volume of the slice.
3. Incubate at 50°C for 5 to 10 minutes, vortexing every 2 to 3 minutes.
4. After the gel slice has completely dissolved, add one volume of isopropanol equal to the original gel slice volume. Mix by inversion or pipetting up and down.
5. Place a spin column in a 2 ml collection tube.
6. Add up to 800 μ l of sample to the spin column. Centrifuge the spin column/collection tube assembly at 6000 - 10000 g for one minute. Discard

- the filtrate and replace the spin column in the same collection tube. If the sample is larger than 800 µl, reload and spin again.
7. Add 750 µl of diluted wash buffer to the spin column. (Combine one part wash buffer concentrate with four parts 95-100% ethanol).
 8. Centrifuge the spin column/collection tube assembly for 1 minute at 6000 - 10000 g
 9. Discard the filtrate and replace the spin column in the same collection tube.
 10. Centrifuge the spin column/collection tube assembly for an additional minute at 6000 - 10000 g to remove any residual diluted wash buffer.
 11. Place the spin column in a fresh 2 ml collection tube.
 12. Add 30 µl of elution buffer or molecular biology grade water to the center of the spin column and centrifuge for 1 minute at 6000 - 10000 g.
 13. Discard the spin column and cap the collection tube, store the DNA at -20°C for future use.

3.2.2.5. DNA sequencing

The DNA sequencing was done using a Beckman CEQ 8000 machine at Bioserve Biotechnologies India, Pvt. Ltd, Hyderabad, India.

3.2.2.5.1. Sequence analysis

The overlapping sequences obtained by sequencing of fifteen *Curcuma* species were edited manually to obtain the partial 18s rRNA nucleotide sequence. These sequences were aligned with the sequences retrieved from NCBI GenBank using ClustalX (Thomson *et al.*, 1997). Phylograms were constructed by Neighbourhood joining boot strap method in Clustal X (version 1.81) and rooted trees were generated using TREE VIEW software (Page, 1996).

3.3. Biochemical characterization

3.3.1. Extraction of volatile oil

The steam volatile oil from *Curcuma* species and turmeric varieties/cultivars were extracted using a modified Clevenger method. The rhizomes were harvested at full maturity and stored at ambient temperature for two weeks before being distilled. Prior to distillation, the fresh rhizomes were washed and chopped into pieces. Equal parts of rhizome from the clump were pooled and used for hydrodistillation. Fresh

matured leaves started yellowing was used for the leaf oil extraction. 25 g of fresh turmeric rhizome or 20 g fresh leaf sample was accurately weighed and transferred to a short neck one litre round bottom flask with 500 ml water. The apparatus was assembled using the proper Clevenger trap (ASTA, 1978) and was boiled for 2 ½ hrs. Oil was collected in the trap until two consecutive readings taken at one hour intervals showed no change of oil volume in the trap, cool to room temperature, and allowed to stand until the oil layer is clear. Read the volume of the oil collected, estimating to the nearest 0.02 ml. The oil extracted was collected into a 2 ml Eppendorf tube, sealed with parafilm and refrigerated until the time of analysis. The percentage of volatile oil was calculated as below.

$$\text{Volatile oil, \% (v/w)} = \frac{\text{Vol. of oil} \times 100}{\text{Wt. of samples (g)}}$$

3.3.1.1. GC MS analysis of essential oil

The analytical system used for GC MS analysis was an HP6890 series 11 gas chromatograph interfaced with a HP 5973 mass spectrometer GCD series11 (GC-EID) detector, equipped with a split less injector (250 °C) and a DB-5 Phenyl methyl siloxane column (30m x 320µm x 0.25µm). The carrier gas was helium at a flow rate of 1.4ml/min while column temperature was programmed at a range of 60°C-246°C with an increase in temperature of 3°C per minute. The mass spectra corresponding to GC peaks were scanned at 70EV under EI conditions. A library search and mass spectral correlations were done using Wiley 275 and NBS 75 databases. The compounds were identified using authentic standards from Sigma chemical Co., USA and by library matching.

The retention indices (RI) were calculated relative to C₈ – C₂₀ n-alkanes using the formula

$$RI_t = RT_x + \left(\left(\frac{RT_t - RT_x}{RT_y - RT_x} \right) \times 100 \right)$$

Where RI_t = Retention indices of test

RT_t = Retention time of test

RT_x = Retention time of first alkane standard

RT_y = Retention time of second alkane standard

The constituents of the oil were identified by a comparison of retention indices with those reported in literature, the mass spectral correlations using Wiley 275 and NBS 75 databases and wherever possible, by co-injection with authentic standards. Compound identifications indicated by the library search being >90% probable being viewed as being likely hits. Spectra for each eluting compounds were then compared to the standard spectrum for the best hit to determine if the molecular ion peaks and the fragmentation did in fact match

3.3.2. Extraction of oleoresin

Oleoresin was extracted from the dried and powdered rhizome of thirty six turmeric varieties/cultivars using acetone (ASTA, 1978). Ten grams of sample was weighed and transferred to a glass column (18 x 450 mm) with stopcock. Fifty ml of acetone was added and allowed to stand overnight or 16 hrs at $25 \pm 2^\circ\text{C}$. The filtrate was extracted through non absorbent cotton and collected to a preweighed 100 ml beaker. Column was washed twice with 15 ml of acetone. The extracts were pooled and evaporated to dry at 80°C over a water bath. The amount of oleoresin was estimated gravimetrically.

$$\text{Acetone extract \% (v/w) = } \frac{\text{Wt. of residues (g)} \times 100}{\text{Wt. of samples (g)}} \text{ (Oleoresin)}$$

3.3.3. Extraction of curcumin

To estimate the curcumin content colorimetrically, ASTA analytical method was used (ASTA, 1978).

3.3.3.1. Procedure

1. Weigh accurately 100 mg of powdered turmeric sample, transfer into a 100 ml round bottom flask with a condenser and water cooled drip tip.
2. Add 30 ml of ethyl alcohol (95%) and reflux for $2\frac{1}{2}$ hours on a boiling water bath.

3. Cool the extract and filter quantitatively into a 100 ml volumetric flask. Transfer the extracted residue to the filter. Wash thoroughly and makeup with alcohol.
4. Pipette 2 ml of the filtered extract into a 25 ml volumetric flask and dilute to volume with alcohol.
5. Measure the absorbance of the extract and the standard solution at 425 nm against an alcohol blank.

3.3.3.2. Quantification using spectrophotometer

The percentage curcumin content in the samples studied was calculated using the following formula.

$$\text{Absorptivity of curcumin, } a = \frac{\text{Absorbance of standard solution at 425 nm}}{\text{Cell length (cm) x Concentration (g l}^{-1}\text{)}}$$

$$\text{Curcumin in turmeric, \%} = \frac{\text{Absorbance of the extract at 425 nm x 125}}{\text{Cell length (cm) x } a \text{ x sample wt. (g)}}$$

3.3.3.3. Separation and quantification of curcuminoids using HPLC

Curcuminoids were separated from the total curcumin using a Shimadzu LC – 10 AT VP high performance liquid chromatographic system and detected using a fluorescence detector RF – 10 A – XL. The stationary phase was Nucleosil NH₂, particle size 5µm, pre packed in a 250 x 4.6 mm I.D column. A rheodyne injector with a 20µl loop was used for injecting the sample. The analyses were carried out at ambient temperature. The method of detection was fluorimetric, the excitation and emission wave lengths were 420 nm & 470 nm, respectively (Tonnessen & Karlsen, 1983).

3.3.3.3.1. Preparation of standard solution

Stock solutions were prepared using the standard curcumin (95 % purity) obtained from Sigma Aldrich, USA, in different concentrations.

3.3.3.3.2. Sample preparation

One hundred μ l of sample from 100 ml stock solution was diluted to one ml (1:10) using methanol in an eppendorf tube just before loading the samples in the HPLC.

3.3.3.3.3. Validation of HPLC method

The HPLC method for isocratic elution and separation of curcuminoids are validated by altering the mobile phase, changing the proportions of the mobile phase (Chloroform: ethanol) used and by changing the flow rate. Chloroform: ethanol in varying proportions (50:50 – 85:15) was used with a flow rate of 0.75 -2 ml minute⁻¹. The minimum detection limit was also recorded by injecting different concentrations of the standard.

3.3.3.3.4. Quantification of curcuminoids

The curcuminoids percentage in the samples was calculated by comparing with the peak area of the three curcuminoids in the standard curcumin using the following formula.

$$\text{Content of curcumin (\%)} = \frac{\text{Sample area of curcumin} \times \text{Standard weight} \times \text{Standard purity}}{\text{Standard area of curcumin} \times \text{Sample weight}}$$

$$\text{Content of demethoxy curcumin (\%)} = \frac{\text{Sample area of demethoxy curcumin} \times \text{Standard weight} \times \text{Standard purity}}{\text{Standard area of curcumin} \times \text{Sample weight}}$$

$$\text{Content of bis demethoxycurcumin (\%)} = \frac{\text{Sample area of bisdemethoxy curcumin} \times \text{Standard weight} \times \text{Standard purity}}{\text{Standard area of curcumin} \times \text{Sample weight}}$$

3.3.4. Extraction of turmerin

Turmerin was isolated using the protocol of Srinivas *et al.* (1992) with modifications.

3.3.4.1. Procedure

1. Three g of dry turmeric powder is dissolved in 90ml of boiling water, mixed thoroughly and stirred for 30 minutes at room temperature.
2. The suspension is centrifuged at 5000rpm for 15 minutes at room temperature.

3. The supernatant is collected and an equal volume of ethyl acetate is added, mixed well and allowed to stand for five hours at room temperature.
4. At the end of five hours, upper ethyl acetate phase, inter phase and water phases are separated. An equal volume of ethyl acetate is added to the separated water phase and mixed well and allowed to stand for five hours at room temperature.
5. The ethyl acetate phase is collected discreetly and pooled with the earlier obtained ethyl acetate and evaporated to dryness at 70°C.
6. The evaporated material is dissolved in a minimum volume of ethyl alcohol and dissolved in about one ml of water.
7. Protein concentration is determined by Brad Ford's method.

3.3.4.2. Protein estimation by Bradford method

3.3.4.2.1. Materials

Dissolve 100 mg of coomassie brilliant blue G 250 in 50 ml of 95% ethanol. Add 100 ml of concentrated ortho phosphoric acid and made up to 200 ml with distilled water, store refrigerated in amber bottles. Mix one volume of the concentrated dye solution with four volumes of distilled water for use.

3.3.4.2.2. Standard solution

Weighed accurately 50 mg of bovine serum albumin and dissolved it in distilled water and made up to 50 ml in a standard flask. Dilute 10 ml of the stock solution to 50ml with distilled water in a standard flask.

3.3.4.2.3. Procedure

- i. Pipette out 0.2, 0.4, 0.6, 0.8 and one ml of working standard into a series of test tubes.
- ii. Pipette out 0.1 and 0.2 ml of the sample extract into other test tubes.
- iii. Make up the volume to 1 ml in all the test tubes.
- iv. Add 5 ml of dye binding solution to each tube
- v. Mix well and allow the colour to develop for 5 minutes
- vi. Read the absorbance at 595 nm
- vii. Plot a standard curve using the standard protein absorbance versus concentration. Calculate the protein in the experimental sample using the standard curve.

3.3.5. Extraction of rhizome proteins

Five grams of fresh matured rhizome was sliced into pieces and grounded in acetone (20 ml) and kept overnight at room temperature for defatting. Centrifuged the samples at 15000g for 15 minutes and the acetone supernatant was removed. Repeated the procedure twice and air dried the rhizome meal at room temperature and the acetone powder was stored at -20°C until use. Proteins were extracted by homogenizing the meal (0.5 g) using a mortar and pestle placed over ice bath with 2 ml of water and 0.5M NaCl, (Mennella *et al.*, 2005).

3.3.5.1. HPLC analysis of rhizome proteins

The chromatographic apparatus consisted of Waters 515 HPLC pump (2 nos.) with a pump control module II, rheodyne injector, temperature control module II, a 2996 photodiode array detector and a personal computer with Empower software for data processing. 100 µl of sample (5 mg ml⁻¹) was filtered through a 0.45 µm membrane filter and 20µl of the sample was injected on a Shodex anionic exchange DEAE-825 column (8×75 mm) with an anionic exchange pre-column cartridge. The mobile phase was a) 20 mM Tris-HCl, pH 8.2 and b) 20 mM Tris-HCl, pH 8.2 + 0.5 M NaCl solutions. Elution was carried out according to the linear gradient: 100% a) → 40% a) → 60% b) in 40 min → 100% b) in 5 min → 100% a) in 5 min. Re-equilibration with 100% solution a) lasted 15 min prior to the next injection. Analysis conditions were: flow rate 1 ml/min, pressure 540 psi ca, detection at 260 and 280 nm, temperature 25 °C.

3.4. Statistical analysis

The quantitative and qualitative morphological characters studied were converted to binary characters and similarity matrices were prepared. The character state with values ≤0 is represented with the binary code '0' and >0 with '1' (Sokal & Sneath, 1963).

Standardized character states are computed as:

$$X'_{ij} = \frac{x_{ij} - \bar{x}_i}{S_i}$$

Where X'_{ij} – is the standardized character state code for 'i' and OTU 'j'

' X_{ij} ' - row score

' $\bar{x}_i$ ' and ' S_i ' are the mean and standard deviation for the character, respectively.

RAPD and ISSR products were scored for the presence (1) and absence (0) of bands between 250 – 2000 bp as products in this range were considered to be the most reliable (Bussel, 1999; Stewart and porter, 1995). The molecular data were analysed based on the Jaccard's, Sorensens Dice and Simple Matching similarity coefficient for binary data via SIMQUAL of the NTSYSpc ver. 2.02i Package (Rohlf, 1993). For constructing Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram SAHN clustering programme of the NTSYSpc Package (Rohlf 1993) was used.

Results

Chapter 4

RESULTS

4. 1. MORPHOLOGICAL CHARACTERIZATION

4.1.1. Morphological characterization of *Curcuma* species

4.1.1.1. Aerial morphological characters

Mean and range of the aerial morphological characters used for characterizing the *Curcuma* species are summarized in Table 17. Characterwise mean and range are given in Table 18.

Table 17. Mean and range of aerial morphological traits of *Curcuma* species

Species	NTC		NLT		NLC		HP (cm)		PG (cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
<i>C. aeruginosa</i>	3.5 ± 0.5	3.0-4.0	5.8 ± 0.3	5.5-6.0	13.5 ± 1.5	12.0-15.0	53.5 ± 2.5	51.0-56.0	10.5 ± 0.5	10.0-11.0
<i>C. amada</i>	3.2 ± 0.9	2.3-4.0	6.0 ± 0.5	5.5-6.5	16.5 ± 0.5	16.0-17.0	61.0 ± 2.0	59.0-63.0	12.3 ± 0.3	12.0-12.5
<i>C. aromatica</i>	4.5 ± 0.5	4.0-5.0	6.5 ± 0.5	6.0-7.0	18.5 ± 0.5	18.0-19.0	62.5 ± 0.5	62.0-63.0	11.9 ± 0.5	11.5-12.4
<i>C. caesia</i>	5.0 ± 1.0	4.0-6.0	5.5 ± 0.5	5.0-6.0	16.5 ± 0.5	16.0-17.0	42.5 ± 1.5	41.0-44.0	10.5 ± 0.5	10.0-11.0
<i>C. comosa</i>	3.0 ± 0.3	2.7-3.3	5.8 ± 0.8	5.0-6.5	13.5 ± 0.5	13.0-14.0	61.5 ± 1.5	60.0-63.0	12.0 ± 0.5	11.5-12.5
<i>C. decipiens</i>	2.5 ± 0.5	2.0-3.0	5.3 ± 0.3	5.0-5.5	9.5 ± 0.5	9.0-10.0	47.5 ± 1.5	46.0-49.0	10.5 ± 0.5	10.0-11.0
<i>Curcuma</i> species	2.5 ± 0.5	2.0-3.0	8.3 ± 1.3	7.0-9.5	14.5 ± 3.5	11.0-18.0	32.3 ± 1.3	31.0-33.5	10.5 ± 0.5	10.0-11.0
<i>C. .haritha</i>	3.0 ± 1.0	2.0-4.0	5.3 ± 0.3	5.0-5.5	10.5 ± 1.5	9.0-12.0	32.0 ± 3.0	29.0-35.0	9.1 ± 0.6	8.5-9.6
<i>C. longa</i>	4.0 ± 0	4.0	6.0 ± 1.0	5.0-7.0	12.5 ± 1.5	11.0-14.0	53.0 ± 3.0	50.0-56.0	10.5 ± 1.5	9.0-12.0
<i>C. malabarica</i>	3.3 ± 0.3	3.0-3.5	6.5 ± 0.5	6.0-7.0	14.0 ± 1.0	13.0-15.0	71.8 ± 1.3	70.5-73.0	13.0 ± 1.0	12.0-14.0
<i>C. montana</i>	2.7 ± 0.4	2.3-3.0	5.8 ± 0.3	5.5-6.0	12.5 ± 0.5	12.0-13.0	71.5 ± 3.5	68.0-75.0	12.0 ± 0.5	11.5-12.5
<i>C. pseudomontana</i>	2.5 ± 0.5	2.0-3.0	5.3 ± 0.3	5.0-5.5	10.5 ± 0.5	10.0-11.0	71.5 ± 7.5	64.0-79.0	10.5 ± 0.5	10.0-11.0
<i>C. raktakanta</i>	3.5 ± 0.5	3.0-4.0	6.3 ± 0.3	6.0-6.5	11.5 ± 0.5	11.0-12.0	62.0 ± 3.0	59.0-65.0	10.2 ± 0.4	9.8-10.5
<i>C. sylvatica</i>	2.7 ± 0.4	2.3-3.0	5.3 ± 0.3	5.0-5.5	11.5 ± 1.5	10.0-13.0	63.5 ± 3.5	60.0-67.0	11.0 ± 1.0	10.0-12.0
<i>C. zedoaria</i>	3.5 ± 0.5	3.0-4.0	6.5 ± 0.5	6.0-7.0	13.5 ± 0.5	13.0-14.0	74.3 ± 0.8	73.5-75.0	12.3 ± 1.3	11.0-13.6

NTC - number of tillers per clump, NLT - number of leaves per tiller, NLC - number of leaves per clump, HP - height of pseudostem, PG - pseudostem girth.

Table 17 (Contd.). Mean and range of aerial morphological and quality traits of *Curcuma* species.

Species	LL (cm)		LW (cm)		CLS	LMC	LT
	Mean	Range	Mean	Range			
<i>C. aeruginosa</i>	70.0 ± 2.0	68.0-72.0	22.0 ± 1.0	21.0-23.0	Purple	Purple	Glabrous
<i>C. amada</i>	71.0 ± 2.0	69.0-73.0	22.0 ± 2.0	20.0-24.0	Green	Green	Glabrous
<i>C. aromatica</i>	75.0 ± 1.0	74.0-76.0	23.5 ± 2.5	21.0-26.0	Green	Green	Pubescent
<i>C. caesia</i>	60.5 ± 1.5	59.0-62.0	18.5 ± 0.5	18.0-19.0	Purple	Purple	Glabrous
<i>C. comosa</i>	70.5 ± 2.5	68.0-73.0	20.0 ± 1.0	19.0-21.0	Purple	Purple	Glabrous
<i>C. decipiens</i>	65.0 ± 3.0	62.0-68.0	19.5 ± 0.5	19.0-20.0	Purple	Green	Glabrous
<i>Curcuma</i> species	43.9 ± 1.6	42.3-45.5	19.1 ± 0.6	18.5-19.6	Green	Green	Glabrous
<i>C. haritha</i>	43.5 ± 2.5	41.0-46.0	15.5 ± 0.5	15.0-16.0	Green	Green	Pubescent
<i>C. longa</i>	70.5 ± 1.5	69.0-72.0	20.0 ± 1.0	19.0-21.0	Green	Green	Glabrous
<i>C. malabarica</i>	73.0 ± 2.0	71.0-75.0	23.8 ± 0.8	23.0-24.5	Green	Purple	Glabrous
<i>C. montana</i>	61.8 ± 1.3	60.5-63.0	23.5 ± 0.5	23.0-24.0	Purple	Green	Glabrous
<i>C. pseudomontana</i>	76.5 ± 7.5	69.0-84.0	24.0 ± 1.0	23.0-25.0	Purple	Purple	Pubescent
<i>C. raktakanta</i>	73.0 ± 5.0	68.0-78.0	20.3 ± 0.8	19.5-21.0	Purple	Green	Glabrous
<i>C. sylvatica</i>	60.5 ± 2.5	58.0-63.0	22.5 ± 0.5	22.0-23.0	Purple	Purple	Glabrous
<i>C. zedoaria</i>	83.8 ± 2.8	81.0-86.5	24.0 ± 1.0	23.0-25.0	Green	Purple	Glabrous

LL - leaf length, LW - leaf width, CLS - colour of leaf sheath, LMC - leaf midrib colour, LT - leaf texture

Table 18. Characterwise mean and range of *Curcuma* species- aerial morphological traits.

Character	NTC	NLT	NLC	HP (cm)	PG (cm)	LL (cm)	LW (cm)
Mean	3.29 ± 0.19	6.01 ± 0.2	13.27 ± 0.65	57.36 ± 3.54	11.12 ± 0.27	66.57 ± 2.89	21.21 ± 0.65
Range	2.5-5.0	5.3-8.3	9.5-18.5	32.3-74.3	9.1-12.3	43.5-83.8	15.5-24.0

NTC - number of tillers per clump, NLT - number of leaves per tiller, NLC - number of leaves per clump, HP - height of pseudostem, PG - pseudostem girth, LL - leaf length, LW - leaf width.

4.1.1.1.1. Height of pseudostem (Plant height)

The height of the pseudostem ranged from 32.0 ± 3.0 cm to 74.3 ± 0.8 cm. The maximum pseudostem height was recorded in *C. zedoaria* followed by *C. malabarica* and the minimum was recorded in *C. haritha*.

4.1.1.1.2. Pseudostem girth

C. malabarica showed maximum pseudostem girth (13.0 ± 1.0 cm) followed by *C. zedoaria* and *C. amada* (12.3 ± 1.3 cm and 12.3 ± 0.3 cm). *C. haritha* showed minimum pseudostem girth (9.1 ± 0.6 cm).

4.1.1.1.3. Number of tillers

Number of tillers per clump varied from 2.5 ± 0.5 (*C. decipiens* and *Curcuma* species) to 5.0 ± 1.0 (*C. caesia*).

4.1.1.1.4. Number of leaves per tiller

Total number of leaves in the main tiller was maximum in *Curcuma* species. (8.3 ± 1.3). *C. zedoaria*, *C. malabarica* and *C. aromatica* showed 6.5 ± 0.5 leaves in

the main tiller while the least number of leaves was recorded by *C. decipiens*, *C. haritha*, *C. pseudomontana* and *C. sylvatica* (5.3 ± 0.3).

4.1.1.1.5. Number of leaves per clump

Maximum number of leaves per clump was recorded in *C. aromatica* (18.5 ± 0.5) followed by *C. amada* and *C. caesia* (16.5 ± 0.5). *C. decipiens* with 9.5 ± 0.5 leaves recorded minimum number of leaves per clump.

4.1.1.1.6. Leaf length

C. zedoaria (83.8 ± 2.8 cm) showed maximum leaf length followed by *C. pseudomontana* (76.5 ± 7.5 cm) and *C. aromatica* (75.0 ± 1.0 cm). *C. haritha* had the least leaf length (43.5 ± 2.5 cm).

4.1.1.1.7. Leaf width

Leaf width varied from 15.5 ± 0.5 cm (*C. haritha*) to 24.0 ± 1.0 cm (*C. zedoaria* and *C. pseudomontana*).

4.1.1.1.8. Colour of leaf sheath

Colour of the leaf sheath varied from green to purple in the different *Curcuma* species studied. The anthocyanin pigmentation of the leaf sheath was high in species like *C. pseudomontana*, *C. sylvatica* and *C. raktakanta*. While the species like *C. amada*, *C. aromatica*, *C. longa* etc. are devoid of anthocyanin pigmentation and the leaf sheaths exhibited green colour (Fig. 4.)



Fig. 4. Leaf sheath colour variation in *Curcuma* species. A). Leaf sheath with anthocyanin pigmentation (*C. pseudomontana*). B). Leaf sheath without anthocyanin pigmentation (*C. zedoaria*).

4.1.1.1.9. Leaf midrib colour

The leaf midrib colour on the ventral side of the leaf varied from purple to light purple and green. Species like *C. zedoaria* and *C. malabarica* had a light purple coloured leaf mid rib, while *C. caesia* had a dark purple coloured leaf mid rib. *C. comosa* and *C. pseudomontana* had a green leaf midrib with the pigmentation fading towards both ends of the leaf. In *C. sylvatica* the purple leaf midrib colour was fading towards the tip of the leaf while in *C. aeruginosa* the pigmentation was found only in the distal half of the leaf. The other species studied showed no anthocyanin pigmentation in the leaf mid rib (Fig. 5).



Fig. 5. Leaf midrib colour variation in *Curcuma* species. A). dark purple coloured leaf mid rib (*C. caesia*), B). pigmentation on distal half of the leaf (*C. aeruginosa*) and C). leaf without anthocyanin pigmentation (*C. longa*).

4.1.1.1.10. Leaf texture

C. aromatica had a highly pubescent leaf lamina followed by *C. haritha* and *C. pseudomontana*. In all other species studied, the leaf lamina was glabrous.

4.1.1.2 Rhizome characters

Mean and range of rhizome characters of different *Curcuma* species studied are summarized in Table 19. Characterwise range and mean are given in Table 20.

Table 19. Mean and range of rhizome characters of *Curcuma* species

Species	CW (g)		NM		WM (g)		NP		WP (g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
<i>C. aeruginosa</i>	366.0 ± 4.0	362.0-370.0	1.0 ± 0.0	1.0	70.0 ± 2.0	68.0-72.0	5.0 ± 1.0	4.0-6.0	158.0 ± 4.0	154.0-162.0
<i>C. amada</i>	754.0 ± 102.0	652.0-856.0	1.0 ± 0.0	1.0	51.5 ± 2.5	49.0-54.0	8.0 ± 1.0	7.0-9.0	182.0 ± 4.0	178.0-186.0
<i>C. aromatica</i>	631.0 ± 14.0	617.0-645.0	1.0 ± 0.0	1.0	62.5 ± 3.5	59.0-66.0	11.5 ± 1.5	10.0-13.0	269.0 ± 2.0	267.0-271.0
<i>C. caesia</i>	446.0 ± 26.0	420.0-472.0	1.5 ± 0.5	1.0-2.0	57.0 ± 19.0	38.0-76.0	10.5 ± 0.5	10.0-11.0	179.0 ± 3.0	176.0-182.0
<i>C. comosa</i>	271.5 ± 18.5	253.0-290.0	1.0 ± 0.0	1.0	34.5 ± 2.5	32.0-37.0	6.5 ± 1.5	5.0-8.0	99.5 ± 10.5	89.0-110.0
<i>C. decipiens</i>	357.5 ± 11.5	346.0-369.0	1.0 ± 0.0	1.0	53.5 ± 2.5	51.0-56.0	5.5 ± 0.5	5.0-6.0	162.5 ± 4.5	58.0-167.0
<i>Curcuma</i> species	171.5 ± 4.5	167.0-176.0	1.0 ± 0.0	1.0	61.5 ± 2.5	59.0-64.0	3.5 ± 0.5	3.0-4.0	67.0 ± 4.0	63.0-71.0
<i>C. haritha</i>	281.5 ± 12.5	269.0-294.0	1.0 ± 0.0	1.0	32.0 ± 2.0	30.0-34.0	3.5 ± 0.5	3.0-4.0	101.0 ± 3.0	98.0-104.0
<i>C. longa</i>	458.0 ± 12.0	446.0-470.0	1.0 ± 0.0	1.0	66.0 ± 2.0	64.0-68.0	8.0 ± 1.0	7.0-9.0	197.0 ± 15.0	182.0-212.0
<i>C. malabarica</i>	291.0 ± 21.0	270.0-312.0	1.0 ± 0.0	1.0	134.0 ± 7.0	127.0-141.0	4.5 ± 0.5	4.0-5.0	119.0 ± 9.0	110.0-128.0
<i>C. montana</i>	377.0 ± 28.0	349.0-405.0	1.5 ± 0.5	1.0-2.0	50.5 ± 11.5	39.0-62.0	7.0 ± 1.0	6.0-8.0	134.5 ± 11.5	123.0-146.0
<i>C. pseudomontana</i>	106.5 ± 3.5	103.0-110.0	1.0 ± 0.0	1.0	32.5 ± 1.5	31.0-34.0	3.5 ± 0.5	3.0-4.0	67.5 ± 5.5	62.0-73.0
<i>C. raktakania</i>	450.5 ± 22.5	428.0-473.0	1.0 ± 0.0	1.0	40.5 ± 1.5	39.0-42.0	11.0 ± 1.0	10.0-12.0	180.5 ± 8.5	172.0-189.0
<i>C. sylvatica</i>	383.5 ± 13.5	370.0-397.0	1.0 ± 0.0	1.0	44.0 ± 2.0	42.0-46.0	5.5 ± 1.5	4.0-7.0	131.0 ± 3.0	128.0-134.0
<i>C. zedoaria</i>	319.0 ± 24.0	295.0-343.0	1.0 ± 0.0	1.0	166.0 ± 5.0	161.0-171.0	4.5 ± 0.5	4.0-5.0	129.0 ± 9.0	120.0-138.0

CW - clump weight, NM - number of mother rhizome, WM - weight of mother rhizome, NP - number of primary rhizome, WP - weight of primary rhizome.

Table 19 (Contd.). Mean and range of rhizome characters and quality traits of *Curcuma* species

Species	NS		WS		INL		RC	AR	F/T
	Mean	Range	Mean	Range	Mean	Range			
<i>C. aeruginosa</i>	6.0 ± 1.0	5.0-7.0	130.0 ± 2.0	128.0-132.0	1.3 ± 0.1	1.2-1.4	Pale blue	Camphoraceous	Bitter
<i>C. amada</i>	19.5 ± 1.5	18.0-21.0	515.0 ± 95.0	420.0-610.0	1.5 ± 0.2	1.3-1.6	Pale yellow	Mango like	Mango/Gingery
<i>C. aromatica</i>	23.0 ± 2.0	21.0-25.0	291.0 ± 19.0	272.0-310.0	1.2 ± 0.1	1.2-1.3	Creamy white	Persisting Camphoraceous	Bitter
<i>C. caesia</i>	22.0 ± 2.0	20.0-24.0	205.0 ± 9.0	196.0-214.0	1.4 ± 0.1	1.3-1.5	Blue black	Camphoraceous	Bitter
<i>C. comosa</i>	15.5 ± 0.5	15.0-16.0	132.0 ± 6.0	126.0-138.0	1.2 ± 0.1	1.1-1.3	Pale yellow	Camphoraceous	Bitter
<i>C. decipiens</i>	7.5 ± 0.5	7.0-8.0	137.0 ± 5.0	132.0-142.0	1.3 ± 0.1	1.2-1.3	Pale yellow	Camphoraceous	Bitter
<i>Curcuma</i> species	3.5 ± 0.5	3.0-4.0	36.5.0 ± 4.5	32.0-41.0	1.3 ± 0.1	1.2-1.4	Creamy white	Camphoraceous	Bitter
<i>C. haritha</i>	7.5 ± 0.5	7.0-8.0	142.0 ± 10.0	132.0-152.0	1.2 ± 0.1	1.1-1.2	Creamy white	Persisting Camphoraceous	Bitter
<i>C. longa</i>	24.0 ± 2.0	22.0-26.0	190.0 ± 4.0	186.0-194.0	1.2 ± 0.1	1.1-1.3	Deep orange yellow	Camphoraceous	Bitter
<i>C. malabarica</i>	3.0 ± 1.0	2.0-4.0	33.0 ± 6.0	27.0-39.0	1.3 ± 0.1	1.2-1.4	Orange yellow	Camphoraceous	Bitter
<i>C. montana</i>	23.5 ± 1.5	22.0-25.0	189.0 ± 7.0	182.0-196.0	1.3 ± 0.1	1.2-1.3	Pale yellow with blue circle	Camphoraceous	Bitter
<i>C. pseudomontana</i>	0.0 ± 0.0	0.0-1.0	0.0 ± 0.0	0.0-9.0	1.0 ± 0.1	0.9-1.0	Pale yellow	Camphoraceous	Bitter
<i>C. raktakania</i>	27.0 ± 1.0	26.0-28.0	225.0 ± 16.0	209.0-241.0	1.3 ± 0.1	1.2-1.3	Pale yellow with bluish green ring	Camphoraceous	Bitter
<i>C. sylvatica</i>	11.0 ± 1.0	10.0-12.0	205.0 ± 13.0	192.0-218.0	1.2 ± 0.1	1.1-1.2	Lemon yellow	Camphoraceous	Bitter
<i>C. zedoaria</i>	2.0 ± 1.0	1.0-3.0	19.5 ± 9.5	10.0-29.0	1.7 ± 0.1	1.7-1.8	Orange yellow	Camphoraceous	Bitter

NS - number of secondary rhizome, WS - weight of secondary rhizome, INL - internode length, RC - rhizome colour, AR - aroma of rhizome, F/T - flavour/taste of rhizome

Table 20.Characterwise range and mean of *Curcuma* species-rhizome characters

Character	CW (g)	NM	WM (g)	NP	WP (g)	NS	WS (g)	INL
Mean	377.63 ± 41.99	1.07 ± 0.05	63.73 ± 9.68	6.53 ± 0.71	145.1 ± 13.75	13.03 ± 2.43	163.63 ± 33.15	1.29 ± 0.04
Range	106.5-754.0	1.0-1.5	32.0-166.0	3.5-11.5	67.0-269.0	0.0-24.0	0.0-515.0	1.0-1.7

CW - clump weight, NM - number of mother rhizome, WM - weight of mother rhizome, NP - number of primary rhizome, WP - weight of primary rhizome, NS - number of secondary rhizome, WS - weight of secondary rhizome, INL - internode length.

4.1.1.2.1. Clump weight /Weight of rhizome

Total weight of the clump (rhizome weight) varied from 106.5 ± 3.5 to 754.0 ± 102.0 g. *C. amada* (754.0 ± 102.0 g) showed maximum clump weight followed by *C. aromatica* (631.0 ± 14.0 g) while *C. pseudomontana* (106.5 ± 3.5 g) recorded minimum weight of the clump.

4.1.1.2.2. Number of mother rhizome

A single mother rhizome was present in most of the species except *C. caesia* and *C. montana* (1.5 ± 0.5 each).

4.1.1.2.3. Weight of mother rhizome

C. zedoaria recorded maximum weight (166.0 ± 5.0 g) of mother rhizome followed by *C. malabarica* (134.0 ± 7.0 g). *C. haritha* had least mother rhizome weight of 32.0 ± 2.0 g.

4.1.1.2.4. Number of primary rhizome

C. aromatica produced maximum number of primary rhizomes (11.5 ± 1.5) followed by *C. raktakanta* (11.0 ± 1.0). Minimum number of primary rhizomes was observed in *C. pseudomontana*, *C. haritha* and *Curcuma species* (3.5 ± 0.5 each).

4.1.1.2.5. Weight of primary rhizome

The weight of primary rhizomes ranged from 67.0 ± 4.0 g to 269.0 ± 2.0 g. Maximum primary rhizome weight was recorded in *C. aromatica* (269.0 ± 2.0 g) followed by *C. longa* (197.0 ± 15.0 g) and *C. amada* (182.0 ± 4.0). *Curcuma sp.* recorded the lowest weight of primary rhizomes (67.0 ± 4.0 g).

4.1.1.2.6. Number of secondary rhizome

Number of secondary rhizome was maximum in *C. raktakanta* (27.0 ± 1.0). *C. longa* and *C. aromatica* produced 24.0 ± 2.0 and 23.0 ± 2.0 numbers of secondary rhizomes each, while *C. zedoaria* and *C. malabarica* produced 2.0 ± 1.0 and 3.0 ± 1.0 secondary rhizomes each. Secondary rhizomes were absent in *C. pseudomontana*.

4.1.1.2.7. Weight of secondary rhizome

Maximum weight of secondary rhizomes was recorded in *C. amada* (515.0 ± 5.0 g). *C. zedoaria* recorded minimum weight of secondary rhizomes (19.5 ± 9.5 g.).

4.1.1.2.8. Internode length

Maximum internode length was recorded in *C. zedoaria* (1.7 ± 0.1 cm) and minimum in *C. pseudomontana* (1.0 ± 0.1 cm).

4.1.1.2.9. Rhizome colour

The rhizome colour (mother/primary) varied from creamy white (*C. aromatica*), pale blue (*C. aeruginosa*), blue black (*C. caesia*), pale yellow (*C. amada*, *C. pseudomontana*, *C. comosa*), pale yellow with bluish green outer ring (*C. raktakanta*), to lemon yellow (*C. sylvatica*) and orange yellow (*C. longa*, *C. zedoaria*, *C. malabarica*) in the different *Curcuma* species studied (Fig. 6).

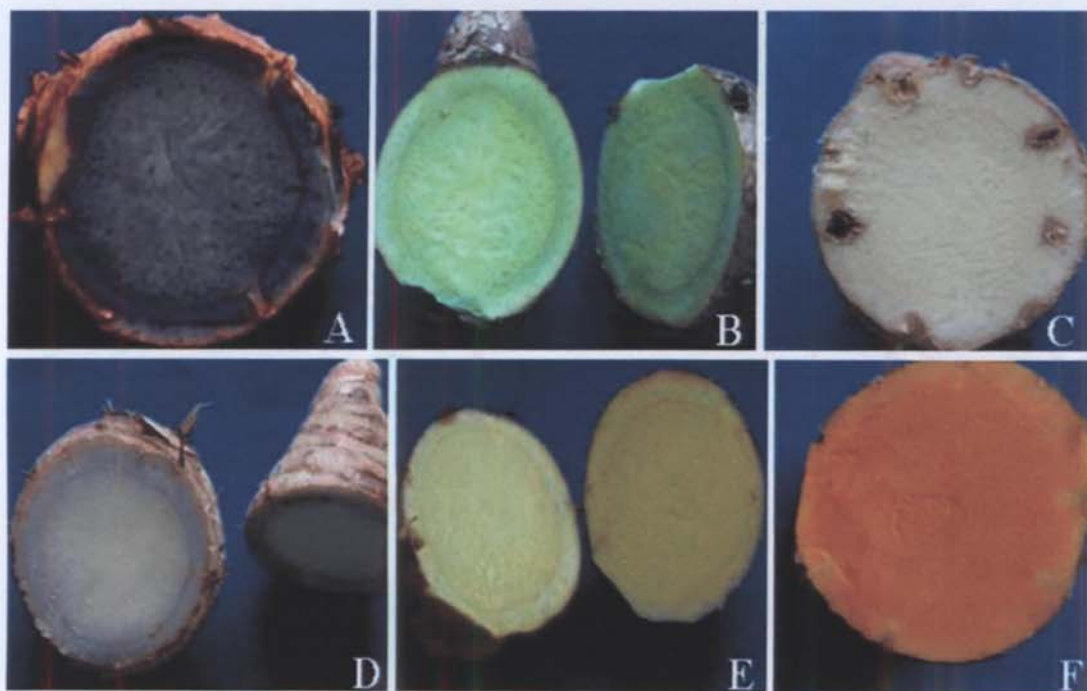


Fig. 6. Rhizome colour variation in the *Curcuma* species. A). blue black (*C. caesia*), B). pale yellow with bluish green outer ring (*C. raktakanta*), C). creamy white (*C. aromatica*), D). pale blue (*C. aeruginosa*), E). pale yellow (*C. pseudomontana*) and F). orange yellow (*C. zedoaria*).

4.1.1.2.10. Aroma of rhizome

Curcuma amada had a characteristic mango aroma. All other species studied had a camphoraceous aroma with a strong persisting note in *C. aromatica* and *C. haritha*.

4.1.1.2.11. Flavour/taste of rhizome

A typical mango flavour with gingery taste was observed in the *C. amada*. All the other species studied had a bitter taste.

4.1.1.3. Floral characters

The floral characters of the different *Curcuma* spp. are summarized in Table 21.

Table 21. Important floral characters of the *Curcuma* species studied

Species	Spike position	Colour of the fertile bract	Colour of the coma bract	Colour of the corolla	Colour of the labellum	Anther spur
<i>C. aeruginosa</i> Roxb.	Lateral	Green with light purple colour at the tip	Pink	Pale yellow	Yellow	Present
<i>C. amada</i> Roxb.	Lateral/terminal*	Green	White with pink tip	White	White with yellow lip	Present
<i>C. aromatica</i> Salisb.	Lateral	Green	Pinkish white	Yellow	Yellow	Present
<i>C. caesia</i> Roxb.	Lateral	Greenish purple	Reddish purple	Red	Yellow	Present
<i>C. comosa</i> Roxb.	Lateral	Green	White with purple tip	White	Yellow	Present
<i>C. decipiens</i> Dalz.	Terminal	Green	Pink	Creamy white	Yellow	Present
<i>Curcuma</i> species	Lateral	Green	Greenish white light purple	White	White with yellow lip	Present
<i>C. haritha</i> Mangaly & Sabu	Lateral	Green	White with dark purple color at the tip	White	White with yellow lip	Present
<i>C. longa</i> L.	Terminal	Green	White, white with pink, green and purple colour at the tip	White	White with yellow lip	Present
<i>C. malabarica</i> Vel et al.	Lateral	Green with light purple colour at the tip	Purple	Pink	Yellow	Present
<i>C. montana</i> Wall.	Lateral	Green	White with light purple streaks towards the tip	Creamy white	Yellow	Present
<i>C. pseudomontana</i> Grah.	Lateral/ Terminal	Greenish purple	Dark purple	Creamy white	Yellow	Present
<i>C. raktakanita</i> Mangaly & Sabu	Lateral	Green with light purple	Purple	White	White with yellow lip	Present
<i>C. sylvatica</i> Val.	Lateral	Greenish purple	Dark purple	Creamy white	Yellow	Present
<i>C. zedoaria</i> Rosc.	Lateral	Green with light purple colour at the tip	Purple	Pink	Yellow	Present

* The entity collected as wild mango ginger (*C. amada*) from Regional Agricultural Research Station, Jagathial (Andhra Pradesh) produced terminal spikes.

4.1.1.3.1. Spike position

Spike position is one of the important floral characters of *Curcuma*. All the species studied had either lateral or terminal spikes except in the case of *C. pseudomontana* and *C. amada* which showed both lateral and central spikes (Fig. 7).



Fig. 7. Spike position in *Curcuma* species. A). lateral (*C. zedoaria*) and B). terminal (*C. longa*)

4.1.1.3.2. Colour of the fertile bract

Fertile bract colour varied from green to green with light purple colour at the tip of the bract in the *Curcuma* species studied. *C. caesia* and *C. pseudomontana* had a distinct greenish purple fertile bract colour (Fig. 8).



Fig. 8. Fertile bract colour variation in *Curcuma*. A). purplish green (*C. caesia*) B). green with purple colour tip (*C. zedoaria*) and C). green (*C. longa*).

4.1.1.3.3. Colour of the coma bract

The coma bract colour varied from reddish purple (*C. caesia*), dark purple (*C. pseudomontana* and *C. sylvatica*), purple (*C. zedoaria*, *C. malabarica* and *C. raktakanta*) to pink (*C. aeruginosa*) to white with pink/purple colour tip (*C. amada*, *C. haritha* and *C. montana*) (Fig. 9). However, *C. longa* showed high variability for coma bract colour. The coma bract colour of *C. longa* varieties varied from white, white with pink, green and purple colour at the tip (Fig. 10).



Fig. 9. Coma bract colour variation in *Curcuma* species. A). white with green colour tip (*C. longa*), B). dark purple (*C. pseudomontana*) and C). white with pink/purple colour tip (*C. haritha*).



Fig. 10. Coma bract colour variation in *C. longa* accessions. A). "Jorhat", B). "Karhadi local", C). "Alleppey" and D). "Kuchipudi"

4.1.1.3.4. Colour of the corolla

Colour of the corolla differed from red, pink, creamy white and white in different *Curcuma* species studied. *C. caesia* showed a characteristic red corolla and the *C. zedoaria* and *C. malabarica* had pink coloured corolla. All other species had white or creamy white corolla (Fig. 11).



Fig. 11. Corolla colour variation in *Curcuma*. A). red (*C. caesia*), B). white (*C. amada*) and C) pink (*C. zedoaria*).

4.1.1.3.5. Colour of the labellum

Most of the species studied had a yellow labellum except *C. amada*, *C. haritha*, *C. longa*, *C. raktakanta* and *Curcuma* species which produced white labellum with yellow lip (Fig. 12).



Fig. 12. Labellum colour variation in *Curcuma*. A) yellow (*C. longa*) and B) white labellum with yellow lip (*C. haritha*)

4.1.1.3.6. Anther spur

4.1.1.3.6. Anther spur

Anther spur was present in all the *Curcuma* species studied (Fig. 13).



Fig. 13. Anther spur in *Curcuma* (*C. zedoaria*)

4.1.1.4. Cluster analysis

The similarity coefficient (paired affinity index) between the species ranged from 0.11 (*C. haritha* and *C. caesia*) to 1.0 (*C. zedoaria* and *C. malabarica*) (Table 22).

Table 22. Paired affinity indices of the *Curcuma* species based on morphological characters

Row\Col	Aeugiososa	Amada	Aromatica	Caesia	Comosa	Decipiens	Species	Haritha	Longa	Malabarica	Montana	Pseudomontana	Raktakanta	Sylratika	Zedoaria
Aeugiososa	1.0000000														
Amada	0.3214286	1.0000000													
Aromatica	0.3333333	0.4642857	1.0000000												
Caesia	0.4074074	0.3000000	0.3548387	1.0000000											
Comosa	0.5454545	0.4583333	0.4074074	0.3333333	1.0000000										
Decipiens	0.4500000	0.2000000	0.1785714	0.2916667	0.3500000	1.0000000									
Species	0.3333333	0.2083333	0.2307692	0.2000000	0.3000000	0.3125000	1.0000000								
Haritha	0.2272727	0.1666667	0.3478261	0.1153846	0.2500000	0.1764706	0.3571429	1.0000000							
Longa	0.4166667	0.4583333	0.4074074	0.3333333	0.3333333	0.2272727	0.2380952	0.1904762	1.0000000						
Malabarica	0.4800000	0.2666667	0.3666667	0.3000000	0.4583333	0.2000000	0.2608696	0.1200000	0.2962963	1.0000000					
Montana	0.3076923	0.3461538	0.3571429	0.4400000	0.4545455	0.4210526	0.2380952	0.1904762	0.2800000	0.2962963	1.0000000				
Pseudomontana	0.3750000	0.2142857	0.2758621	0.2962963	0.5500000	0.3684211	0.1363636	0.2000000	0.1923077	0.3600000	0.3478261	1.0000000			
Raktakanta	0.4074074	0.4444444	0.4482759	0.4285714	0.3846154	0.2400000	0.2500000	0.1600000	0.5000000	0.4444444	0.3846154	0.2962963	1.0000000		
Sylratika	0.2800000	0.1785714	0.2413793	0.4166667	0.3636364	0.3157895	0.1428571	0.1500000	0.2000000	0.2692308	0.4285714	0.6111111	0.3076923	1.0000000	
Zedoaria	0.4800000	0.2666667	0.3666667	0.3000000	0.4583333	0.2000000	0.2608696	0.1200000	0.2962963	1.0000000	0.2962963	0.3600000	0.4444444	0.2692308	1.0000000

The UPGMA dendrogram constructed using the Jaccard's similarity coefficients splits into seven groups at coefficient 0.44 (Fig. 14).

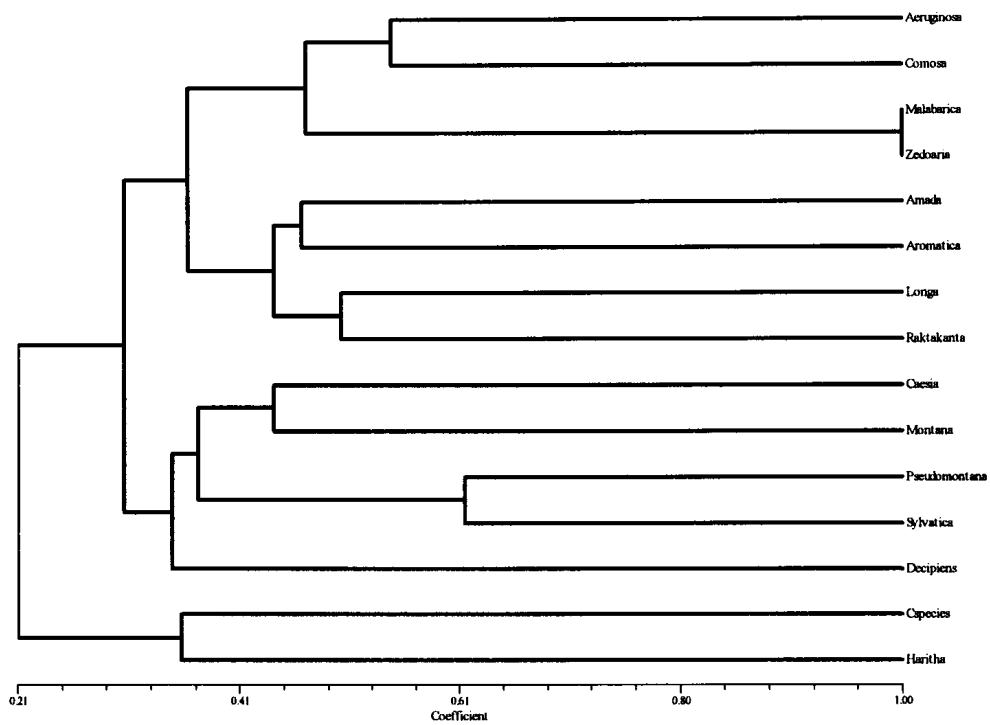


Fig.14. Clustering patterns of 15 *Curcuma* species based on Jaccard's similarity coefficients using morphological traits.

- Group I - *C. aeruginosa*, *C. comosa*, *C. malabarica* and *C. zedoaria*
- Group II - *C. amada*, *C. aromatica*, *C. longa* and *C. raktakanta*
- Group III - *C. caesia* and *C. montana*
- Group IV - *C. pseudomontana* and *C. sylvatica*
- Group V - *C. decipiens*
- Group VI - *Curcuma* species
- Group VII - *C. haritha*

C. aeruginosa, *C. comosa*, *C. malabarica* and *C. zedoaria* formed the first group in the cluster in which *C. malabarica* and *C. zedoaria* showed a maximum similarity (1.00) between them. *C. amada*, *C. aromatica*, *C. longa* and *C. raktakanta* formed the second group. In this group *C. raktakanta* and *C. longa* showed maximum similarity (0.50). *C. caesia* & *C. montana* and *C. pseudomontana* & *C. sylvatica* formed the third and fourth group of the cluster respectively. *C. haritha*, *C. species* and *C. decipiens* were the sole members of the dendrogram.

The UPGMA dendrogram constructed using the key morphological characters showed a different clustering pattern. The paired affinity indices between the species ranged from 0.11 (*C. caesia* and *C. amada*) to 1.0 (*C. zedoaria* and *C. malabarica*) (Table 23).

Table 23. Paired affinity indices of the *Curcuma* species based on key morphological characters

Row/Col	Aeruginosa	Amada	Aromatica	Caesia	Comosa	Decipiens	Species	Haritha	Longa	Malabarica	Montana	Pseudomontana	Raktakanta	Sylvatica	Zedoaria
Aeruginosa	1.0000000														
Amada	0.2666667	1.0000000													
Aromatica	0.3125000	0.2000000	1.0000000												
Caesia	0.4666667	0.1176471	0.2352941	1.0000000											
Comosa	0.7500000	0.3846154	0.3333333	0.5000000	1.0000000										
Decipiens	0.5384615	0.2142857	0.2666667	0.3333333	0.5833333	1.0000000									
Species	0.3846154	0.2500000	0.3076923	0.2000000	0.4166667	0.3333333	1.0000000								
Haritha	0.3333333	0.3076923	0.7272727	0.1764706	0.3571429	0.2000000	0.4545455	1.0000000							
Longa	0.3846154	0.3636364	0.2142857	0.2000000	0.4166667	0.3333333	0.5555556	0.3333333	1.0000000						
Malabarica	0.4000000	0.1250000	0.2500000	0.4000000	0.4285714	0.2666667	0.2142857	0.1875000	0.3076923	1.0000000					
Montana	0.5384615	0.2142857	0.3571429	0.4285714	0.5833333	0.6363636	0.3333333	0.2857143	0.3333333	0.3571429	1.0000000				
Pseudomontana	0.4375000	0.1764706	0.2941176	0.5333333	0.5714286	0.5000000	0.1875000	0.2352941	0.1875000	0.3750000	0.5000000	1.0000000			
Raktakanta	0.4285714	0.2142857	0.1875000	0.3333333	0.4615385	0.2857143	0.3333333	0.2857143	0.3333333	0.4615385	0.3846154	0.3125000	1.0000000		
Sylvatica	0.4666667	0.1176471	0.2352941	0.5714286	0.5000000	0.4285714	0.2000000	0.1764706	0.2000000	0.4000000	0.5384615	0.7692308	0.3333333	1.0000000	
Zedoaria	0.4000000	0.1250000	0.2500000	0.4000000	0.4285714	0.2666667	0.2142857	0.1875000	0.3076923	1.0000000	0.3571429	0.3750000	0.4615385	0.4000000	1.0000000

The dendrogram was split into seven groups at Jaccard's similarity coefficient of 0.52. (Fig. 15).

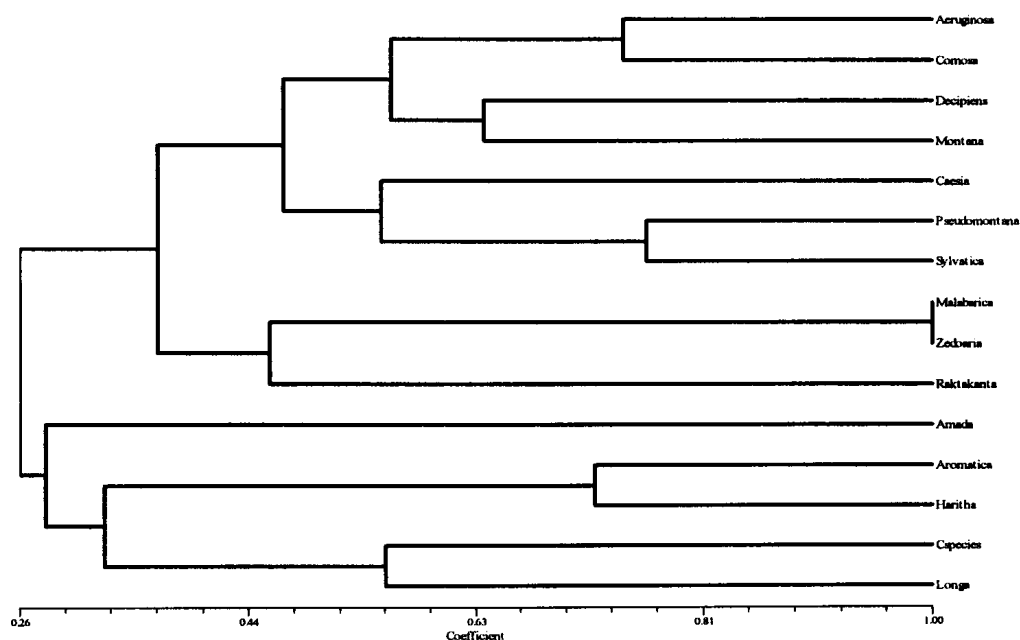


Fig.15. Clustering patterns of 15 *Curcuma* species based on Jaccard's similarity coefficients using the key morphological traits.

Group I	- <i>C. aeruginosa</i> , <i>C. comosa</i> , <i>C. decipiens</i> and <i>C. montana</i>
Group II	- <i>C. caesia</i> , <i>C. pseudomontana</i> and <i>C. sylvatica</i> ,
Group III	- <i>C. malabarica</i> and <i>C. zedoaria</i>
Group IV	- <i>C. raktakanta</i>
Group V	- <i>C. amada</i>
Group VI	- <i>C. aromatica</i> , <i>C. haritha</i>
Group VII	- <i>Curcuma</i> species and <i>C. longa</i>

C. aeruginosa, *C. comosa*, *C. decipiens* and *C. montana* formed the first group in the cluster in which *C. aeruginosa*, *C. comosa*, showed a maximum similarity (0.75) between them. *C. caesia*, *C. pseudomontana* and *C. sylvatica* formed the second group. In this group *C. pseudomontana* and *C. sylvatica* showed maximum similarity (0.76). *C. malabarica* and *C. zedoaria* formed the third of the cluster and shared a maximum similarity (1.00). *C. raktakanta* and *C. amada* formed the fourth and fifth groups of the cluster respectively. *C. aromatica* and *C. haritha* shared a similarity of 0.72 and formed the sixth group. The last group of the dendrogram was formed by *C. species* and *C. longa*.

4.1.2. Intraspecific characterization of *Curcuma*

4.1.2.1. Aerial morphological characters

Mean and range of aerial morphological characters of turmeric studied are summarized in Table 24. Characterwise range and mean are given in Table 25.

Table 24. Mean and range of aerial morphological characters of turmeric (*C. longa* L.) varieties/cultivars

Cultivar/Variety	HP (cm)		PG (cm)		NLT		NTC	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
'Alleppey'	35.8 ± 6.7	26.0-48.5	9.4 ± 0.9	7.0-12.3	7.8 ± 1.0	6.0-9.5	2.3 ± 0.4	1.5-3.0
'Amalapuram'	43.3 ± 5.2	36.5-53.5	9.8 ± 1.5	7.5-11.5	8.7 ± 1.7	6.5-12.0	2.5 ± 0.2	2.0-3.3
'Amrithapani'	50.1 ± 3.9	45.5-57.8	8.6 ± 0.3	6.8-11.8	8.7 ± 1.2	7.0-11.0	2.8 ± 0.0	2.3-3.7
'Armoor'	47.3 ± 0.9	42.5-55.0	10.2 ± 0.8	9.0-11.0	8.2 ± 1.4	6.5-11.0	2.8 ± 0.2	2.3-3.3
'Jabedi'	42.4 ± 6.7	31.0-54.3	8.3 ± 0.8	6.5-10.5	9.0 ± 1.8	7.0-12.5	2.5 ± 0.2	2.0-3.3
'Dhagi'	41.5 ± 6.9	29.5-53.5	9.6 ± 0.0	8.5-11.8	8.3 ± 1.2	6.5-10.5	2.5 ± 0.2	2.0-3.3
'Katergia'	32.8 ± 8.1	38.0-54.5	9.1 ± 1.3	6.5-11.8	7.8 ± 1.6	5.5-11.0	3.5 ± 0.9	2.0-4.7
'Chayapasupu'	43.8 ± 5.6	37.5-55.0	9.3 ± 0.7	7.5-11.5	8.8 ± 1.1	7.5-11.0	3.6 ± 0.4	3.0-4.0
'Avanigadda'	46.2 ± 5.1	39.0-56.0	9.8 ± 0.5	9.5-10.5	8.5 ± 0.8	7.5-10.0	2.9 ± 0.4	2.0-4.0
'Dindigam'	47.3 ± 4.5	41.0-56.0	8.9 ± 0.6	7.3-10.8	9.0 ± 0.5	8.5-10.0	2.9 ± 0.5	2.0-3.7
'Ethamkula'	38.8 ± 8.1	30.5-55.0	8.9 ± 0.2	8.0-10.5	8.3 ± 1.2	6.5-10.5	3.0 ± 0.2	2.3-4.0
'GL Puram'	37.0 ± 7.2	27.0-51.0	10.4 ± 0.4	9.5-11.5	8.5 ± 1.0	7.0-10.5	2.8 ± 0.2	2.3-3.3
'Karhadi local'	45.0 ± 3.9	39.0-52.5	9.8 ± 0.0	8.5-11.3	9.0 ± 1.0	7.5-11.0	2.8 ± 0.7	2.0-3.3
'Kasturi'	41.2 ± 6.1	31.0-52.0	9.4 ± 0.6	7.8-11.3	8.3 ± 0.6	7.5-9.5	2.9 ± 0.0	2.7-3.3
'Kodur'	42.3 ± 6.6	31.0-54.0	10.2 ± 1.3	8.5-11.0	7.7 ± 0.4	7.0-8.5	2.5 ± 0.2	2.0-3.3
'Kuchipudi'	39.0 ± 6.3	29.0-50.5	8.9 ± 1.9	5.0-11.5	8.7 ± 0.4	8.0-9.5	3.0 ± 0.9	2.0-3.7
'Mamomthody'	39.0 ± 9.1	23.0-54.5	9.0 ± 1.9	5.0-13.3	6.8 ± 1.3	5.5-9.5	2.7 ± 0.0	2.7-3.2
'Muvatupuzha'	47.2 ± 5.2	39.5-57.0	9.8 ± 1.0	8.5-10.5	8.2 ± 0.9	6.5-9.5	3.4 ± 0.5	2.7-3.7
'Nandyal type'	45.0 ± 2.8	40.5-50.0	10.2 ± 0.9	8.5-11.8	8.2 ± 0.9	7.0-10.0	3.2 ± 0.3	2.7-3.7
'Rajapuri'	39.2 ± 6.2	31.5-51.5	9.2 ± 1.4	6.8-11.5	8.0 ± 0.5	7.0-8.5	2.9 ± 0.2	2.3-3.7
'Sugandham'	46.3 ± 5.2	39.5-56.5	9.4 ± 0.1	8.8-10.3	8.2 ± 0.7	7.0-9.5	2.9 ± 0.0	2.7-3.3
'Vondimita'	39.2 ± 8.3	28.5-55.5	10.0 ± 1.3	8.5-11.0	8.0 ± 1.0	6.5-10.0	3.1 ± 0.2	2.7-3.7
'Wynad' local'	39.7 ± 6.9	30.0-53.0	8.9 ± 0.5	8.0-9.8	8.7 ± 0.8	7.0-9.5	2.6 ± 0.0	2.3-3.3
'Aizwal'	48.7 ± 5.3	41.5-51.0	9.6 ± 0.4	8.5-10.5	7.3 ± 0.7	6.8-8.5	2.4 ± 0.0	2.0-3.3
Ayur	43.2 ± 6.7	35.0-56.5	9.0 ± 0.6	8.3-9.5	8.2 ± 0.9	6.5-9.5	3.1 ± 0.5	2.3-3.7
'Basar Along'	38.5 ± 9.4	22.5-55.0	8.8 ± 0.0	8.3-9.0	6.5 ± 1.5	4.5-9.6	3.2 ± 0.2	3.0-3.3
'Gaspani'	40.8 ± 4.3	33.0-48.0	9.5 ± 1.0	8.5-10.5	8.2 ± 1.2	6.5-10.5	2.7 ± 0.2	2.3-3.0
'Hajo'	43.2 ± 4.8	36.5-52.5	10.6 ± 0.3	10.1-11.0	8.5 ± 0.8	7.5-10.0	2.9 ± 0.0	2.7-3.3
'Jorhat'	44.2 ± 4.3	38.5-55.5	8.7 ± 0.8	7.0-10.5	8.8 ± 1.2	7.0-11.0	3.0 ± 0.5	2.3-3.3
'Suvarna'	40.5 ± 5.9	31.5-51.5	9.1 ± 1.0	7.5-10.3	7.5 ± 1.0	6.5-9.5	3.4 ± 0.9	2.3-4.0
'Sudarsana'	35.7 ± 11.2	15.5-54.0	7.6 ± 1.4	4.8-10.5	8.2 ± 0.7	7.5-9.5	3.8 ± 1.2	3.0-5.3
'Kedaram'	35.8 ± 7.8	23.0-50.0	8.1 ± 1.8	5.0-10.8	7.5 ± 1.0	6.0-9.5	2.4 ± 0.2	2.0-3.0
'Suguna'	38.2 ± 8.6	22.5-52.0	8.3 ± 1.5	6.0-10.0	7.8 ± 1.8	4.5-10.5	2.5 ± 0.5	1.7-3.0
'Prathibha'	35.5 ± 8.4	19.5-48.0	8.3 ± 1.5	5.5-10.8	8.3 ± 0.6	7.5-9.5	2.3 ± 0.5	1.3-3.3
'Prabha'	39.0 ± 7.4	26.5-52.0	8.9 ± 0.9	7.0-10.8	7.5 ± 1.0	6.0-9.5	2.6 ± 0.7	1.7-3.0
'Alleppey Supreme'	39.7 ± 6.4	29.5-51.5	8.9 ± 0.8	7.3-10.5	7.3 ± 1.4	5.5-10.0	2.4 ± 0.4	1.5-3.3

HP - height of pseudostem, PG - pseudostem girth, NLT - number of leaves per tiller, NTC - number of tillers per clump.

Table 24. (Contd.). Mean and range of aerial morphological characters of turmeric (*C. longa* L.) varieties/cultivars

Cultivar/Variety	NLC		LL (cm)		LW (cm)	
	Mean	Range	Mean	Range	Mean	Range
'Alleppey'	14.2 ± 2.1	14.0-26.0	31.2 ± 0.7	27.3-59.3	12.7 ± 0.8	10.7-15.2
'Amalapuram'	14.7 ± 2.9	12.0-22.0	44.5 ± 0.5	50.0-63.0	16.8 ± 0.5	16.0-17.7
'Amrithapani'	14.3 ± 2.9	13.0-23.0	50.4 ± 2.7	60.0-67.5	15.2 ± 0.2	15.0-15.3
'Armoor'	13.3 ± 3.4	13.0-24.0	46.1 ± 0.5	57.0-59.3	14.7 ± 0.9	14.2-15.8
'Jabedi'	18.0 ± 2.5	16.0-24.0	46.3 ± 6.1	51.0-63.5	17.7 ± 0.7	16.0-19.8
'Dhagi'	16.7 ± 1.0	16.0-22.0	38.6 ± 8.8	36.7-54.3	16.3 ± 0.2	15.7-17.2
'Katergia'	18.4 ± 1.2	18.0-22.0	42.2 ± 2.2	44.0-67.3	16.1 ± 0.5	15.8-16.7
'Chayapasupu'	19.3 ± 0.6	19.0-21.0	44.9 ± 0.7	52.7-63.3	17.6 ± 1.3	16.0-18.5
'Avanigadda'	15.3 ± 3.2	12.0-22.0	47.8 ± 0.4	57.0-66.7	17.4 ± 0.1	17.0-18.0
'Dindigam'	15.3 ± 2.6	14.0-23.0	48.3 ± 4.9	52.3-70.0	17.3 ± 2.3	15.3-19.8
'Ethamkula'	19.0 ± 3.0	16.0-25.0	39.3 ± 1.5	41.7-61.7	16.5 ± 0.0	16.3-17.0
'GL Puram'	17.7 ± 2.7	17.0-26.0	40.1 ± 0.2	44.0-61.7	17.2 ± 0.1	16.7-18.0
'Karhadi local'	18.3 ± 1.2	17.0-25.0	43.1 ± 7.5	42.3-63.0	16.4 ± 0.1	16.0-17.0
'Kasturi'	16.7 ± 2.2	15.0-26.0	38.3 ± 0.3	41.7-60.0	17.4 ± 0.6	16.8-18.0
'Kodur'	15.7 ± 2.1	15.0-23.0	39.8 ± 0.9	44.3-58.7	17.5 ± 0.3	16.7-18.7
'Kuchipudi'	17.7 ± 1.5	16.0-23.0	37.9 ± 2.2	43.7-51.0	16.9 ± 0.3	16.3-17.7
'Mamomthody'	14.3 ± 1.7	14.0-19.0	36.3 ± 5.9	36.0-52.3	13.9 ± 1.9	10.8-16.3
'Muvatupuzha'	18.7 ± 2.9	18.0-24.0	43.9 ± 0.0	47.3-71.3	15.1 ± 0.9	13.5-16.5
'Nandyal type'	18.3 ± 2.3	16.0-23.0	49.3 ± 3.7	51.7-76.3	16.6 ± 0.5	16.2-17.2
'Rajapuri'	15.7 ± 0.7	15.0-19.0	42.1 ± 0.3	45.7-67.3	16.8 ± 0.1	15.7-17.5
'Sugandham'	14.3 ± 2.4	14.0-24.0	44.9 ± 1.4	50.3-67.0	16.2 ± 0.6	15.7-16.8
'Vondimita'	18.0 ± 3.6	13.0-25.0	45.1 ± 0.7	49.0-71.0	15.9 ± 2.0	13.7-17.7
'Wynad' local'	14.3 ± 3.5	13.0-25.0	41.3 ± 4.4	42.3-63.0	15.9 ± 0.3	15.7-16.2
'Aizwal'	15.0 ± 2.1	14.0-21.0	45.3 ± 5.2	45.0-71.3	16.3 ± 0.9	15.3-17.0
Ayur	15.3 ± 1.3	15.0-23.0	43.7 ± 0.5	51.0-62.7	16.6 ± 0.1	16.0-17.0
'Basar Along'	15.7 ± 2.7	14.0-22.0	43.6 ± 0.5	52.7-59.3	15.7 ± 0.8	15.2-16.7
'Gaspani'	15.0 ± 2.0	14.0-22.0	45.7 ± 1.2	51.3-68.3	15.0 ± 1.1	13.5-15.7
'Hajo'	16.3 ± 3.5	14.0-26.0	43.6 ± 5.0	42.7-68.3	14.4 ± 1.0	13.0-15.2
'Jorhat'	15.3 ± 2.7	16.0-25.0	44.2 ± 2.0	51.0-62.3	14.5 ± 0.8	13.5-15.0
'Suvama'	16.3 ± 2.0	16.0-23.0	43.3 ± 4.0	44.0-68.0	15.5 ± 0.2	14.7-16.0
'Sudarsana'	17.3 ± 1.7	16.0-21.0	36.3 ± 13.5	24.0-62.7	15.2 ± 1.3	13.7-16.2
'Kedaram'	13.3 ± 2.0	18.0-21.0	36.5 ± 42.0	34.3-60.7	14.7 ± 2.5	11.5-15.0
'Suguna'	13.0 ± 2.6	14.0-23.0	39.3 ± 5.4	38.3-61.7	15.3 ± 1.0	13.3-17.2
'Prathibha'	13.0 ± 3.8	12.0-25.0	37.1 ± 10.7	28.0-62.7	14.3 ± 1.6	11.5-16.7
'Prabha'	14.2 ± 0.7	24.0-26.0	37.2 ± 11.0	27.7-62.7	14.7 ± 2.9	10.2-18.0
'Alleppey Supreme'	13.3 ± 4.1	10.0-24.0	42.7 ± 1.4	48.0-63.3	15.8 ± 0.1	15.3-16.7

NLC - number of leaves per clump, LL - leaf length, LW - leaf width.

Table 25. Characterwise mean and range of aerial morphological characters of turmeric (*C. longa* L.) varieties/cultivars.

Character	HP (cm)	PG (cm)	NTC	NLT	NLC	LL (cm)	LW (cm)
Mean	41.45 ± 1.22	9.24 ± 0.16	2.85 ± 0.09	8.138 ± 0.11	15.14 ± 0.51	42.23 ± 1.28	15.89 ± 0.35
Range	32.8 - 50.1	7.6- 10.6	2.3 - 3.8	6.5 - 9.0	12.0 - 19.0	31.2 - 50.4	12.7 - 17.7

HP - height of pseudostem, PG - pseudostem girth, NLT - number of leaves per tiller, NTC - number of tillers per clump, NLC - number of leaves per clump, LL - leaf length, LW - leaf width.

4.1.2.1.1. Height of pseudostem (Plant height)

The height of the pseudostem ranged from 32.8 ± 8.1 cm to 50.1 ± 3.9 cm in different cultivars/varieties of turmeric studied. Maximum pseudostem height was recorded in the cultivar 'Amrithapani' followed by 'Aizwal', 'Armoor' and 'Dindigam' with 48.7 ± 5.3 cm, 47.3 ± 0.9 cm and 47.3 ± 4.5 cm, respectively. Minimum plant height was recorded in the cultivar 'Katergia' (32.8 ± 8.1 cm).

4.1.2.1.2. Pseudostem girth

The cultivar 'Hajo' collected from Assam showed maximum pseudostem girth (10.6 ± 0.3 cm) followed by 'GL Puram', 'Kodur' and 'Armoor' collected from Andhra Pradesh with 10.4 ± 0.4 cm, 10.2 ± 1.3 cm and 10.2 ± 0.8 cm, respectively. The improved variety 'Sudarsana' showed minimum pseudostem girth (7.6 ± 1.4 cm).

4.1.2.1.3. Number of tillers

Number of tillers per clump varied from 2.3 ± 0.4 ('Alleppey') to 3.8 ± 1.2 ('Sudarsana').

4.1.2.1.4. Number of leaves per tiller

Total number of leaves in the main tiller was maximum in cultivar 'Jabedi' (9.0 ± 1.8) and the least number of leaves per tiller was recorded in 'Basar Along' (6.5 ± 1.5).

4.1.2.1.5. Number of leaves per clump

Highest number of leaves per clump was recorded by 'Ethamkula' (19.0 ± 3.0) followed by 'Chayapasupu' (19.0 ± 0.6) and 'Muvatupuzha' (18.7 ± 2.9). Least number of leaves per clump was recorded in 'Aizwal' (12.0 ± 2.1).

4.1.2.1.6. Leaf length

'Amrithapani' (50.4 ± 2.7 cm) showed maximum leaf length followed by 'Nandyal type' (49.3 ± 3.7 cm) and 'Dindigam' (48.3 ± 4.9 cm). Cultivar 'Alleppey' had the least leaf length (31.2 ± 0.7 cm).

4.1.2.1.7. Leaf width

The leaf width varied from 17.7 ± 0.7 cm (“Jabedi”) to 12.7 ± 0.8 cm (‘Alleppey’).

4.1.2.2. Rhizome characters

Mean and range of rhizome characters and dry recovery are summarized in Table 26. Characterwise range and mean are given in Table 27.

Table 26. Mean and range of rhizome traits of turmeric varieties/cultivars

Cultivar/Variety	CW (g)		NM		WM (g)		NP		WP (g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
‘Alleppey’	695.3 $\pm$ 4.0	548.0-982.0	1.3 $\pm$ 0.0	1.0-2.0	52.7 $\pm$ 2.0	38.0-78.0	8.0 $\pm$ 1.5	4.0-13.0	256.7 $\pm$ 7.0	132.0-492.0
‘Amalapuram’	784.3 $\pm$ 15.0	690.0-943.0	1.0 $\pm$ 0.0	1.0	43.0 $\pm$ 3.0	30.0-63.0	11.3 $\pm$ 3.5	5.0-17.0	272.7 $\pm$ 12.0	148.0-498.0
‘Amrithapani’	554.3 $\pm$ 14.5	483.0-668.0	1.0 $\pm$ 0.0	1.0	51.0 $\pm$ 3.5	39.0-68.0	7.0 $\pm$ 2.0	4.0-9.0	136.7 $\pm$ 3.0	82.0-240.0
‘Armoor’	517.0 $\pm$ 16.0	342.0-835.0	1.0 $\pm$ 0.0	1.0	48.3 $\pm$ 6.5	35.0-62.0	9.3 $\pm$ 5.0	4.0-14.0	221.7 $\pm$ 4.0	146.0-365.0
‘Jabedi’	695.3 $\pm$ 9.0	585.0-898.0	1.0 $\pm$ 0.0	1.0	42.7 $\pm$ 4.0	31.0-58.0	7.0 $\pm$ 0.5	3.0-14.0	232.0 $\pm$ 0.0	92.0-512.0
‘Dhagi’	296.7 $\pm$ 55.5	119.0-541.0	1.0 $\pm$ 0.0	1.0	44.0 $\pm$ 3.5	31.0-63.0	5.3 $\pm$ 0.0	3.0-10.0	100.0 $\pm$ 4.0	56.0-180.0
‘Katergia’	777.0 $\pm$ 12.5	586.0-1134.0	1.3 $\pm$ 0.0	1.0-2.0	48.0 $\pm$ 4.5	32.0-71.0	12.7 $\pm$ 3.0	6.0-20.0	367.3 $\pm$ 5.0	186.0-720.0
‘Chayapasupu’	579.3 $\pm$ 11.0	410.0-896.0	1.0 $\pm$ 0.0	1.0	58.7 $\pm$ 6.0	45.0-74.0	9.0 $\pm$ 1.0	4.0-17.0	250.7 $\pm$ 0.0	121.0-510.0
‘Avanigadda’	511.0 $\pm$ 3.5	233.0-1060.0	1.6 $\pm$ 0.0	1.0-3.0	84.7 $\pm$ 5.0	46.0-152.0	9.3 $\pm$ 0.0	4.0-20.0	268.7 $\pm$ 0.0	98.0-610.0
‘Dindigam’	567.0 $\pm$ 12.0	426.0-825.0	1.0 $\pm$ 0.0	1.0	62.3 $\pm$ 6.0	51.0-73.0	7.3 $\pm$ 0.0	4.0-14.0	208.0 $\pm$ 3.0	89.0-440.0
‘Ethamkula’	530.3 $\pm$ 5.5	405.0-770.0	1.6 $\pm$ 0.0	1.0-3.0	69.3 $\pm$ 2.0	28.0-148.0	8.7 $\pm$ 1.5	5.0-13.0	199.3 $\pm$ 3.0	126.0-340.0
‘GL Puram’	667.7 $\pm$ 7.0	584.0-821.0	1.3 $\pm$ 0.0	1.0-2.0	57.3 $\pm$ 3.5	42.0-81.0	9.0 $\pm$ 2.5	5.0-12.0	250.7 $\pm$ 5.0	146.0-450.0
‘Karhadi local’	452.7 $\pm$ 11.0	212.0-912.0	1.6 $\pm$ 0.0	1.0-3.0	70.3 $\pm$ 6.0	26.0-147.0	8.3 $\pm$ 0.0	5.0-15.0	237.0 $\pm$ 0.0	98.0-515.0
“‘Kasturi”	453.0 $\pm$ 14.0	261.0-809.0	1.3 $\pm$ 0.0	1.0-2.0	43.7 $\pm$ 5.5	24.0-72.0	6.7 $\pm$ 0.0	4.0-12.0	193.3 $\pm$ 3.5	74.0-425.0
‘Kodur’	617.0 $\pm$ 12.5	508.0-810.0	2.0 $\pm$ 0.0	2.0	104.7 $\pm$ 5.0	68.0-168.0	8.7 $\pm$ 0.0	7.0-12.0	154.7 $\pm$ 1.0	91.0-280.0
‘Kuchipudi’	479.3 $\pm$ 20.0	435.0-528.0	1.3 $\pm$ 0.5	1.0-2.0	79.3 $\pm$ 5.5	72.0-83.0	7.7 $\pm$ 1.0	7.0-9.0	150.7 $\pm$ 5.0	131.0-180.0
‘Mamomthody’	415.7 $\pm$ 9.0	278.0-673.0	1.6 $\pm$ 0.0	1.0-3.0	63.7 $\pm$ 5.5	21.0-138.0	6.7 $\pm$ 0.0	4.0-12.0	154.7 $\pm$ 1.5	83.0-295.0
‘Muvatupuzha’	532.7 $\pm$ 11.5	506.0-563.0	1.3 $\pm$ 0.5	1.0-2.0	67.0 $\pm$ 4.5	62.0-71.0	9.3 $\pm$ 3.5	5.0-12.0	178.3 $\pm$ 4.0	121.0-285.0
‘Nandyal type’	423.3 $\pm$ 12.0	302.0-642.0	1.0 $\pm$ 0.0	1.0	48.7 $\pm$ 8.0	33.0-64.0	8.0 $\pm$ 1.5	5.0-11.0	172.0 $\pm$ 2.0	101.0-310.0
‘Rajapuri’	531.3 $\pm$ 10.5	349.0-875.0	1.6 $\pm$ 0.0	1.0-3.0	75.3 $\pm$ 6.5	39.0-135.0	7.7 $\pm$ 0.0	5.0-13.0	178.3 $\pm$ 0.5	97.0-340.0
‘Sugandham’	435.3 $\pm$ 15.5	288.0-699.0	1.3 $\pm$ 0.0	1.0-2.0	62.7 $\pm$ 4.5	54.0-71.0	5.7 $\pm$ 0.0	4.0-9.0	137.0 $\pm$ 7.5	63.0-270.0
‘Vondimita’	395.0 $\pm$ 16.0	248.0-657.0	1.3 $\pm$ 0.0	1.0-2.0	56.3 $\pm$ 10.0	38.0-73.0	6.0 $\pm$ 0.0	5.0-8.0	126.7 $\pm$ 7.0	47.0-272.0
“‘Wynad’ local’	582.3 $\pm$ 8.5	499.0-732.0	1.0 $\pm$ 0.0	1.0	53.3 $\pm$ 3.5	42.0-69.0	8.7 $\pm$ 2.5	5.0-11.0	184.3 $\pm$ 2.5	114.0-320.0
Aizwal	636.0 $\pm$ 8.0	572.0-676.0	1.6 $\pm$ 0.0	1.0-2.0	75.0 $\pm$ 3.0	67.0-82.0	10.3 $\pm$ 2.0	7.0-14.0	205.3 $\pm$ 5.0	198.0-210.0
Ayur	475.3 $\pm$ 7.5	419.0-573.0	1.0 $\pm$ 0.0	1.0	46.7 $\pm$ 5.0	31.0-68.0	5.7 $\pm$ 0.0	5.0-7.0	138.0 $\pm$ 0.5	95.0-223.0
‘Basar Along’	528.0 $\pm$ 13.0	453.0-652.0	1.3 $\pm$ 0.0	1.0-2.0	53.3 $\pm$ 5.0	39.0-72.0	6.0 $\pm$ 0.0	5.0-8.0	138.7 $\pm$ 2.0	72.0-268.0
Gaspani	342.7 $\pm$ 11.0	210.0-586.0	1.3 $\pm$ 0.0	1.0-2.0	62.0 $\pm$ 7.5	46.0-79.0	5.3 $\pm$ 0.0	5.0-6.0	117.3 $\pm$ 2.0	78.0-192.0
Hajo	482.0 $\pm$ 9.0	357.0-714.0	1.3 $\pm$ 0.0	1.0-2.0	65.7 $\pm$ 5.5	52.0-82.0	8.0 $\pm$ 1.0	6.0-10.0	186.0 $\pm$ 1.0	132.0-292.0
‘Jorhat’	307.7 $\pm$ 1.0	137.0-647.0	1.0 $\pm$ 0.0	1.0	47.0 $\pm$ 2.5	34.0-68.0	6.7 $\pm$ 0.0	5.0-10.0	140.3 $\pm$ 3.5	62.0-290.0
‘Suvama’	355.3 $\pm$ 67.5	164.0-603.0	1.0 $\pm$ 0.0	1.0	39.7 $\pm$ 7.0	18.0-69.0	8.7 $\pm$ 0.0	8.0-10.0	148.7 $\pm$ 2.0	78.0-286.0
‘Sudarsana’	820.7 $\pm$ 69.5	701.0-950.0	1.6 $\pm$ 0.5	1.0-2.0	82.3 $\pm$ 5.0	76.0-86.0	10.3 $\pm$ 2.0	7.0-13.0	327.0 $\pm$ 6.5	310.0-348.0
‘Kedaram’	822.7 $\pm$ 11.0	740.0-875.0	1.6 $\pm$ 0.5	1.0-2.0	89.0 $\pm$ 4.5	80.0-98.0	8.7 $\pm$ 0.5	8.0-9.0	280.3 $\pm$ 0.5	271.0-298.0
‘Suguna’	423.3 $\pm$ 10.0	295.0-660.0	1.0 $\pm$ 0.0	1.0	37.3 $\pm$ 4.0	21.0-62.0	6.7 $\pm$ 1.0	4.0-10.0	184.7 $\pm$ 2.0	121.0-308.0
‘Prathibha’	770.0 $\pm$ 9.0	737.0-818.0	1.3 $\pm$ 0.0	1.0-2.0	57.7 $\pm$ 1.5	49.0-72.0	7.3 $\pm$ 0.5	5.0-11.0	212.3 $\pm$ 5.0	152.0-323.0
‘Prabha’	809.7 $\pm$ 5.0	803.0-813.0	1.3 $\pm$ 0.0	1.0-2.0	59.7 $\pm$ 0.5	50.0-78.0	8.7 $\pm$ 1.0	6.0-12.0	219.3 $\pm$ 1.5	168.0-319.0
‘Alleppey’ Supreme	818.7 $\pm$ 7.5	795.0-838.0	1.3 $\pm$ 0.0	1.0-2.0	57.3 $\pm$ 0.5	48.0-75.0	8.7 $\pm$ 2.0	5.0-12.0	228.7 $\pm$ 2.0	178.0-326.0

CW - clump weight, NM - number of mother rhizome, WM - weight of mother rhizome, NP - number of primary rhizome, WP - weight of primary rhizome.

Table 26. (Contd.). Mean and range of rhizome traits of turmeric varieties/cultivars

Cultivar/Variety	NS		WS (g)		INL (cm)		Dry (%)		RC
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
'Alleppey'	27.0 ± 0.5	22.0-36.0	386.0 ± 5.0	368.0-412.0	1.3 ± 0.0	1.2-1.6	20.2 ± 0.4	19.8-20.6	Reddish yellow
'Amalapuram'	32.3 ± 1.5	26.0-42.0	468.7 ± 0	382.0-512.0	1.3 ± 0.0	1.2-1.6	19.6 ± 0.4	19.2-20.0	Reddish yellow
'Amrithapani'	24.7 ± 1.5	21.0-28.0	366.7 ± 8.0	360.0-378.0	1.1 ± 0.0	1.0-1.3	19.4 ± 0.4	19.0-19.8	Reddish yellow
'Armoor'	25.7 ± 0.5	16.0-44.0	247.0 ± 5.5	161.0-408.0	1.1 ± 0.0	1.0-1.3	19.7 ± 0.4	19.3-20.0	Yellow
'Jabedi'	33.0 ± 3.0	24.0-45.0	420.7 ± 5.0	328.0-472.0	1.3 ± 0.0	1.2-1.6	19.1 ± 0.3	18.8-19.4	Yellow
'Dhagi'	18.7 ± 0.0	14.0-28.0	179.3 ± 8.0	112.0-298.0	1.4 ± 0.0	1.3-1.7	19.3 ± 0.2	19.1-19.4	Yellow
'Katergia'	43.0 ± 1.5	36.0-54.0	361.3 ± 3.0	342.0-374.0	1.2 ± 0.0	1.1-1.4	19.6 ± 0.4	19.2-20.0	Yellow
'Chayapasupu'	28.0 ± 1.0	20.0-42.0	270.0 ± 5.0	244.0-312.0	1.4 ± 0.0	1.3-1.7	18.7 ± 0.4	18.3-19.0	Yellow
'Avanigadda'	23.0 ± 0.0	9.0-51.0	157.7 ± 1.5	86.0-298.0	1.0 ± 0.0	0.9-1.2	18.9 ± 0.3	18.6-19.2	Yellow
'Dindigam'	24.0 ± 0.0	18.0-36.0	296.7 ± 3.0	286.0-312.0	1.1 ± 0.0	1.0-1.4	19.9 ± 0.4	19.5-20.2	Yellow
'Ethamkula'	33.7 ± 1.5	32.0-36.0	261.7 ± 0.5	251.0-282.0	1.1 ± 0.0	1.0-1.4	18.8 ± 0.2	18.6-19.0	Yellow
'GL Puram'	26.7 ± 3.0	20.0-30.0	359.7 ± 1.5	290.0-396.0	1.2 ± 0	1.1-1.5	19.2 ± 0.4	18.8-19.6	Yellow
'Karhadi local'	23.0 ± 0.0	20.0-29.0	145.3 ± 5.0	88.0-250.0	1.5 ± 0.0	1.3-1.6	19.5 ± 0.3	19.2-19.8	Reddish yellow
'Kasturi'	20.3 ± 0.0	15.0-31.0	216.0 ± 5.0	163.0-312.0	1.0 ± 0.0	0.9-1.2	19.2 ± 0.2	19.0-19.4	Yellow
'Kodur'	35.3 ± 1.0	34.0-36.0	357.7 ± 6.5	349.0-362.0	1.3 ± 0.0	1.2-1.6	19.1 ± 0.1	19.0-19.2	Yellow
'Kuchipudi'	29.3 ± 0.5	29.0-30.0	256.0 ± 9.5	232.0-285.0	1.3 ± 0.0	1.2-1.6	18.8 ± 0.2	18.6-19.0	Yellow
'Mamomthody'	16.7 ± 0.0	13.0-24.0	193.3 ± 5.0	171.0-240.0	1.3 ± 0.0	1.2-1.6	18.6 ± 0.3	18.3-18.8	Reddish yellow
'Muvatupuzha'	20.7 ± 1.5	18.0-23.0	287.3 ± 3.0	210.0-329.0	1.1 ± 0.0	1.0-1.3	18.7 ± 0.3	18.4-19.0	Yellow
'Nandyal type'	19.3 ± 0.0	15.0-28.0	202.7 ± 2.0	168.0-268.0	1.1 ± 0.0	1.0-1.3	18.7 ± 0.4	18.3-19.0	Yellow
'Rajapuri'	24.0 ± 0.0	20.0-32.0	277.7 ± 4.5	212.0-400.0	1.3 ± 0.0	1.2-1.6	21.8 ± 0.8	21.0-22.6	Yellow
'Sugandham'	14.0 ± 1.5	8.0-23.0	235.7 ± 3.5	171.0-358.0	1.3 ± 0.0	1.2-1.6	18.1 ± 0.3	17.8-18.4	Yellow
'Vondimita'	19.0 ± 0.0	16.0-25.0	215.3 ± 4.0	163.0-312.0	1.1 ± 0.0	1.0-1.3	18.5 ± 0.4	18.1-18.9	Yellow
'Wynad' local'	27.0 ± 0.5	25.0-30.0	344.7 ± 2.5	343.0-348	1.3 ± 0.0	1.2-1.6	19.9 ± 0.9	19.0-20.8	Reddish Yellow
'Aizwal'	35.7 ± 3.0	23.0-45.0	355.7 ± 0.0	295.0-386.0	1.2 ± 0.0	1.1-1.5	19.1 ± 0.1	19.0-19.2	Reddish yellow
Ayur	21.0 ± 0.0	16.0-26.0	290.7 ± 3.0	282.0-298.0	0.9 ± 0.0	0.8-1.1	19.1 ± 0.5	18.6-19.5	Reddish yellow
'Basar Along'	16.7 ± 4.0	10.0-22.0	336.0 ± 6.0	312.0-354.0	1.4 ± 0.0	1.3-1.7	17.1 ± 0.2	16.9-17.2	Reddish yellow
'Gaspani'	14.7 ± 0.0	9.0-26.0	163.3 ± 1.5	86.0-315	1.1 ± 0.0	1.0-1.3	17.9 ± 0.1	17.8-18.0	Yellow
'Hajo'	21.3 ± 0.0	17.0-30.0	230.3 ± 2.5	173.0-340.0	1.4 ± 0.0	1.3-1.7	18.3 ± 0.1	18.2-18.3	Yellow
'Jorhat'	13.7 ± 0.0	5.0-31.0	120.3 ± 2.0	34.0-289.0	1.4 ± 0.0	1.3-1.7	18.3 ± 0.2	18.1-18.4	Reddish yellow
'Suvarna'	23.3 ± 0.0	21.0-28.0	129.0 ± 1.5	68.0-248.0	1.2 ± 0.0	1.1-1.4	18.2 ± 0.2	18.0-18.3	Reddish yellow
'Sudarsana'	29.3 ± 0.0	26.0-36.0	411.3 ± 58.0	268.0-541.0	1.6 ± 0.0	1.5-1.8	18.0 ± 0.2	17.8-18.2	Reddish yellow
'Kedaram'	34.0 ± 0.0	30.0-36.0	453.3 ± 7.0	362.0-506.0	1.3 ± 0.0	1.2-1.6	20.5 ± 0.5	20.0-21.0	Reddish yellow
'Suguna'	17.3 ± 0.0	10.0-32.0	201.3 ± 4.0	153.0-290.0	1.5 ± 0.0	1.4-1.7	18.4 ± 0.2	18.2-18.6	Reddish yellow
'Prathibha'	28.0 ± 0.0	26.0-32.0	500.0 ± 2.5	423.0-54.0	1.3 ± 0.0	1.3-1.5	20.0 ± 0.2	19.8-20.2	Reddish yellow
'Prabha'	31.7 ± 0.5	29.0-36.0	497.3 ± 54.0	416.0-592.0	1.3 ± 0.0	1.2-1.6	19.8 ± 0.2	19.6-20.0	Reddish yellow
'Alleppey Supreme'	31.7 ± 5.5	25.0-36.0	532.7 ± 6.0	394.0-608.0	1.4 ± 0.0	1.3-1.5	20.4 ± 0.6	19.8-21.0	Reddish yellow

NS - number of secondary rhizome, WS - weight of secondary rhizome, INL - internode length, RC - rhizome colour

Table 27. Characterwise mean and range of rhizome traits of turmeric varieties/cultivars

Character	CW (cm)	NM	WM (cm)	NP	WP (cm)	NS	WS (cm)	INL	Dry %
Mean	557.9 ± 33.9	1.3 ± 0.1	60.0 ± 4.5	8.0 ± 0.5	198.8 ± 16.5	25.19 ± 1.67	298 ± 25.49	1.27 ± 0.04	19.1 ± 0.32
Range	296.7-822.7	1.0-1.6	37.3-104.7	5.3-12.7	100.0-367.3	13.7-43.0	120.3 -532.7	0.9-1.6	17.1-21.8

CW - clump weight, *NM* - number of mother rhizome, *WM* - weight of mother rhizome, *NP* - number of primary rhizome, *WP* - weight of primary rhizome, *NS* - number of secondary rhizome, *WS* - weight of secondary rhizome, *INL* - internode length.

4.1.2.2.1. Clump weight /Weight of rhizome

Total weight of the clump (rhizome weight) varied from 296.7 ± 55.5 to 822.7 ± 11.0 g. The improved varieties like 'Kedaram' (822.7 ± 11.0 g) followed by 'Sudarsana' (820.7 ± 69.5 g) and 'Alleppey Supreme' (818.7 ± 7.5 g) showed maximum clump weight while 'Dhagi', a cultivar collected from Andhra Pradesh recorded minimum weight. (296.7 ± 55.5 g)

4.1.2.2.2. Number of mother rhizome

Number of mother rhizome was maximum in the cultivar "Kodur" (2.0 ± 0). In all other varieties/cultivars either 1.0 ± 0 , 1.3 ± 0 or 1.6 ± 0 numbers of mother rhizomes were observed.

4.1.2.2.3. Weight of mother rhizome

Maximum weight of mother rhizomes was recorded in the cultivar 'Kodur' (104.7 ± 5.0 g) followed by the improved variety 'Kedaram' (89.0 ± 4.5 g). Improved variety 'Suguna' had the least mother rhizome weight of 37.3 ± 4.0 g.

4.1.2.2.4. Number of primary rhizome

'Katergia' produced maximum number of primary rhizomes (12.7 ± 3.0) followed by 'Amalapuram' (11.3 ± 3.5). Minimum number of primary rhizome was observed in 'Dhagi' and 'Gaspani' (5.3 ± 0 each).

4.1.2.2.5. Weight of primary rhizome

The weight of primary rhizomes ranged from 100.0 ± 4.0 to 367.3 ± 5.0 g. Maximum primary rhizome weight was recorded in 'Katergia' (367.3 ± 5.0 g) followed by 'Sudarsana' (327.0 ± 6.5 g) and 'Kedaram' (280.3 ± 0.5 g). 'Dhagi' recorded the least weight of primary rhizomes (100.0 ± 4.0 g).

4.1.2.2.6. Number of secondary rhizome

Number of secondary was maximum in 'Katergia' (43.0 ± 1.5) followed by 'Aizwal' (35.7 ± 3.0). The improved variety 'Kedaram' produced 34.0 ± 0 numbers of secondary rhizome while 'Jorhat' had the least value for this trait (13.7 ± 0).

4.1.2.2.7. Weight of secondary rhizome

Maximum weight of secondary rhizomes was recorded in the improved varieties of turmeric viz. 'Alleppey Supreme', 'Prathibha', and 'Prabha' with 532.7 ± 6.0 g, 500.0 ± 2.5 g and 497.3 ± 54.0 g respectively while 'Jorhat' recorded minimum weight of secondary rhizomes (120.3 ± 2.0 g).

4.1.2.2.8. Internode length

Maximum internode length was recorded in the improved variety 'Sudarsana' (1.6 ± 0 cm) and minimum in the cultivar 'Ayur' (0.9 ± 0 cm).

4.1.2.2.9. Rhizome colour

The rhizome colour (mother/primary) varied from reddish yellow to orange yellow in the different cultivars/varieties studied. All the improved varieties and cultivars like 'Alleppey', 'Aizwal', 'Ayur', 'Amalapuram', 'Amrithapani', 'Basar Along', 'Jorhat', 'Karhadi local' and 'Manaonthody' had a characteristic reddish yellow colour while all other cultivars studied showed yellow colour of the rhizome inner core.

4.1.2.2.10. Dry recovery

Dry recovery was found to be maximum in the cultivar 'Rajapuri' (21.8 ± 0.8 %) while 'Basar Along' gave minimum dry recovery (17.1 ± 0.2 %).

4.1.2.3. Cluster analysis

The paired affinity index of the different traits in the turmeric cultivars/varieties studied and the UPGMA dendrogram constructed using these indexes are presented in Table 28 and Fig. 16.

The UPGMA dendrogram constructed using the Jaccard's similarity coefficients splits into eleven groups at coefficient 0.48, showing maximum similarity between 'Alleppey' and 'Prabha' (1.0)

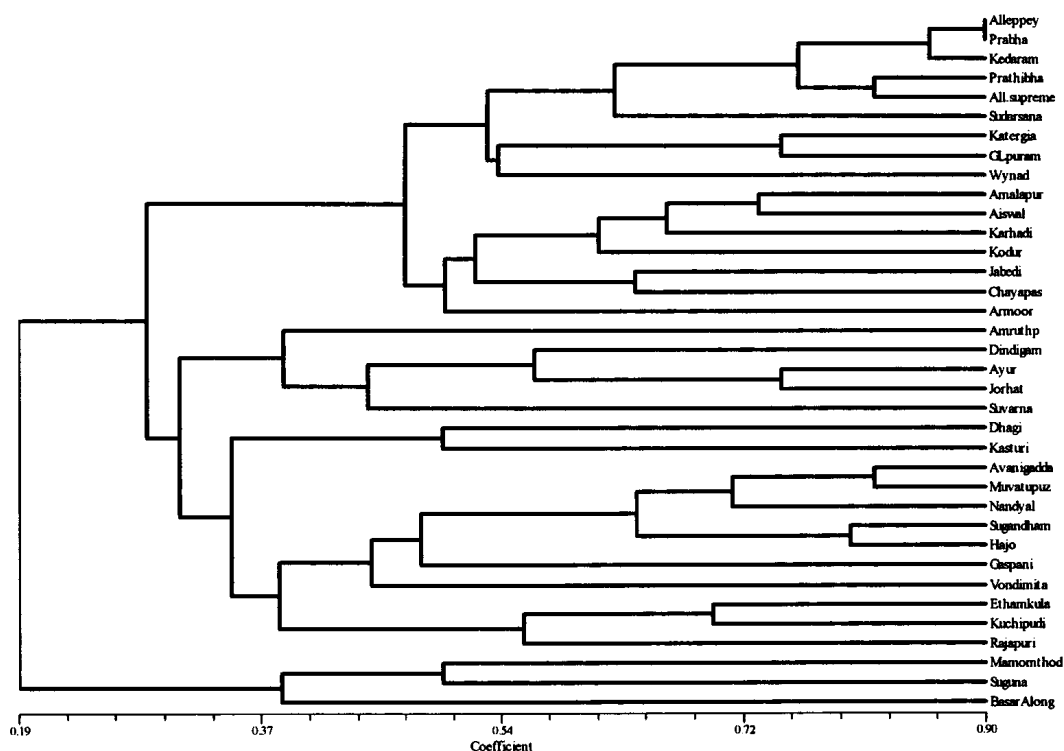


Fig.16. Clustering patterns of 36 turmeric varieties/cultivars based on Jaccard's similarity coefficients using morphological traits.

- Group I - 'Alleppey', 'Prabha', 'Kedaram', 'Prathibha', 'Alleppey Supreme', 'Sudarsana', 'Katergia', 'GL Puram' and 'Wynad'
- Group II - 'Amalapuram', 'Aizwal', 'Karhadi local', 'Kodur', 'Jabedi', 'Chayapasupu' and 'Armoor'
- Group III - 'Amrithpani'
- Group IV - 'Dindigam', 'Ayur' and 'Jorhat'
- Group V - 'Suvarna'
- Group VI - 'Dhagi' and 'Kasturi'
- Group VII - 'Avanigadda', 'Muvatupuzha', 'Nandyal', 'Sugandham', 'Hajo' and 'Gaspani'
- Group VIII - 'Vondimitta'
- Group IX - 'Ethamkula', 'Kuchipudi' and 'Rajapuri'
- Group X - 'Mamomthody' and 'Suguna'
- Group XI - 'Basar Along'

The group I of the dendrogram accommodated maximum number (nine) of varieties/cultivars studied. Members of this group showed highest similarity in the dendrogram ('Alleppey' and 'Prabha' (1.0)). Seven accessions formed the II group of the cluster with maximum similarity between 'Amalapuram' and 'Aizwal' (0.73). Four groups in the cluster viz. Group III, V, VIII and XI were formed by single individuals ('Amruthpani', 'Suvarna', 'Vondimita' and 'Basar Along', respectively). 'Dindigam', 'Ayur' and 'Jorhat' formed the fourth group with maximum similarity between 'Ayur' and 'Jorhat' (0.75). 'Dhagi' and 'Kasturi' & 'Mamomthody' and 'Suguna' formed sixth and tenth groups of the cluster. 'Avanigadda', 'Muvatupuzha', 'Nandyal', 'Sugandham', 'Hajo' and 'Gaspani' formed the seventh group of the cluster with maximum similarity between 'Avanigadda' and 'Muvatupuzha' (0.82). 'Ethamkula', 'Kuchipudi' and 'Rajapuri' were the members of the group IX in the dendrogram.

4.1.3. Characterization of open pollinated progenies of turmeric.

4.1.3.1. Aerial morphological characters

Mean and range of the characters used for characterization of the open pollinated progenies of turmeric (*C. longa* L.) are summarized in Table 29.

Table 29. Mean and range of aerial morphological characters in open pollinated progenies of turmeric

Accession	HP (cm)		PG (cm)		NLT		NTC	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
OP 'Muvatupuzha'	61.7 ± 2.7	58.0-67.0	11.6 ± 1.5	9.4-14.5	6.7 ± 0.3	6.0-7.0	3.0 ± 0.6	2.0-4.0
OP 'Alleppey'	74.2 ± 2.7	69.0-78.0	12.2 ± 1.6	10.0-15.4	10.0 ± 0.6	9.0-11.0	2.3 ± 0.3	2.0-3.0
OP 3/ 'Amrithapani'	51.0 ± 3.5	45.0-57.0	9.1 ± 0.6	8.0-10.2	6.0 ± 0.6	5.0-7.0	2.3 ± 0.3	2.0-3.0
OP 'Katergia'	52.3 ± 3.8	48.0-60.0	11.0 ± 0.6	10.0-12.0	8.7 ± 0.3	8.0-9.0	3.0 ± 0.6	2.0-4.0
OP 'Dindigam'	66.3 ± 2.7	61.0-70.0	11.2 ± 0.8	9.8-12.7	10.0 ± 0.6	9.0-11.0	2.7 ± 0.7	2.0-4.0
OP 'Kasturi'	42.7 ± 2.7	39.0-48.0	9.1 ± 0.7	8.0-10.4	7.3 ± 0.9	6.0-9.0	3.7 ± 0.9	2.0-5.0
OP 23/ Ayur	63.7 ± 6.1	53.0-74.0	10.5 ± 0.4	9.8-11.0	9.0 ± 0.6	8.0-10.0	2.3 ± 0.3	2.0-3.0
OP 'Sugandham'	62.0 ± 1.5	59.0-64.0	10.4 ± 0.3	10.0-11.0	9.3 ± 0.3	9.0-1.0	2.7 ± 0.7	2.0-4.0
OP 'Aizwal'	58.0 ± 2.1	54.0-61.0	10.7 ± 0.9	9.0-12.0	9.7 ± 0.9	8.0-11.0	2.0 ± 0.6	1.0-3.0
OP 'Amalapuram'	52.7 ± 3.5	47.0-59.2	11.0 ± 0.6	10.0-12.0	9.0 ± 0.6	8.0-10.0	2.3 ± 0.3	2.0-3.0
OP 2/ 'Jabedi'	50.8 ± 4.2	45.0-59.2	10.1 ± 0.2	9.8-10.4	7.3 ± 0.9	6.0-9.0	2.0 ± 0.6	1.0-3.0
OP 'Avanigadda'	60.0 ± 5.0	54.0-70.0	10.2 ± 0.6	9.0-11.0	8.3 ± 0.7	7.0-9.0	3.0 ± 0.6	2.5-4.0

HP - height of pseudostem, PG - pseudostem girth, NLT - number of leaves per tiller, NTC - number of tillers per clump.

Table 29 (Contd.). Mean and range of aerial morphological characters in open pollinated progenies of turmeric

Accession	NLC		LL (cm)		LW (cm)	
	Mean	Range	Mean	Range	Mean	Range
OP 'Muvatupuzha'	15.7 ± 1.5	13.0-18.0	64.5 ± 2.9	61.0-70.2	17.9 ± 0.5	17.2-18.9
OP 'Alleppey'	14.3 ± 1.5	12.0-17.0	69.0 ± 5.0	63.0-79.0	18.9 ± 0.2	18.6-19.3
OP 3/ 'Amrithapani'	11.0 ± 1.7	8.0-14.0	55.7 ± 0.9	54.0-57.0	18.7 ± 0.9	17.0-20.0
OP 'Katergia'	17.7 ± 1.9	14.0-20.0	58.6 ± 1.2	56.8-61.0	17.6 ± 1.4	15.0-19.7
OP 'Dindigam'	14.7 ± 0.7	14.0-16.0	66.3 ± 2.5	63.2-71.3	16.4 ± 0.6	15.5-17.6
OP 'Kasturi'	14.7 ± 1.2	13.0-17.0	56.2 ± 1.9	52.3-58.7	16.9 ± 0.5	16.0-17.6
OP 23/ Ayur	13.7 ± 1.2	12.0-16.0	62.8 ± 3.5	57.0-69.0	17.4 ± 1.3	15.0-19.3
OP Sugandham'	14.3 ± 0.9	13.0-16.0	61.4 ± 2.8	56.0-65.2	19.3 ± 0.7	18.4-20.6
OP 'Aizwal'	12.0 ± 1.2	10.0-14.0	68.7 ± 1.8	65.4-71.5	15.5 ± 0.5	14.5-16.0
OP 'Amalapuram'	13.3 ± 0.3	13.0-14.0	60.9 ± 4.2	52.8-67.0	16.9 ± 0.5	16.0-17.6
OP 2/ 'Jabedi'	10.7 ± 2.4	6.0-14.0	53.3 ± 2.4	49.0-57.3	18.3 ± 0.7	17.0-19.0
OP 'Avanigadda'	15.0 ± 1.5	13.0-18.0	64.3 ± 3.3	58.0-69.0	17.5 ± 0.9	16.0-19.0

NLC - number of leaves per clump, LL - leaf length, LW - leaf width

Table 30. Characterwise mean and range of aerial morphological characters in open pollinated progenies of turmeric

Character	HP (cm)	PG (cm)	NLT	NTC	NLC	LL (cm)	LW (cm)
Mean	58.0 ± 2.5	10.6 ± 0.3	8.4 ± 0.4	2.6 ± 0.1	13.9 ± 0.6	61.8 ± 1.5	17.6 ± 0.3
Range	51.0-74.2	9.1-12.2	6.7-10.0	2.0-3.7	10.7-17.7	53.3-69.0	15.5-19.3

HP - height of pseudostem, PG - pseudostem girth, NLT - number of leaves per tiller, NTC - number of tillers per clump, NLC - number of leaves per clump, LL - leaf length, LW - leaf width.

4.1.3.1.1. Height of pseudostem (Plant height)

The height of the pseudostem ranged from 50.8 ± 4.2 cm to 74.2 ± 2.7 cm. Maximum pseudostem height was recorded in the OP progeny of 'Alleppey' and the minimum plant height was recorded in the OP 2 progeny of 'Jabedi'.

4.1.3.1.2. Pseudostem girth

The OP 'Alleppey' showed maximum pseudostem girth (12.2 ± 1.6 cm) and the OP 3/ 'Amrithapani' showed minimum pseudostem girth (9.1 ± 0.6 cm).

4.1.3.1.3. Number of tillers

Number of tillers per clump varied from 2.0 ± 0.6 (OP 'Aizwal', OP 2/ 'Jabedi') to 3.7 ± 0.9 (OP 'Kasturi').

4.1.3.1.4. Number of leaves per tiller

Total number of leaves in the main tiller was maximum in the OP 'Alleppey' and OP 'Dindigam' (10.0 ± 0.6) and the least number of leaves was recorded by OP 3/ 'Amrithapani' (6.0 ± 0.6).

4.1.3.1.5. Number of leaves per clump

Highest number of leaves per clump was recorded by OP 'Katergia' (17.7 ± 1.9) followed by OP 'Muvatupuzha' (15.7 ± 1.5) and OP 'Avanigadda' (15.0 ± 1.5). Least number of leaves per clump was recorded in OP 2/ 'Jabedi' (10.7 ± 2.4).

4.1.3.1.6. Leaf length

OP 'Alleppey' (69.0 ± 5.0 cm) showed maximum leaf length followed by OP 'Aizwal' (68.7 ± 1.8 cm) and OP 'Dindigam' (66.3 ± 2.5 cm). OP 2/ 'Jabedi' had the least leaf length (53.3 ± 2.4 cm).

4.1.3.1.7. Leaf width

The leaf width varied from 15.5 ± 0.5 cm (OP 'Aizwal') to 19.3 ± 0.7 cm (OP 'Sugandham').

4.1.3.2. Rhizome characters

The mean and range of rhizome characters and dry recovery are summarized in Table.31. Characterwise range and mean are given in Table 32.

Table 31. Mean and range of rhizome traits of open pollinated progenies of turmeric

Acc. No.	CW (g)		NM		WM (g)		NP		WP (g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
OP 'Muvatupuzha'	413.3 $\pm$ 21.1	374.0-446.0	1.3 $\pm$ 0.3	1.0-2.0	63.3 $\pm$ 3.5	58.0-70.0	9.3 $\pm$ 0.9	8.0-11.0	164.0 $\pm$ 7.8	149.0-175.0
OP 'Alleppey'	534.3 $\pm$ 64.7	445.0-660.0	1.3 $\pm$ 0.3	1.0-2.0	64.7 $\pm$ 4.7	59.0-74.0	7.7 $\pm$ 0.9	6.0-9.0	230.0 $\pm$ 26.5	190.0-280.0
OP 3/ 'Amrithapani'	478.0 $\pm$ 63.6	372.0-592.0	1.0 $\pm$ 0.0	1.0-1.0	65.3 $\pm$ 3.8	61.0-73.0	7.0 $\pm$ 0.6	6.0-8.0	147.3 $\pm$ 13.7	120.0-162.0
OP 'Katergia'	605.3 $\pm$ 64.9	512.0-730.0	1.3 $\pm$ 0.3	1.0-2.0	58.7 $\pm$ 6.1	48.0-69.0	10.7 $\pm$ 0.9	9.0-12.0	265.7 $\pm$ 7.6	254.0-280.0
OP 'Dindigam'	425.0 $\pm$ 47.7	345.0-510.0	1.0 $\pm$ 0.0	1.0-1.0	70.7 $\pm$ 8.7	60.0-88.0	6.7 $\pm$ 0.9	5.0-8.0	192.0 $\pm$ 28.9	140.0-240.0
OP 'Kasturi'	610.0 $\pm$ 60.8	510.0-720.0	1.0 $\pm$ 0.0	1.0-1.0	64.7 $\pm$ 4.7	59.0-74.0	11.3 $\pm$ 1.2	9.0-13.0	294.0 $\pm$ 22.0	254.0-330.0
OP 23/ 'Ayur'	536.0 $\pm$ 73.7	422.0-674.0	1.0 $\pm$ 0.0	1.0-1.0	62.7 $\pm$ 4.1	55.0-69.0	11.3 $\pm$ 1.2	9.0-13.0	209.7 $\pm$ 14.7	184.0-235.0
OP 'Sugandham'	558.3 $\pm$ 73.6	430.0-685.0	1.0 $\pm$ 0.0	1.0-1.0	73.7 $\pm$ 3.3	69.0-80.0	9.7 $\pm$ 1.2	8.0-12.0	231.0 $\pm$ 30.9	178.0-285.0
OP 'Aizwal'	379.0 $\pm$ 21.1	380.0-415.0	1.3 $\pm$ 0.3	1.0-2.0	51.3 $\pm$ 1.8	48.0-54.0	6.0 $\pm$ 0.6	5.0-7.0	120.7 $\pm$ 12.5	96.0-136.0
OP 'Amalapuram'	336.7 $\pm$ 10.7	324.0-358.0	1.0 $\pm$ 0.0	1.0-1.0	60.3 $\pm$ 1.5	58.0-63.0	7.0 $\pm$ 0.6	6.0-8.0	124.0 $\pm$ 6.7	112.0-135.0
OP 2/ 'Jabedi'	567.0 $\pm$ 33.9	524.0-634.0	1.0 $\pm$ 0.0	1.0-1.0	56.3 $\pm$ 2.4	53.0-61.0	9.3 $\pm$ 1.2	7.0-11.0	223.7 $\pm$ 13.9	196.0-240.0
OP 'Avanigadda'	665.8 $\pm$ 72.9	530.0-780.0	1.3 $\pm$ 0.3	1.0-2.0	55.7 $\pm$ 2.4	51.0-59.0	12.7 $\pm$ 2.0	9.0-16.0	285.7 $\pm$ 36.9	215.0-340.0

CW - clump weight, NM - number of mother rhizome, WM - weight of mother rhizome, NP - number of primary rhizome, WP - weight of primary rhizome.

Table 31. (contd.). Mean and range of rhizome traits and dry recovery of open pollinated progenies of turmeric

Acc. No.	NS		WS (g)		INL		Dry (%)		RC
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
OP 'Muvatupuzha'	25.7 ± 2.0	22.0-29.0	181.0 ± 10.8	161.0-198.0	1.2 ± 0.1	1.1-1.4	18.7 ± 0.4	17.3-19.6	Yellow
O P 'Alleppey'	24.0 ± 3.6	19.0-31.0	231.7 ± 32.3	189.0-295.0	1.3 ± 0.1	1.2-1.5	19.9 ± 0.9	19.1-20.3	Reddish yellow
OP 'Amrithapani'	29.0 ± 4.6	21.0-37.0	261.7 ± 48.8	190.0-355.0	1.3 ± 0.1	1.2-1.5	19.5 ± 0.3	19.0-21.1	Orange
OP 'Katergia'	29.3 ± 5.2	21.0-39.0	272.7 ± 54.5	189.0-375.0	1.3 ± 0.1	1.2-1.5	19.4 ± 0.4	18.6-21.6	Yellow
OP 'Dindigam'	18.3 ± 3.4	14.0-25.0	155.0 ± 11.8	134.0-175.0	1.3 ± 0.1	1.2-1.5	19.7 ± 0.4	19.0-22.1	22Yellow
OP 'Kasturi'	30.0 ± 5.5	21.0-40.0	244.3 ± 35.3	189.0-310.0	1.4 ± 0.1	1.3-1.6	19.1 ± 0.1	17.8-19.5	Yellow
OP 23/ 'Ayur'	31.0 ± 5.5	22.0-41.0	257.0 ± 56.2	176.0-365.0	1.3 ± 0.1	1.2-1.4	19.2 ± 0.5	18.0-20.0	Orange
OP 'Sugandham'	27.7 ± 5.5	19.0-38.0	242.7 ± 38.7	176.0-310.0	1.2 ± 0.1	1.1-1.4	18.4 ± 0.2	17.8-19.6	Yellow
OP 'Aizwal'	16.3 ± 0.9	15.0-18.0	203.3 ± 13.4	187.0-230.0	1.2 ± 0.1	1.2-1.3	19.5 ± 0.3	18.5-21.0	Orange
OP 'Amalapuram'	19.7 ± 3.5	14.0-26.0	144.3 ± 13.1	120.0-165.0	1.2 ± 0	1.2	19.3 ± 0.2	17.6-21.7	Orange
OP 2/ 'Jabedi'	27.0 ± 6.6	19.0-40.0	301.0 ± 42.3	250.0-385.0	1.2 ± 0.1	1.1-1.2	18.9 ± 0.3	17.8-20.6	Yellow
OP 'Avanigadda'	27.7 ± 4.1	21.0-35.0	318.7 ± 34.7	254.0-373.0	1.3 ± 0.1	1.2-1.5	19.2 ± 0.2	18.3-21.0	Yellow

NS - number of secondary rhizome, WS - weight of secondary rhizome, INL - internode length, RC - rhizome colour

Table 32. Characterwise mean and range of rhizome characters in open pollinated progenies of turmeric

Character	CW	NM	WM	NP	WP	NS	WS	INL
Mean	509.1 ± 29.5	1.1 ± 0.1	62.3 ± 1.83	9.1 ± 0.6	207.3 ± 17.1	25.5 ± 1.4	234.4 ± 15.7	1.3 ± 0.0
Range	336.7-665.8	1.0-1.3	51.3-73.7	6.0-12.7	120.7-294.0	16.3-31.0	144.3-318.7	1.2-1.4

CW - clump weight, NM - number of mother rhizome, WM - weight of mother rhizome, NP - number of primary rhizome, WP - weight of primary rhizome, NS - number of secondary rhizome, WS - weight of secondary rhizome, INL - internode length.

4.1.3.2.1. Clump weight /Weight of rhizome

Fresh rhizome weight varied from 336.7 ± 10.7g (OP 'Amalapuram') to 665.8 ± 72.9 g (OP 'Avanigadda').

4.1.3.2.2. Number of mother rhizome

In all the progenies studied either 1.0 ± 0 or 1.3 ± 0 number of mother rhizomes was recorded.

4.1.3.2.3. Weight of mother rhizome

The total weight of mother rhizomes recorded was maximum in OP 'Sugandham' (73.7 ± 3.3 g) followed by OP 'Dindigam' (70.7 ± 8.7g). OP 'Aizwal' had the least mother rhizome wt .of 51.3 ± 1.8 g.

4.1.3.2.4. Number of primary rhizome

OP 'Avanigadda' had maximum number of primary rhizomes (12.7 ± 2.0) followed by OP 'Kasturi' and OP 23/ 'Ayur' (11.3 ± 1.2 each). Minimum number of primary rhizomes was observed in OP 'Aizwal' (6.0 ± 0.6).

4.1.3.2.5. Weight of primary rhizome

The weight of primary rhizomes ranged from 120.7 ± 12.5 g. to 294.0 ± 22.0 g. The highest primary rhizome weight was recorded in OP 'Kasturi' (294.0 ± 22.0 .) followed by OP 'Avanigadda' (285.7 ± 36.9 g.). OP 'Aizwal' recorded the lowest weight of primary rhizomes (120.7 ± 12.5 g).

4.1.3.2.6. Number of secondary rhizome

Number of secondary was maximum in OP 23/ 'Ayur' (31.0 ± 5.5) followed by OP 'Kasturi' (30.0 ± 5.5). The number of secondary rhizomes was minimum in OP 'Aizwal' (16.3 ± 0.9).

4.1.3.2.7. Weight of secondary rhizome

Maximum weight of secondary rhizomes was recorded in OP 'Avanigadda' and OP 'Jabedi' (318.7 ± 34.7 g and 301.0 ± 42.3 g respectively). OP 'Amalapuram' recorded minimum weight of secondary rhizomes (144.3 ± 13.1 g).

4.1.3.2.8. Internode length

The internode length was recorded maximum in the OP 'Kasturi' (1.4 ± 0.1 cm) and minimum in OP 'Amalapuram' (1.2 ± 0 cm).

4.1.3.2.9. Rhizome colour

The open pollinated progenies studied showed an orange/ yellow rhizome colour. OP 'Alleppey', OP 3/ 'Amrithapani', OP 23/ 'Ayur', OP 'Aizwal', OP 'Amalapuram' had an orange inner core while the others had a yellow rhizome colour.

4.1.3.2.10. Dry recovery

Dry recovery was found to be maximum in OP 'Alleppey' (19.9 ± 0.9 %) while OP 'Sugandham' gave minimum dry yield (18.4 ± 0.2 %).

4.1.3.3. Cluster analysis

The paired affinity index of the different traits in the turmeric open pollinated progenies studied and the UPGMA dendrogram constructed using these indices are presented in Table 33 and Fig. 17.

Table 33. Paired affinity indices of the turmeric open pollinated progenies alongwith their parents based on morphological characters

Row/Col	Allepe	Amalapur	Amruthapan	Jabedi	Katengia	Avanigadda	Dindigam	Kasturi	Muvatupuz	Sugantham	Aiswal	Ayur	OPMuvatup	OPAllepe	OPAmruth	OPKatengia	OPDindiga	OPKasturi	OPAyur	OPSuganth	OPAiswal	OPAmalap	OPJabedi	OPAvaniga
Allepe	1.0000000																							
Amalapur	0.6363636	1.0000000																						
Amruthapan	0.4000000	0.4545455	1.0000000																					
Jabedi	0.5454545	0.7272727	0.3636364	1.0000000																				
Katengia	0.5454545	0.5833333	0.3636364	0.5000000	1.0000000																			
Avanigadda	0.1428571	0.2857143	0.1666667	0.3076923	0.4166667	1.0000000																		
Dindigam	0.2307692	0.3846154	0.5555556	0.5454545	0.4166667	0.4545455	1.0000000																	
Kasturi	0.1818182	0.1538462	0.2222222	0.2727273	0.4000000	0.4444444	0.4444444	1.0000000																
Muvatupuz	0.1666667	0.2307692	0.2000000	0.1538462	0.5000000	0.5555556	0.4000000	0.3750000	1.0000000															
Sugantham	0.2000000	0.0769231	0.1111111	0.0833333	0.1818182	0.3333333	0.2000000	0.2857143	0.4285714	1.0000000														
Aiswal	0.7000000	0.5833333	0.3636364	0.3846154	0.5000000	0.2142857	0.3076923	0.1666667	0.3636364	0.3000000	1.0000000													
Ayur	0.2000000	0.2727273	0.4285714	0.1818182	0.3000000	0.2000000	0.3333333	0.2857143	0.4285714	0.1428571	0.1818182	1.0000000												
OPMuvatup	0.1250000	0.1764706	0.0666667	0.1875000	0.2666667	0.5000000	0.2857143	0.3636364	0.4545455	0.4000000	0.2666667	0.1666667	1.0000000											
OPAllepe	0.3571429	0.3125000	0.2142857	0.3333333	0.1764706	0.3571429	0.2666667	0.2307692	0.1333333	0.2500000	0.3333333	0.0714286	0.5000000	1.0000000										
OPAmruth	0.3333333	0.2857143	0.1666667	0.3076923	0.1333333	0.1428571	0.2307692	0.1818182	0.0769231	0.2000000	0.4166667	0.0909091	0.3846154	0.5833333	1.0000000									
OPKatengia	0.3750000	0.5000000	0.2500000	0.5333333	0.5333333	0.4666667	0.3750000	0.3571429	0.2500000	0.2000000	0.3529412	0.1250000	0.6000000	0.5625000	0.3750000	1.0000000								
OPDindiga	0.1428571	0.2857143	0.1666667	0.3076923	0.1333333	0.2307692	0.3333333	0.1818182	0.1666667	0.2000000	0.2142857	0.0909091	0.5000000	0.5833333	0.4545455	0.4666667	1.0000000							
OPKasturi	0.3076923	0.4615385	0.1538462	0.3846154	0.5000000	0.4166667	0.3076923	0.1666667	0.3636364	0.3000000	0.3846154	0.1818182	0.4615385	0.2500000	0.3076923	0.5333333	0.3076923	1.0000000						
OPAyur	0.2000000	0.3333333	0.1428571	0.2666667	0.1176471	0.2857143	0.2000000	0.0714286	0.1428571	0.1666667	0.3571429	0.0769231	0.5384615	0.6153846	0.6363636	0.5000000	0.5000000	0.3571429	1.0000000					
OPSuganth	0.2666667	0.4000000	0.1333333	0.4285714	0.2500000	0.3571429	0.2666667	0.0666667	0.1333333	0.1538462	0.3333333	0.0000000	0.5000000	0.5714286	0.4615385	0.6666667	0.4615385	0.5384615	0.7500000	1.0000000				
OPAiswal	0.3333333	0.2857143	0.2727273	0.2142857	0.1333333	0.1428571	0.1428571	0.1818182	0.0769231	0.2000000	0.3076923	0.0909091	0.3846154	0.7272727	0.4545455	0.4666667	0.6000000	0.1333333	0.5000000	0.3571429	1.0000000			
OPAmalap	0.1538462	0.2142857	0.3000000	0.1428571	0.0666667	0.1538462	0.2500000	0.0909091	0.0833333	0.1000000	0.2307692	0.1000000	0.3076923	0.6363636	0.5000000	0.3125000	0.6666667	0.1428571	0.5454545	0.3846154	0.6666667	1.0000000		
OPJabedi	0.3076923	0.3571429	0.1538462	0.3846154	0.3846154	0.2142857	0.2142857	0.0769231	0.1538462	0.0000000	0.2857143	0.0833333	0.3571429	0.3333333	0.3076923	0.5333333	0.2142857	0.3846154	0.4615385	0.6666667	0.2142857	0.2307692	1.0000000	
OPAvaniga	0.4000000	0.4375000	0.1875000	0.4666667	0.5714286	0.4000000	0.3125000	0.2857143	0.3571429	0.2142857	0.3750000	0.2142857	0.6428571	0.4117647	0.3125000	0.8000000	0.3125000	0.5714286	0.4375000	0.6000000	0.3125000	0.1764706	0.6923077	1.0000000

4.2. MOLECULAR CHARACTERISATION

4.2.1. Isolation of DNA from fresh leaves

4.2.1.1. *Curcuma* spp.

The yield of DNA isolated from fresh leaves of *Curcuma* ranged from 93.0-153.0 $\mu\text{g g}^{-1}$ (Table 34). Among the fifteen *Curcuma* species studied *C. aromatica* gave the highest yield of DNA per gram tissue (153 $\mu\text{g g}^{-1}$) followed by *C. caesia* (147.0 $\mu\text{g g}^{-1}$). *C. zedoaria* (93.0 $\mu\text{g g}^{-1}$) had the lowest yield of DNA per gram tissue.

Table 34. Yield of DNA from different *Curcuma* species.

Sl. No.	<i>Curcuma</i> species	DNA yield ($\mu\text{g g}^{-1}$ fresh tissue)
1	<i>C. aeruginosa</i> Roxb.	103.0
2	<i>C. amada</i> Roxb.	130.0
3 a	* <i>C. aromatica</i> ^a Salisb. (<i>C. aromatica</i> old)	142.0
3 b	* <i>C. aromatica</i> ^b Salisb. (<i>C. aromatica</i> new)	153.0
4	<i>C. caesia</i> Roxb.	147.0
5	<i>C. comosa</i> Roxb.	119.0
6	<i>C. decipiens</i> Dalz.	123.0
7	<i>Curcuma</i> species	99.0
8	<i>C. haritha</i> Mangaly & Sabu	124.0
9	<i>C. longa</i> L.	120.0
10	<i>C. malabarica</i> Vel <i>et al.</i>	96.0
11	<i>C. montana</i> Wall.	104.0
12	<i>C. pseudomontana</i> Grah.	120.0
13	<i>C. raktakanta</i> Mangaly & Sabu	109.0
14	<i>C. sylvatica</i>	116.0
15	<i>C. zedoaria</i> Rosc.	93.0

* *C. aromatica*^a collected from NBPGR, Trissur and *C. aromatica*^b collected from 'Wynad' are maintained as separate entities in the IISR field genebank, Peruvannamuzhi, Calicut, Kerala.

A spectrophotometric value (A_{260}/A_{280}) of 1.80 – 1.95 and a conspicuous band in 0.70 % agarose gel indicated the high quality of the DNA. Fig. 18 shows the DNA isolated from leaves of *Curcuma* species.



Fig.18. Genomic DNA isolated from *Curcuma* species. M – Marker (Human Genomic DNA, Bangalore Genei, India), Lane 1 – 16 are *C. aeruginosa*, *C. amada*, *C. aromatica*^a, *C. aromatica*^b, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. malabarica*, *C. montana*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica*, and *C. zedoaria*, respectively

4.2.1.2. Turmeric varieties/cultivars and open pollinated progenies

Out of the thirty six varieties/ cultivars of turmeric studied, the highest yield of DNA was recorded in the cultivar ‘Ayur’ (152.0 $\mu\text{g g}^{-1}$) followed by variety ‘Sudarsana’ (149.0 $\mu\text{g g}^{-1}$). The lowest yield of DNA per gram tissues was recorded in the cultivar ‘Nandyal type’ (95.0 $\mu\text{g g}^{-1}$) and ‘GL Puram’ (98.0 $\mu\text{g g}^{-1}$) (Table 35). The seedling progeny of cultivar ‘Alleppey’ gave maximum yield (158.0 $\mu\text{g g}^{-1}$) of DNA per gram tissue among the twelve open pollinated progenies studied. The quality of the isolated DNA ranged from 1.76-1.89.

Table 35. Yield of DNA from different turmeric varieties/cultivars and open pollinated progenies.

Sl. No.	Cultivar/variety	DNA yield ($\mu\text{g g}^{-1}$ fresh tissue)	Sl. No.	Cultivar/variety	DNA yield ($\mu\text{g g}^{-1}$ fresh tissue)
1	‘Alleppey’	112.0	25	‘Ayur’	152.0
2	‘Amalapuram’	121.0	26	‘Basar Along’	118.0
3	‘Amrithapani’	116.0	27	‘Gaspani’	143.0
4	‘Armoor’	132.0	28	‘Hajo’	112.0
5	‘Jabedi’	100.0	29	‘Jorhat’	119.0
6	‘Dhagi’	131.0	30	‘Suvama’	126.0
7	‘Katergia’	125.0	31	‘Sudarsana’	149.0
8	‘Chayapasupu’	116.0	32	‘Kedaram’	131.0
9	‘Avanigadda’	129.0	33	‘Suguna’	109.0
10	‘Dindigam’	142.0	34	‘Prathibha’	119.0
11	‘Ethamkula’	135.0	35	‘Prabha’	138.0
12	‘GL Puram’	98.0	36	‘Alleppey Supreme’	143.0
13	‘Karhadi local’	110.0	37	OP ‘Muvatupuzha’	127.0
14	‘Kasturi’	143.0	38	OP ‘Alleppey’	158.0
15	‘Kodur’	139.0	39	OP 3/ ‘Amrithapani’	132.0
16	‘Kuchipudi’	128.0	40	OP ‘Katergia’	114.0
17	‘Mamomthody’	147.0	41	OP ‘Dindigam’	138.0
18	‘Muvatupuzha’	99.0	42	OP ‘Kasturi’	123.0
19	‘Nandyal type’	95.0	43	OP 23/ ‘Ayur’	135.0
20	‘Rajapuri’	120.0	44	OP ‘Sugandham’	129.0
21	‘Sugandham’	136.0	45	OP ‘Aizwal’	146.0
22	‘Vondimitha’	142.0	46	OP ‘Amalapuram’	132.0
23	‘Wynad’ local’	137.0	47	OP 2/ ‘Jabedi’	110.0
24	‘Aizwal’	99.0	48	OP ‘Avanigadda’	106.0

Fig. 19 shows the DNA isolated from the leaves of thirty six cultivar/varieties and twelve open pollinated progenies of turmeric.



Fig.19. Genomic DNA isolated from turmeric varieties/cultivars and open pollinated progenies. M – Marker (Eco RI/Hind III double digest, Bangalore Genei, India), Lane 1 – 36 are ‘Alleppey’, ‘Amalapuram’, ‘Amrithapani’, ‘Armoor’, ‘Jabedi’, ‘Dhagi’, ‘Katergia’, ‘Chayapasupu’, ‘Avanigadda’, ‘Dindigam’, ‘Ethamkula’, ‘GL Puram’, ‘Karhadi local’, ‘Kasturi’, ‘Kodur’, ‘Kuchipudi’, ‘Mamomthody’, ‘Muvatupuzha’, ‘Nandyal type’, ‘Rajapuri’, ‘Sugandham’, ‘Vondimita’, ‘Wynad local’, ‘Aizwal’, ‘Ayur’, ‘Basar Along’, ‘Gaspani’, ‘Hajo’, ‘Jorhat’, ‘Suvarna’, ‘Sudarsana’, ‘Kedaram’, ‘Suguna’, ‘Prathibha’, ‘Prabha’, ‘Alleppey Supreme’ and 37 – 46 are OP ‘Muvatupuzha’, OP ‘Alleppey’, OP 3/ ‘Amrithapani’, OP ‘Katergia’, OP ‘Dindigam’, OP ‘Kasturi’, OP 23/ ‘Ayur’, OP ‘Sugandham’, OP ‘Aizwal’, OP ‘Amalapuram’, OP 2/ ‘Jabedi’ and OP ‘Avanigadda’, respectively.

4.2.2. Isolation of DNA from fresh rhizomes

The yield of the DNA ranged from 10.75 – 14.44 $\mu\text{g g}^{-1}$ of fresh mature rhizome of turmeric with maximum yield in the variety ‘Sudarsana’ and minimum in the cultivar ‘Katergia’ (Table 36). DNA purity was in the range of 1.83-1.87(A_{260}/A_{280}) in all the samples (Fig. 20).

Table 36. Yield and purity of DNA from mature turmeric rhizome.

Sl. No	Accession/variety	DNA yield ($\mu\text{g g}^{-1}$ fresh tissue)
1	‘Alleppey’	11.67
2	‘Amalapuram’	11.67
3	‘Armoor’	11.67
4	‘Amrithapani’	14.38
5	‘Dhagi’	10.78
6	‘Jabedi’	13.75
7	‘Prabha’	14.09
8	‘Katergia’	10.75
9	‘Prathibha’	10.83
10	‘Suvarna’	13.78
11	‘Suguna’	12.92
12	‘Sudarsana’	14.44



Fig.20. Genomic DNA isolated from fresh rhizomes of turmeric varieties/cultivars. M – Marker (Human genomic DNA, Bangalore Genei, India), Lane 1 – 12 are ‘Alleppey’, ‘Amalapuram’, ‘Armoor’, ‘Amrithapani’, ‘Dhagi’, ‘Jabedi’, ‘Prabha’, ‘Katergia’, ‘Prathibha’, ‘Suvarna’, ‘Suguna’ and ‘Sudarsana’ respectively.

4.2.3. Characterization of *Curcuma* species using RAPD markers

Forty eight random decamer primers were used for the molecular characterization of fifteen Indian *Curcuma* species. A total of four hundred and fourty nine scorable bands were produced in the fifteen species studied, out of which four hundred and nine were polymorphic. The percentage of polymorphism ranged from a maximum of 100 percent showed by twenty two random primers (OPA 05, OPA 15, OPA 17, OPA 19, OPB 12, OPB 16, OPC 07, OPC 08, OPC 10, OPC 11, OPC 12, OPC 13, OPC 14, OPC 18, OPC 20, OPD 01, OPD 03, OPD 04, OPD 05, OPD 16, OPJ 10 and OPJ 16) (i.e. 56.41% of the total RAPD primers used) to a minimum of 44 percent showed by primer OPA 03 (Fig. 21 – 26). Sequence of RAPD primers, number of bands produced by each primer number of polymorphic bands and percentage polymorphism is given in Table 37.

Table 37. Sequence of RAPD primers, number of bands produced by each primer number of polymorphic bands and percentage polymorphism generated in different species of *Curcuma*

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	OPA 03	AGTCAGCCAC	09	04	44.44
2	OPA 05	AGGGGTCTTG	10	10	100.00
3	OPA 07	GAAACGGGTG	12	10	83.33
4	OPA 08	GTGACGTAGG	13	11	84.62
5	OPA 10	GTGATCGCAG	11	10	90.91
6	OPA 11	CAATCGCCGT	12	09	75.00
7	OPA 12	TCGGCGATAG	11	10	90.91
8	OPA 14	TCTGTGCTGG	09	08	88.89
9	OPA 15	TTCCGAACCC	09	09	100.00
10	OPA 17	GACCGTTGT	09	09	100.00
11	OPA 18	AGGTGACCGT	11	10	90.91
12	OPA 19	CAAACGTCGG	11	11	100.00
13	OPB 07	GGTGACGCAG	08	07	87.50
14	OPB 08	GTCCACACGG	09	08	88.89
15	OPB 09	TGGGGGACTC	08	07	87.50
16	OPB 11	GTAGACCCGT	09	08	88.89
17	OPB 12	CCTTGACGCA	08	08	100.00
18	OPB 14	TCCGCTCTGG	06	05	83.33
19	OPB 16	TTTGCCCGGA	06	06	100.00
20	OPC 07	GTCCCGACGA	09	09	100.00
21	OPC 08	TGGACCGGTG	13	13	100.00
22	OPC 09	CTCACCGTCC	11	10	90.91
23	OPC 10	TGTCTGGGTG	09	09	100.00
24	OPC 11	AAAGCTGCGG	06	06	100.00
25	OPC 12	TGTCATCCCC	04	04	100.00
26	OPC 13	AAGCCTCGTC	09	09	100.00
27	OPC 14	TGCGTGCTTG	05	05	100.00
28	OPC 16	CACACTCCAG	13	12	92.31
29	OPC 18	TGAGTGGGTG	08	08	100.00
30	OPC 20	ACTTCGCCAC	08	08	100.00
31	OPD 01	ACCGCGAAGG	11	11	100.00
32	OPD 02	GGACCCAACC	10	08	80.00
33	OPD 03	GTCGCCGTCA	10	10	100.00
34	OPD 04	TCTGGTGAGG	09	09	100.00
35	OPD 05	TGAGCGGACA	09	09	100.00
36	OPD 07	TTGGCACGGG	13	10	76.92
37	OPD 08	GTGTGCCCCA	12	11	91.67
38	OPD 13	GGGGTGACGA	09	08	88.89
39	OPD 14	CTTCCCCAAG	10	09	90.00
40	OPD 15	CATCCGTGCT	14	12	85.71
41	OPD 16	AGGGCGTAAG	11	11	100.00
42	OPD 18	GAGAGCCAAC	05	03	60.00
43	OPJ 10	AAGCCCGAGG	07	07	100.00
44	OPJ 11	ACTCCTGCGA	07	05	71.43
45	OPJ 13	CCACACTACC	10	09	90.00
46	OPJ 16	CTGCTTAGGG	09	09	100.00
47	OPJ 17	ACGCCAGTTC	08	07	87.50
48	OPJ 18	TGGTCGCAGA	09	08	88.89
Total			449	409	91.23

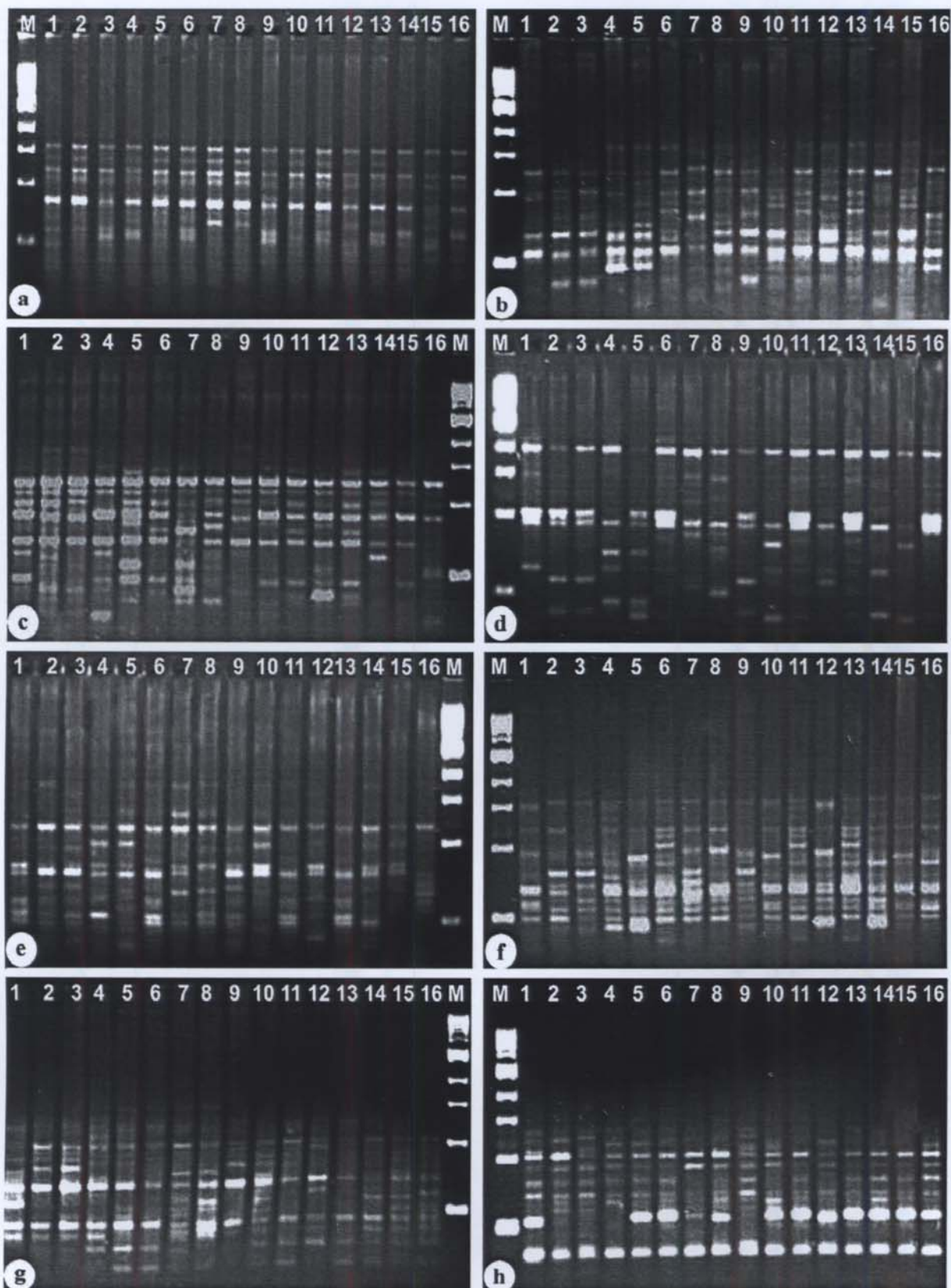


Fig. 21. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPA 03, OPA 05, OPA 07, OPA 08, OPA 10, OPA 11, OPA 12 and OPA 14. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica*^a, *C. aromatica*^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria* , respectively.

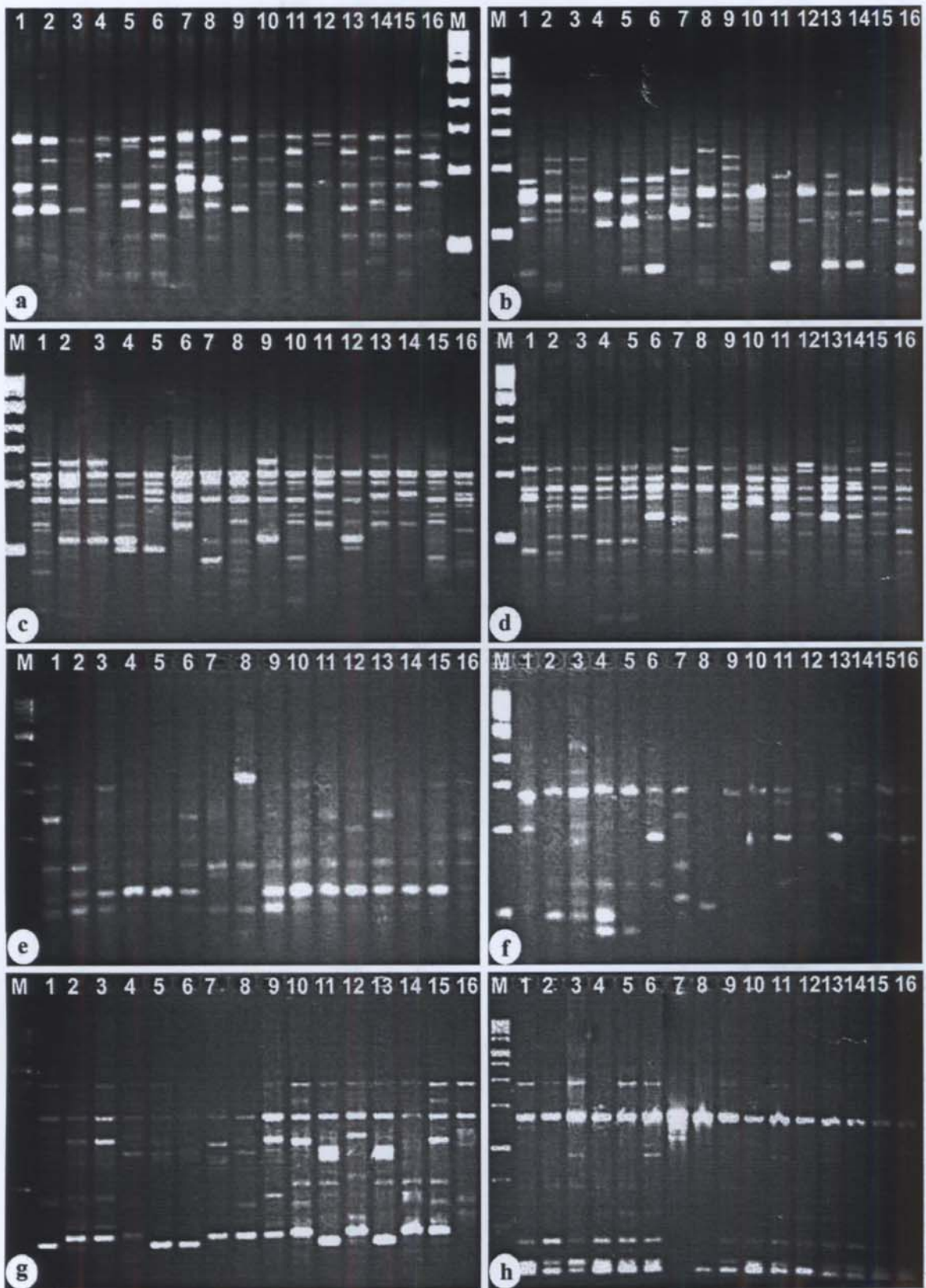


Fig. 22. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPA 15, OPA 17, OPA 18, OPA 19, OPB 07, OPB 08, OPB 09 and OPB 11. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica* ^a, *C. aromatica* ^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

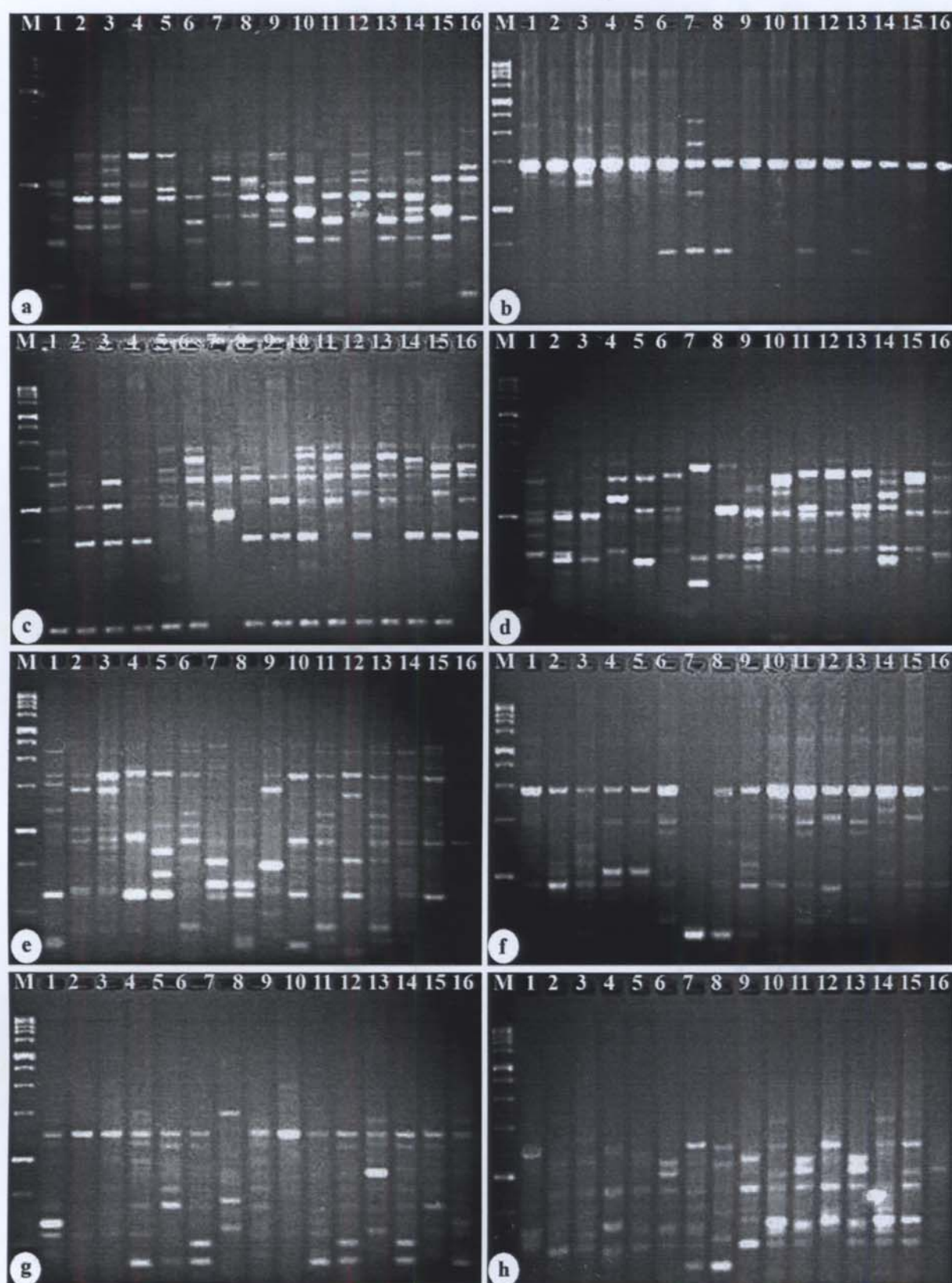


Fig. 23. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPB 12, OPB 14, OPB 16, OPC 07, OPC 08, OPC 09, OPC 10 and OPC 11. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica*^a, *C. aromatica*^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

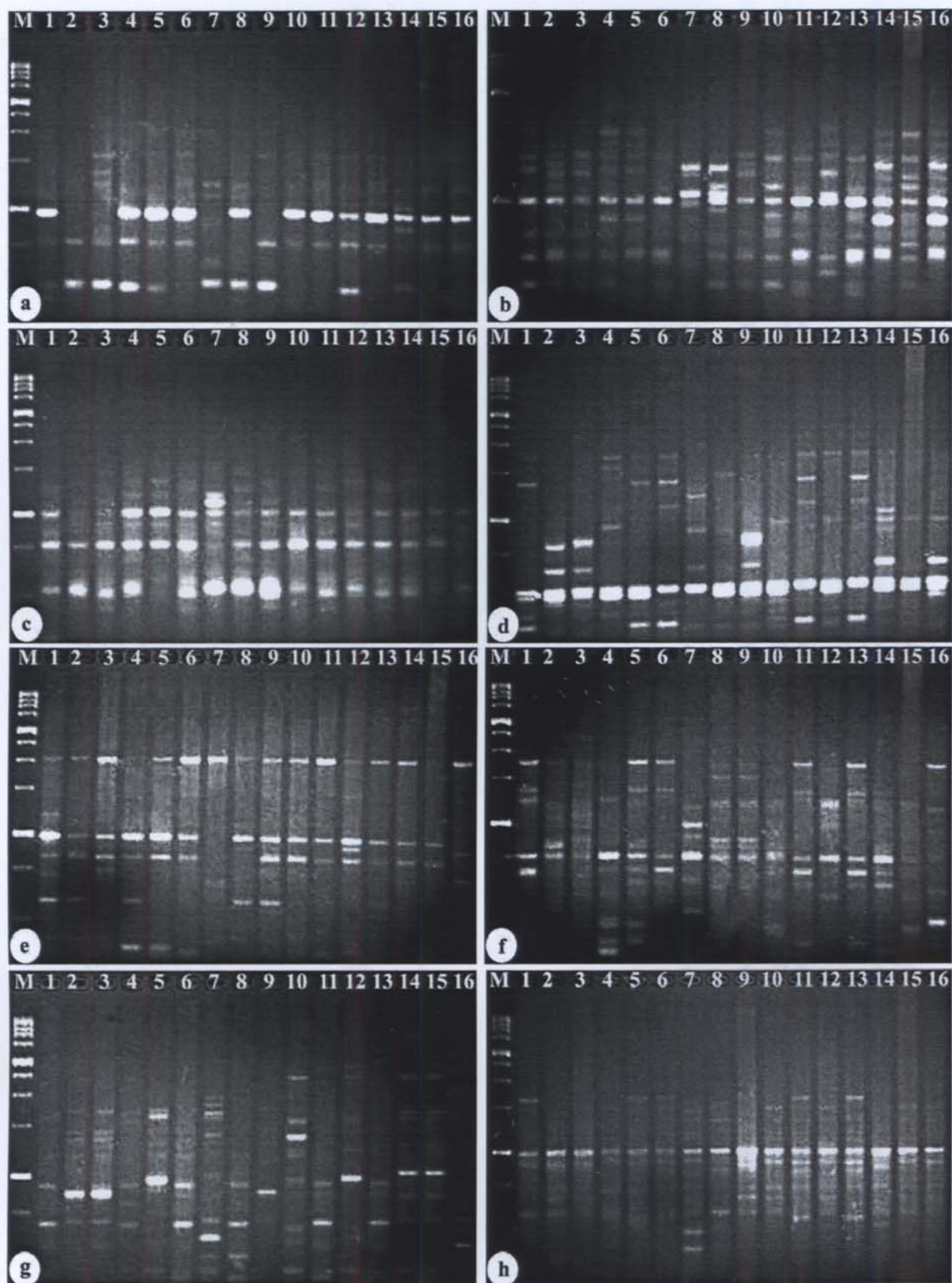


Fig. 24. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPC 12, OPC 13, OPC 14, OPC 16, OPC 18, OPC 20, OPD 01 and OPD 02. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica* ^a, *C. aromatica* ^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria* , respectively.

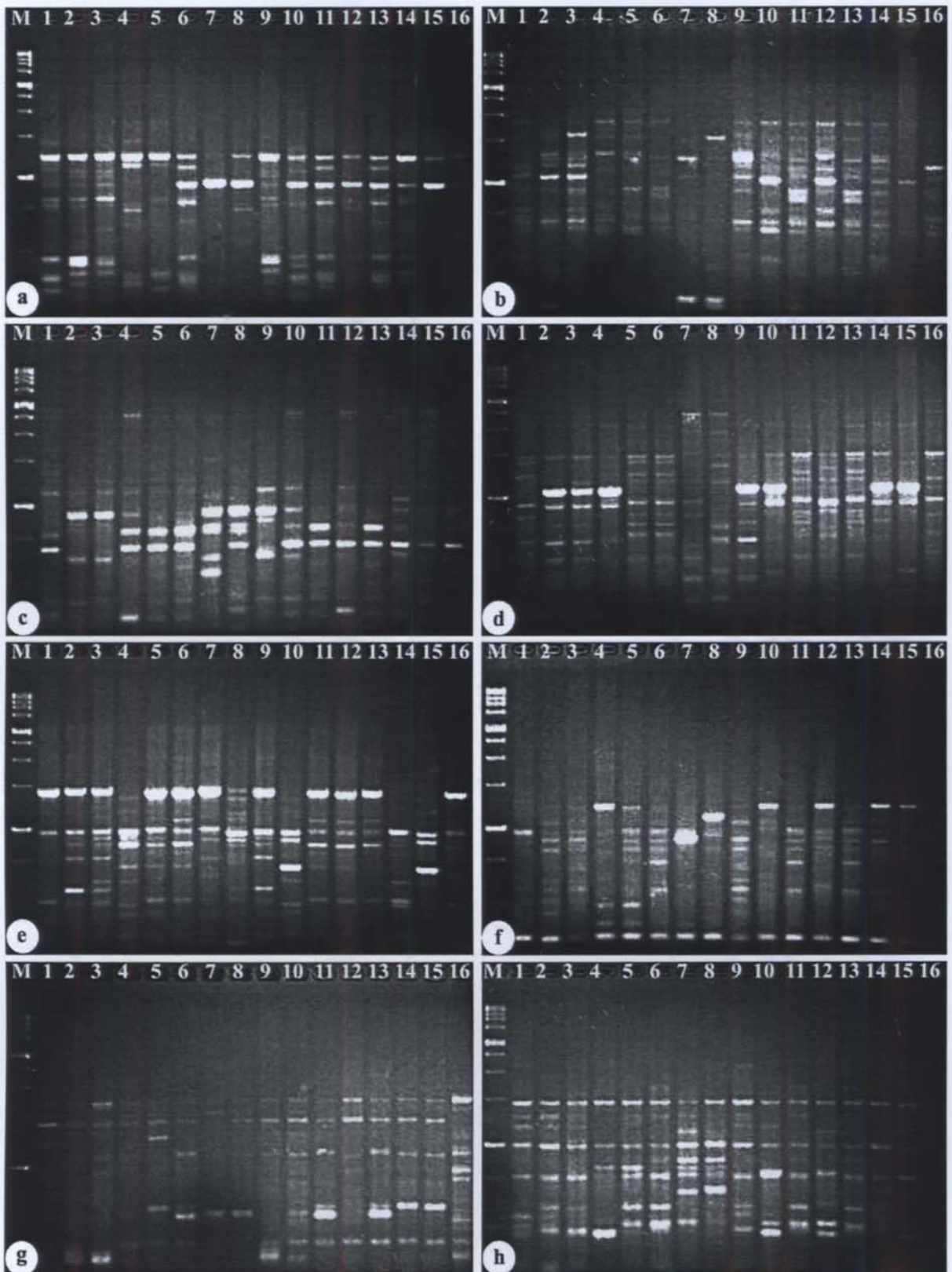


Fig. 25. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPD 03, OPD 04, OPD 05, OPD 07, OPD 08, OPD 13, OPD 14 and OPD 15. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica*^a, *C. aromatica*^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

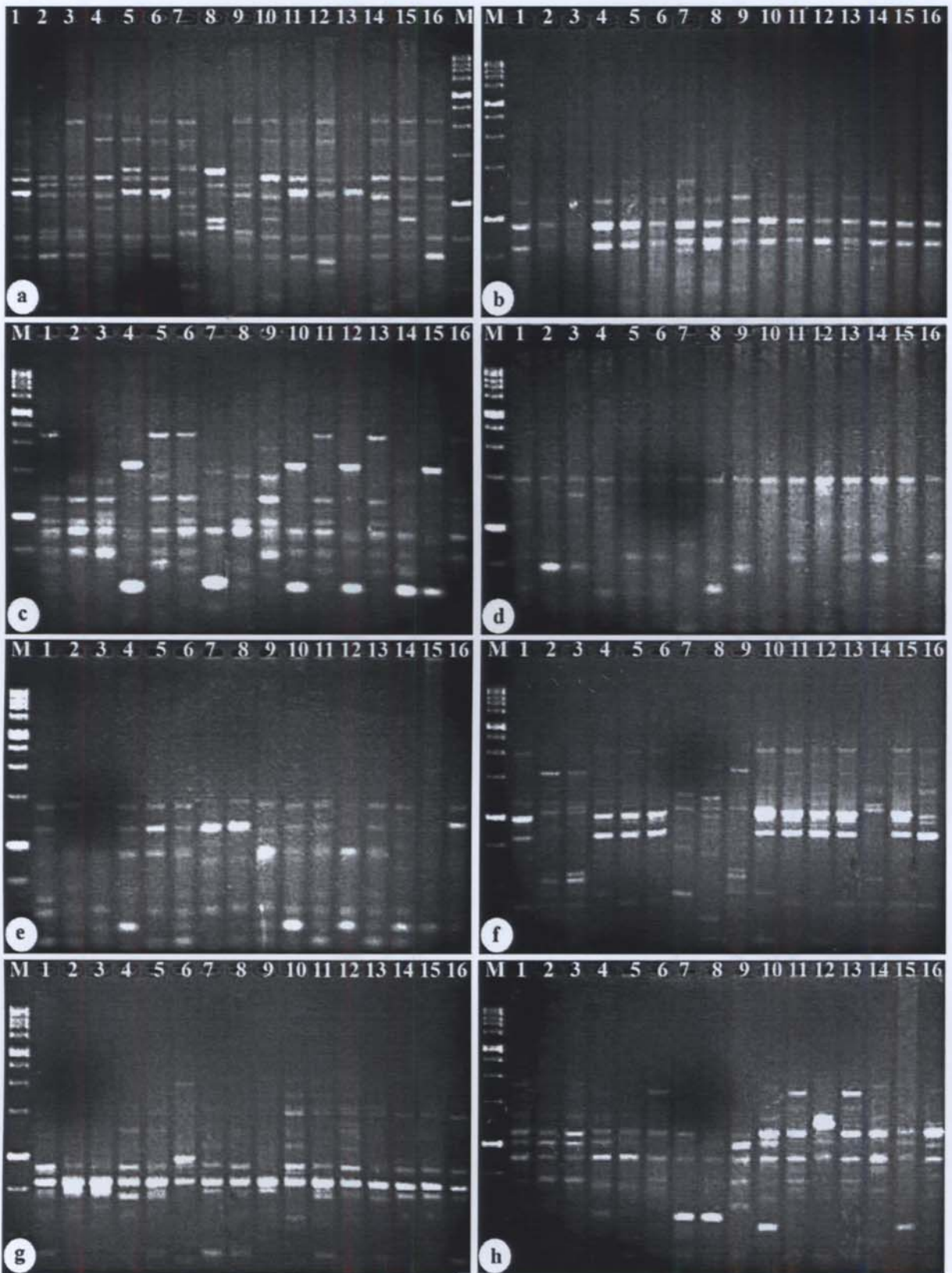


Fig. 26. a - h are the RAPD profiles of *Curcuma* species DNA using primers OPD 16, OPD 18, OPJ 10, OPJ 11, OPJ 13, OPJ 16, OPJ 17 and OPJ 18. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica* ^a, *C. aromatica* ^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria* , respectively.

4.2.3.1. Cluster analysis

The genetic similarity was calculated using Jaccard's, Sorensen - Dice and Simple Matching coefficients. The similarity matrices obtained are represented in Tables 38-40. In all the similarity matrices *C. raktakanta* and *C. montana* showed maximum similarity (0.83, 0.91 and 0.90 in Jaccard's, Sorensen - Dice and Simple Matching, respectively) and the least similarity between *C. montana* and *C. decipiens* (0.32, 0.48 and 0.49 in Jaccard's, Sorensen - Dice and Simple Matching, respectively).

Table 38. Similarity matrix of different species of *Curcuma* obtained from the RAPD data using Jaccard's similarity coefficients

Row/Col	amada	aromacul	aromanew	aeruginosa	caesia	comosa	decipiens	Cpecies	harita	malabarica	montana	pseudomontana	raktakanta	rythatica	indonesia	longa
amada	1.0000000															
aromacul	0.5306122	1.0000000														
aromanew	0.4939759	0.7696078	1.0000000													
aeruginosa	0.4837398	0.4274194	0.3873518	1.0000000												
caesia	0.5341365	0.4214559	0.3782772	0.5232068	1.0000000											
comosa	0.6072874	0.4456929	0.4237918	0.4296578	0.5595238	1.0000000										
decipiens	0.3394834	0.3750000	0.3579767	0.3307393	0.3395522	0.3453237	1.0000000									
Cpecies	0.4291498	0.4190871	0.4066390	0.3775934	0.3740157	0.3735849	0.4537445	1.0000000								
harita	0.4645669	0.6635945	0.6651163	0.4210526	0.4045802	0.4185185	0.3794466	0.4008264	1.0000000							
malabarica	0.5060241	0.4736842	0.4556452	0.5000000	0.4780876	0.4676806	0.3538462	0.3680000	0.4212598	1.0000000						
montana	0.5742972	0.4377358	0.4264151	0.4269231	0.4980695	0.7521739	0.3178571	0.3702290	0.4210526	0.4767442	1.0000000					
pseudomontana	0.5140562	0.4640000	0.4637097	0.5411255	0.4979920	0.4531835	0.3720930	0.3870968	0.4235294	0.5508475	0.4507576	1.0000000				
raktakanta	0.5436508	0.4104478	0.4044944	0.4375000	0.5157480	0.7621145	0.3309091	0.3798450	0.4204545	0.4372624	0.8287037	0.4359623	1.0000000			
rythatica	0.5195313	0.5059761	0.4822134	0.4959350	0.4638783	0.5094340	0.3863636	0.3960784	0.5000000	0.5750000	0.5250965	0.5322581	0.5252918	1.0000000		
indonesia	0.4285714	0.4000000	0.3991416	0.4500000	0.3884298	0.3928571	0.3432203	0.3464912	0.4173913	0.5446009	0.4180328	0.5114155	0.4115226	0.5176991	1.0000000	
longa	0.4780876	0.4342629	0.4571429	0.3671875	0.4280156	0.4636015	0.3975904	0.3966942	0.4053118	0.4754098	0.4784314	0.4538153	0.4666667	0.4552529	0.3598326	1.0000000

Table 39. Similarity matrix of different species of *Curcuma* obtained from the RAPD data using Sorensen - Dice similarity coefficients

Row/Col	amada	aromacul	aromanew	aeruginosa	caesia	comosa	decipiens	Cpecies	harita	malabarica	montana	pseudomontana	raktakanta	rythatica	indonesia	longa
amada	1.0000000															
aromacul	0.6933333	1.0000000														
aromanew	0.6612903	0.8698061	1.0000000													
aeruginosa	0.6520548	0.5988701	0.5584046	1.0000000												
caesia	0.6963351	0.5928919	0.5489130	0.6869806	1.0000000											
comosa	0.7556675	0.6165803	0.5953003	0.6010638	0.7175573	1.0000000										
decipiens	0.5068871	0.5454545	0.5272206	0.4970760	0.5069638	0.5133690	1.0000000									
Cpecies	0.6005666	0.5906433	0.5781711	0.5481928	0.5444126	0.5439560	0.6242424	1.0000000								
harita	0.6344086	0.7977839	0.7988827	0.5925926	0.5760870	0.5900783	0.5501433	0.5722714	1.0000000							
malabarica	0.6720000	0.6428571	0.6360388	0.6666667	0.6469003	0.6373057	0.5227273	0.5380117	0.5927978	1.0000000						
montana	0.7295918	0.6089239	0.5978836	0.5983827	0.6649485	0.8585608	0.4823848	0.5403900	0.5925926	0.6456693	1.0000000					
pseudomontana	0.6790451	0.6338798	0.6336088	0.7022472	0.6648794	0.6237113	0.5423729	0.5581395	0.5950413	0.7103825	0.6214099	1.0000000				
raktakanta	0.7043702	0.5820106	0.5760000	0.6086957	0.6805195	0.8650000	0.4972678	0.5505618	0.5920000	0.6084656	0.9063291	0.6052632	1.0000000			
rythatica	0.6838046	0.6719577	0.6506667	0.6630435	0.6337662	0.6750000	0.5573770	0.5674157	0.6666667	0.7301587	0.6886076	0.6947368	0.6887735	1.0000000		
indonesia	0.6000000	0.5714286	0.5705521	0.6206897	0.5595238	0.5641026	0.5110410	0.5146580	0.3889571	0.7051672	0.5895954	0.6767372	0.5830904	0.6822157	1.0000000	
longa	0.6469003	0.6055536	0.6274510	0.5371429	0.5994550	0.6335079	0.5689655	0.5680473	0.5770308	0.6444444	0.6472149	0.6243094	0.6363636	0.6256684	0.5392308	1.0000000

Table 40. Similarity matrix of different species of *Curcuma* obtained from the RAPD data using Simple Matching similarity coefficients

Row/Cols	amada	aromaid	aromaneu	aeruginosa	caesia	comosa	decipiens	Cypecias	harita	malabarica	montana	pseudomontana	raktakanta	rylvatica	zedoaria	longa
amada	1.0000000															
aromaid	0.6941489	1.0000000														
aromaneu	0.6648936	0.8750000	1.0000000													
aeruginosa	0.6622340	0.6223404	0.5877660	1.0000000												
caesia	0.6914894	0.5984043	0.5385106	0.6994681	1.0000000											
comosa	0.7420213	0.6063830	0.5877660	0.6010638	0.7047872	1.0000000										
decipiens	0.5239362	0.5744681	0.5611702	0.5425532	0.5292553	0.5159574	1.0000000									
Cypecias	0.6250000	0.6276596	0.6196809	0.6010638	0.5771277	0.5385106	0.6702128	1.0000000								
harita	0.6382979	0.8058511	0.8085106	0.6196809	0.5851064	0.5824468	0.5824468	0.6143617	1.0000000							
malabarica	0.6728723	0.6542553	0.6409574	0.6861702	0.6515957	0.6276596	0.5531915	0.5797872	0.6090426	1.0000000						
montana	0.7180851	0.6037234	0.5957447	0.6037234	0.6542553	0.8484043	0.4920213	0.5611702	0.5904255	0.6409574	1.0000000					
pseudomontana	0.6781915	0.6436170	0.6462766	0.7180851	0.6675532	0.6117021	0.5691489	0.5957447	0.6090426	0.7180851	0.6143617	1.0000000				
raktakanta	0.6941489	0.5797872	0.5771277	0.6170213	0.6728723	0.8563830	0.5106383	0.5744681	0.5930851	0.6063830	0.9015957	0.6010638	1.0000000			
rylvatica	0.6728723	0.6702128	0.6515957	0.6702128	0.6250000	0.6542553	0.5691489	0.5904255	0.6675532	0.7287234	0.6728723	0.6914894	0.6755319	1.0000000		
zedoaria	0.6382979	0.6250000	0.6276596	0.6781915	0.6063830	0.5930851	0.5877660	0.6037234	0.6436170	0.7420213	0.6223404	0.7154255	0.6196809	0.7101064	1.0000000	
longa	0.6515957	0.6223404	0.6462766	0.5691489	0.6090426	0.6276596	0.6010638	0.6117021	0.5984043	0.6595745	0.6462766	0.6382979	0.6382979	0.6276596	0.5930851	1.0000000

The dendrograms constructed using UPGMA (unweighted pair-group mean arithmetic) method from the similarity matrices of genetic distances obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients are given in Figs. 27 – 29. The dendrograms constructed using Jaccard's and Sorensen- Dice coefficients splits at coefficient 0.53 and 0.67, respectively showed a similar clustering pattern with seven groups as shown below.

- Group I - *C. amada*, *C. comosa*, *C. montana*, *C. raktakanta* and *C. caesia*
- Group II - *C. aeruginosa*
- Group III - *C. malabarica*, *C. sylvatica*, *C. pseudomontana* and *C. zedoaria*
- Group IV - *C. longa*
- Group V - *C. aromatica*^b, *C. aromatica*^a and *C. haritha*,
- Group VI - *C. decipiens*
- Group VII - *Curcuma* species

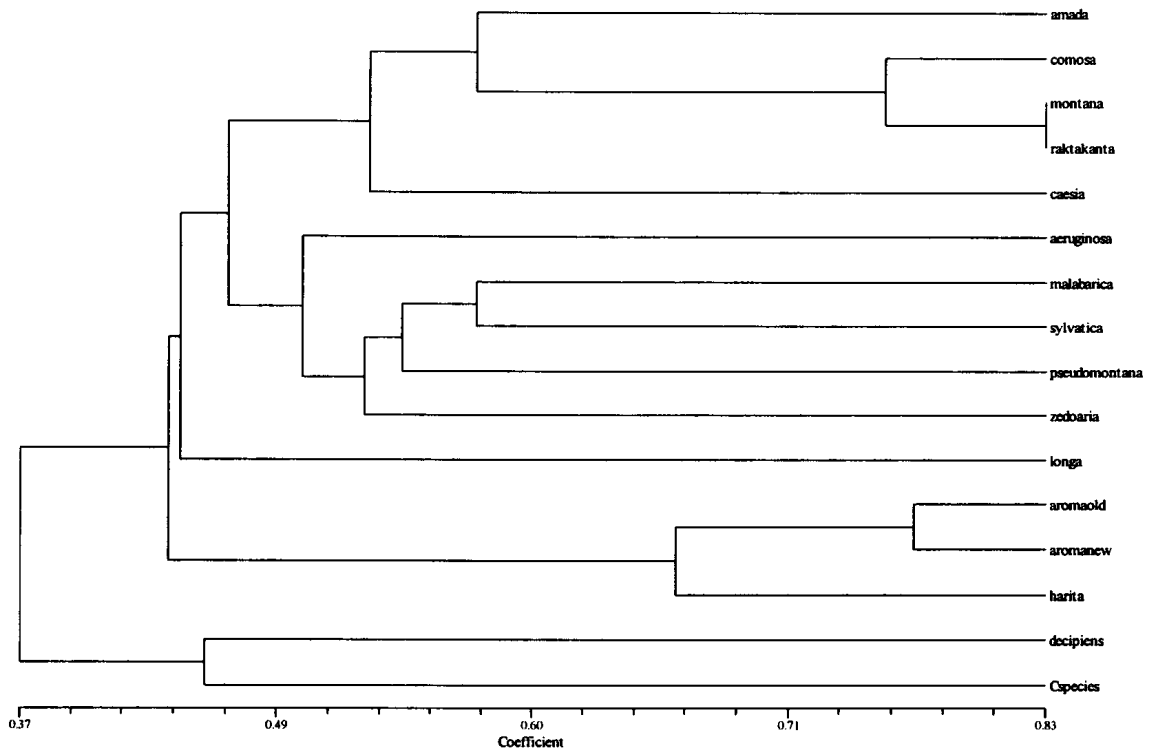


Fig. 27. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on RAPD analysis using Jaccard's similarity coefficients.

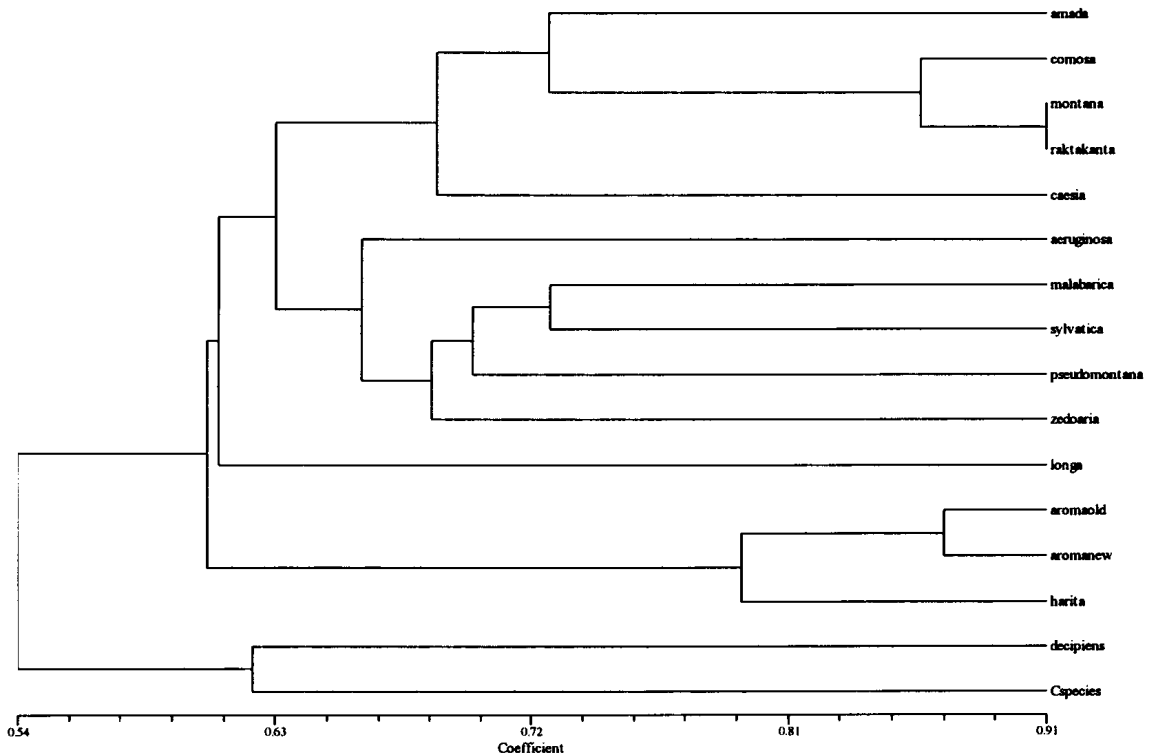


Fig. 28. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on RAPD analysis using Sorensen - Dice similarity coefficients.

However the dendrogram constructed using simple matching coefficients splits at coefficient 0.68 revealed only six groups. Clustering of the lone member of

the group II, *C. aeruginosa* with the members of group III (Figs. 27 & 28) resulted in the shortage of one group in the dendrogram constructed using simple matching coefficient (Fig. 29). The different members of the groups are given below.

- Group I - *C. amada*, *C. comosa*, *C. montana*, *C. raktakanta* and *C. caesia*
 Group II - *C. aeruginosa*, *C. pseudomontana*, *C. Malabarica*, *C. zedoaria* and *C. sylvatica*.
 Group III - *C. longa*
 Group IV - *C. aromatica*^b, *C. aromatica*^a and *C. haritha*
 Group V - *C. decipiens*
 Group VI - *Curcuma* species

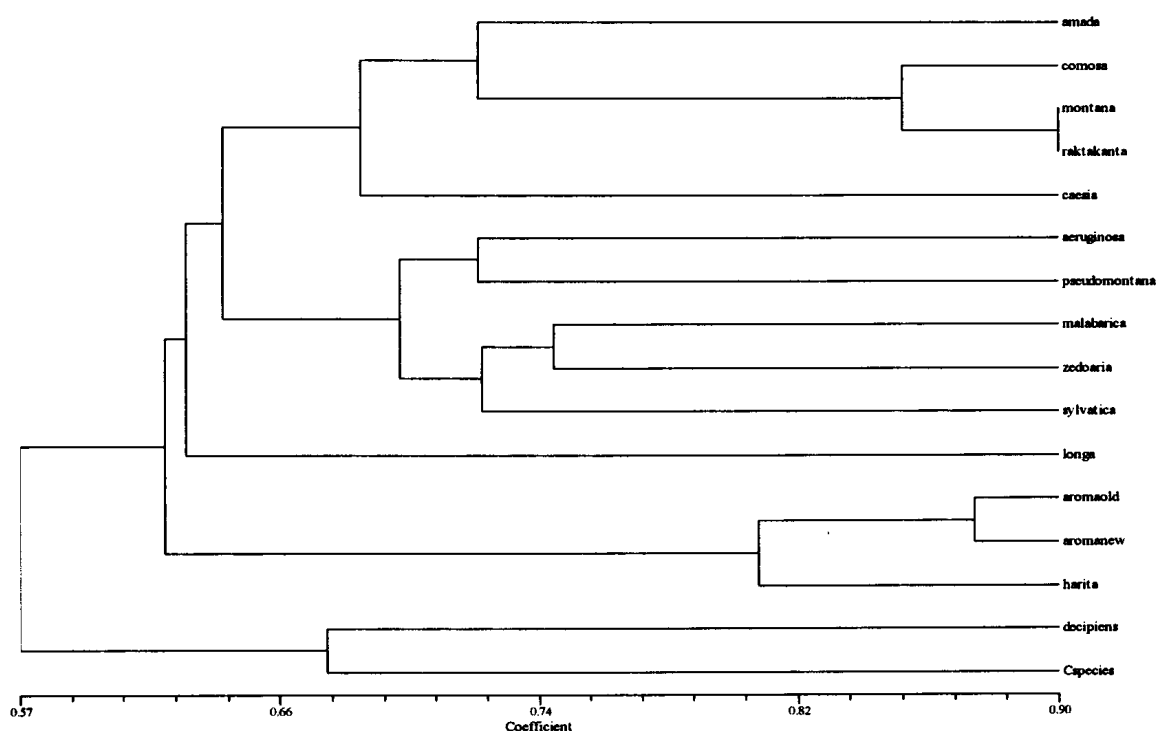


Fig. 29. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on RAPD analysis using Simple Matching similarity coefficients.

Highest and lowest similarities between different species of *Curcuma* in the different groups of the dendrogram produced by three similarity coefficients using RAPD marker data is given in Table 41.

Table 41. Highest and lowest similarities between different species of *Curcuma* in the different groups of the dendrogram produced by three similarity coefficients using RAPD marker data.

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	<i>C. montana</i> & <i>C. raktakanta</i> (0.83)	<i>C. montana</i> & <i>C. raktakanta</i> (0.91)	<i>C. montana</i> & <i>C. raktakanta</i> (0.90)
	Lowest	<i>C. amada</i> & <i>C. caesia</i> (0.53)	<i>C. amada</i> & <i>C. caesia</i> (0.66)	<i>C. amada</i> & <i>C. caesia</i> (0.69)
II	Highest	<i>C. aeruginosa</i>	<i>C. aeruginosa</i>	<i>C. malabarica</i> & <i>C. zedoaria</i>
	Lowest	-	-	<i>C. aeruginosa</i> & <i>C. sylvatica</i>
III	Highest	<i>C. malabarica</i> & <i>C. sylvatica</i> (0.58)	<i>C. malabarica</i> & <i>C. sylvatica</i> (0.57)	<i>C. longa</i>
	Lowest	<i>C. zedoaria</i> & <i>C. pseudomontana</i> (0.51)	<i>C. zedoaria</i> & <i>C. pseudomontana</i>	-
IV	Highest	<i>C. longa</i>	<i>C. longa</i>	<i>C. aromatica</i> ^a & <i>C. aromatica</i> ^b (0.88)
	Lowest	-	-	<i>C. haritha</i> & <i>C. aromatica</i> ^a ()
V	Highest	<i>C. aromatica</i> ^a & <i>C. aromatica</i> ^b (0.77)	<i>C. aromatica</i> ^a & <i>C. aromatica</i> ^b (0.87)	<i>C. decipiens</i>
	Lowest	<i>C. haritha</i> & <i>C. aromatica</i> ^a (0.66)	<i>C. haritha</i> & <i>C. aromatica</i> ^a ()	-
VI	Highest	<i>C. decipiens</i>	<i>C. decipiens</i>	<i>Curcuma</i> species
	Lowest	-	-	-
VII	Highest	<i>Curcuma</i> species	<i>Curcuma</i> species	-
	Lowest	-	-	-

4.2.4. Characterization of *Curcuma* species using ISSR markers

Eight ISSR primers produced a total of ninety one scorable bands in the 15 *Curcuma* species studied. Six primers (ISSR 1, ISSR 2, ISSR 4, ISSR 5, ISSR 6 and ISSR 8) produced 100 percent polymorphic bands among the species and two primers namely ISSR 3 and ISSR 7 gave 70 and 90.91 percent polymorphism, respectively (Table 42) and (Fig. 30).

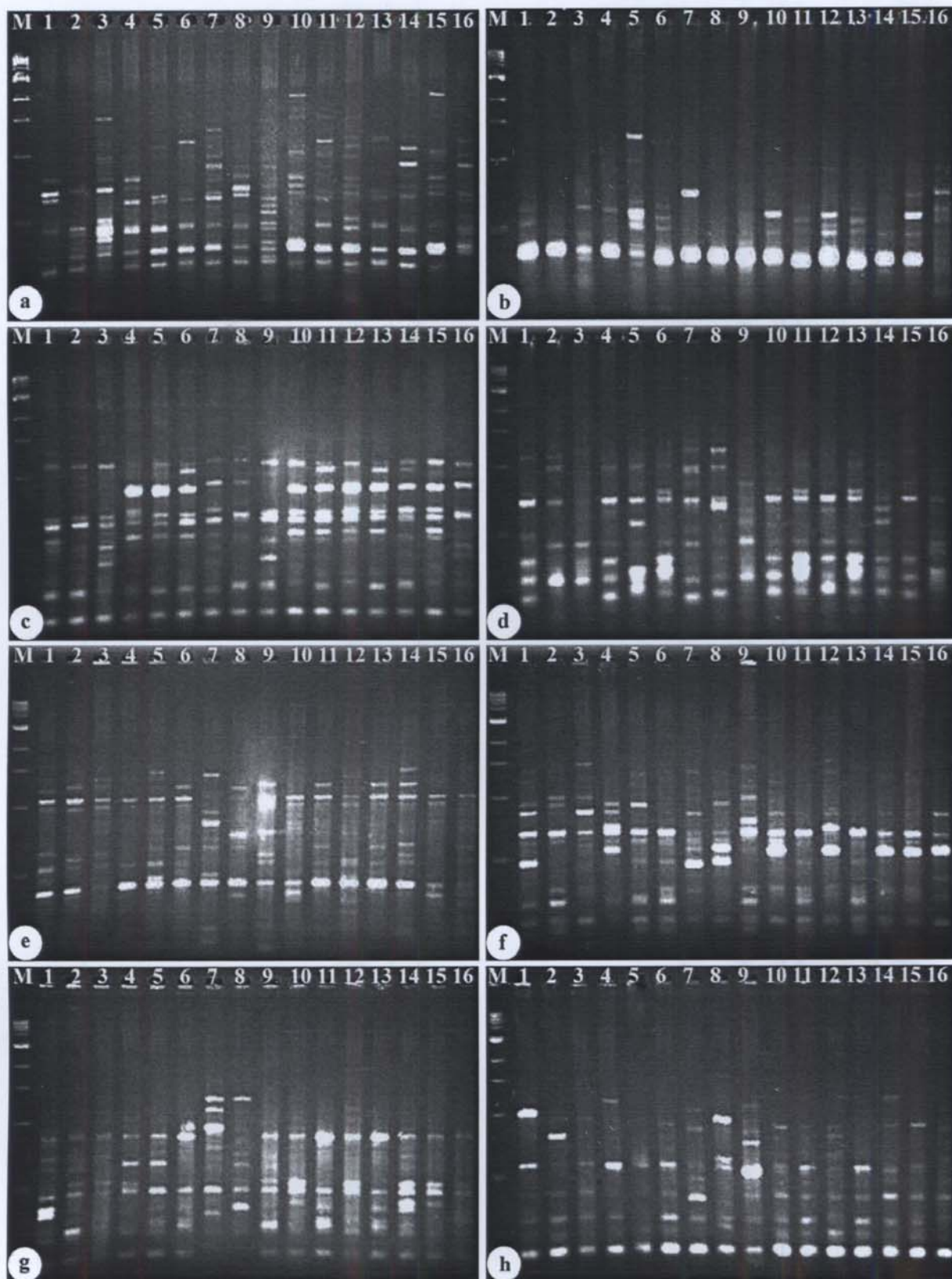


Fig. 30. a - h are the ISSR profiles of *Curcuma* species DNA using primers ISSR 01, ISSR 02, ISSR 03, ISSR 04, ISSR 05, ISSR 06, ISSR 07 and ISSR 08. M - Marker (1 Kb ladder, New England Biolab), Lane 1 - 16 are *C. amada*, *C. aromatica*^a, *C. aromatica*^b, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

Table 42. Sequence of ISSR primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in different species of *Curcuma*

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	ISSR 1	(CT) ₈ TG	17	17	100.00
2	ISSR 2	(CT) ₈ AC	05	05	100.00
3	ISSR 3	(TCC) ₅ AG	10	07	70.00
4	ISSR 4	(AGC) ₄ GT	12	12	100.00
5	ISSR 5	(CAC) ₃ GC	14	14	100.00
6	ISSR 6	(CTC) ₃ GC	13	13	100.00
7	ISSR 7	(GACA) ₃	11	10	90.91
8	ISSR 8	(GACA) ₃ GC	09	09	100.00
Total			91	87	95.60

4.2.4.1. Cluster analysis

In case of the ISSR primers also three similarity coefficients viz, Jaccard's, Sorensen – Dice and Simple Matching coefficients were used for calculating the genetic similarity. The similarity matrices obtained are represented in Tables 43 – 45. In all the similarity matrices produced by the ISSR markers *C. zedoaria* and *C. malabarica* showed maximum similarity (0.85, 0.92 and 0.92 in Jaccard's, Sorensen - Dice and simple matching, respectively) followed by *C. raktakanta* and *C. montana* (0.84, 0.92 and 0.92 in Jaccard's, Sorensen - Dice and Simple Matching, respectively). The least similarity was showed by *C. decipiens* and *C. aromatica*^b (0.26, 0.41 and 0.48 in Jaccard's, Sorensen - Dice and Simple Matching, respectively) between them.

Table 43. Similarity matrix of different species of *Curcuma* obtained from the ISSR data using Jaccard's similarity coefficients

Row\Col	amada	aromaid	aromanaw	aruginosa	casia	comosa	decipiens	Cipensis	harita	malabarica	montana	pseudomontana	raktakanta	syriatica	zedoaria	longa
amada	1.0000000															
aromaid	0.4814815	1.0000000														
aromanaw	0.4035088	0.5416667	1.0000000													
aruginosa	0.4814815	0.5102041	0.3962264	1.0000000												
casia	0.5272727	0.4444444	0.2786885	0.5600000	1.0000000											
comosa	0.5344828	0.5090909	0.4067797	0.4821429	0.5818182	1.0000000										
decipiens	0.3750000	0.3442623	0.2615385	0.3225806	0.3870968	0.4444444	1.0000000									
Cipensis	0.4310345	0.3750000	0.2622951	0.3750000	0.4464286	0.3870968	0.4406780	1.0000000								
harita	0.4333333	0.6666667	0.5094340	0.4285714	0.3770492	0.4354839	0.3333333	0.2769231	1.0000000							
malabarica	0.4745763	0.4210526	0.5283019	0.4727273	0.4166667	0.5000000	0.3906250	0.4000000	0.4032258	1.0000000						
montana	0.4655172	0.5800000	0.4107143	0.4905660	0.5370370	0.7600000	0.3593750	0.3666667	0.4655172	0.5087719	1.0000000					
pseudomontana	0.4590164	0.4821429	0.5370370	0.5660377	0.4745763	0.5593220	0.4218750	0.3437500	0.4590164	0.6666667	0.5714286	1.0000000				
raktakanta	0.5555556	0.5294118	0.3928571	0.5294118	0.6400000	0.8125000	0.3650794	0.3965517	0.4482759	0.4912281	0.8444444	0.5263158	1.0000000			
syriatica	0.5000000	0.5000000	0.4262295	0.6111111	0.5166667	0.6000000	0.3571429	0.4285714	0.4761905	0.5161290	0.6428571	0.5737705	0.6851852	1.0000000		
zedoaria	0.4915254	0.4137931	0.5185185	0.5185185	0.4098361	0.5166667	0.4062500	0.3492063	0.4193548	0.8541687	0.5000000	0.6545455	0.5087719	0.5322581	1.0000000	
longa	0.3728814	0.4150943	0.4705882	0.4423077	0.4107143	0.4736842	0.3833333	0.3000000	0.3965517	0.4385965	0.4285714	0.5000000	0.4629630	0.5172414	0.5090909	1.0000000

Table 44. Similarity matrix of different species of *Curcuma* obtained from the ISSR data using Sorensen - Dice similarity coefficients

Row\Col	amada	aromaold	aromanew	aeruginosa	caesia	comosa	decipiens	Cypries	harita	malabarica	montana	pseudomontana	raktakanta	rylvatica	medoaria	longa
amada	1.0000000															
aromaold	0.6500000	1.0000000														
aromanew	0.5750000	0.7027027	1.0000000													
aeruginosa	0.6500000	0.6756757	0.5675676	1.0000000												
caesia	0.6904762	0.6153846	0.4358974	0.7179487	1.0000000											
comosa	0.6966292	0.6746988	0.5783133	0.6506024	0.7356322	1.0000000										
decipiens	0.5454545	0.5121951	0.4146341	0.4878049	0.5581395	0.6153846	1.0000000									
Cypries	0.6024096	0.5454545	0.4153844	0.5454545	0.6172840	0.5581395	0.6117647	1.0000000								
harita	0.6046512	0.8000000	0.6750000	0.6000000	0.5476190	0.6067416	0.5000000	0.4337349	1.0000000							
malabarica	0.6436782	0.5925926	0.6913580	0.6419753	0.5882353	0.6666667	0.5617978	0.5714286	0.5747126	1.0000000						
montana	0.6352941	0.7341772	0.5822785	0.6582278	0.6987952	0.8636364	0.5287356	0.5365854	0.6352941	0.6744186	1.0000000					
pseudomontana	0.6292135	0.6506024	0.6987952	0.7228916	0.6436782	0.7173913	0.5934066	0.5116279	0.6292135	0.8000000	0.7272727	1.0000000				
raktakanta	0.7142857	0.6923077	0.5641026	0.6923077	0.7804878	0.8965317	0.5348837	0.5679012	0.6190476	0.6588235	0.9156627	0.6896352	1.0000000			
rylvatica	0.6666667	0.6666667	0.5977011	0.7586207	0.6813187	0.7500000	0.5263158	0.6000000	0.6451613	0.6808511	0.7826087	0.7291667	0.8131868	1.0000000		
medoaria	0.6590909	0.5853659	0.6829268	0.6829268	0.5813953	0.6813187	0.5777778	0.5176471	0.5909091	0.9213483	0.6666667	0.7912088	0.6744186	0.6947368	1.0000000	
longa	0.5452099	0.5866667	0.6400000	0.6133333	0.5822785	0.6428571	0.5542169	0.4615385	0.5679012	0.6097561	0.6000000	0.6666667	0.6329114	0.6818182	0.6746988	1.0000000

Table 45. Similarity matrix of different species of *Curcuma* obtained from the RAPD data using Jaccard's similarity coefficients

Row\Col	amada	aromaold	aromanew	aeruginosa	caesia	comosa	decipiens	Cypries	harita	malabarica	montana	pseudomontana	raktakanta	rylvatica	medoaria	longa
amada	1.0000000															
aromaold	0.6956522	1.0000000														
aromanew	0.6304348	0.7608696	1.0000000													
aeruginosa	0.6956522	0.7391304	0.6521739	1.0000000												
caesia	0.7173913	0.6739130	0.5217391	0.7608696	1.0000000											
comosa	0.7065217	0.7065217	0.6195652	0.6847826	0.7500000	1.0000000										
decipiens	0.5652174	0.5652174	0.4782609	0.5434783	0.5869565	0.6195652	1.0000000									
Cypries	0.6413043	0.6195652	0.5108696	0.6195652	0.6630435	0.5869565	0.6413043	1.0000000								
harita	0.6304348	0.8260870	0.7173913	0.6521739	0.5869565	0.6195652	0.5217391	0.4891304	1.0000000							
malabarica	0.6630435	0.6413043	0.7282609	0.6847826	0.6195652	0.6739130	0.5760870	0.6086957	0.5978261	1.0000000						
montana	0.6630435	0.7717391	0.6413043	0.7065217	0.7282609	0.8695652	0.5434783	0.5869565	0.6630435	0.6956522	1.0000000					
pseudomontana	0.6413043	0.6847826	0.7282609	0.7500000	0.6630435	0.7173913	0.5978261	0.5434783	0.6413043	0.8043478	0.7391304	1.0000000				
raktakanta	0.7391304	0.7391304	0.6304348	0.7391304	0.8043478	0.9021739	0.5652174	0.6195652	0.6521739	0.6847826	0.9239130	0.7065217	1.0000000			
rylvatica	0.6630435	0.6847826	0.6195652	0.7717391	0.6847826	0.7391304	0.5108696	0.6086957	0.6413043	0.6739130	0.7826087	0.7173913	0.8152174	1.0000000		
medoaria	0.6739130	0.6304348	0.7173913	0.7173913	0.6086957	0.6847826	0.5869565	0.5543478	0.6086957	0.9239130	0.6847826	0.7934783	0.6956522	0.6847826	1.0000000	
longa	0.5978261	0.6630435	0.7065217	0.6847826	0.6413043	0.6739130	0.5978261	0.5434783	0.6195652	0.6521739	0.6521739	0.6956522	0.6847826	0.6956522	0.7065217	1.0000000

The dendrograms constructed using UPGMA method from matrices of genetic distances obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on ISSR markers are given in Figs. 31 – 33. The fifteen *Curcuma* species studied were splits at coefficients 0.52, 0.67 and 0.69, respectively placed them in six different groups in all the dendrograms. The clustering pattern in the dendrograms generated using the Simple Matching similarity coefficient of the ISSR markers was slightly different from the dendrograms generated using the Jaccard's similarity coefficient and the Sorensen - Dice similarity coefficients. The members of the group were also alike except for *C. aromatica*. The clustering pattern in the

dendrograms generated using Jaccard's similarity coefficient and Sorensen - Dice similarity coefficient are as below.

- Group I - *C. amada*, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. montana*, *C. raktakanta* and *C. sylvatica*.
- Group II - *C. aromatica*^a, *C. haritha*
- Group III - *C. aromatica*^b, *C. malabarica*, *C. zedoaria* and *C. pseudomontana*
- Group IV - *C. longa*
- Group V - *C. decipiens*
- Group VI - *Curcuma* species

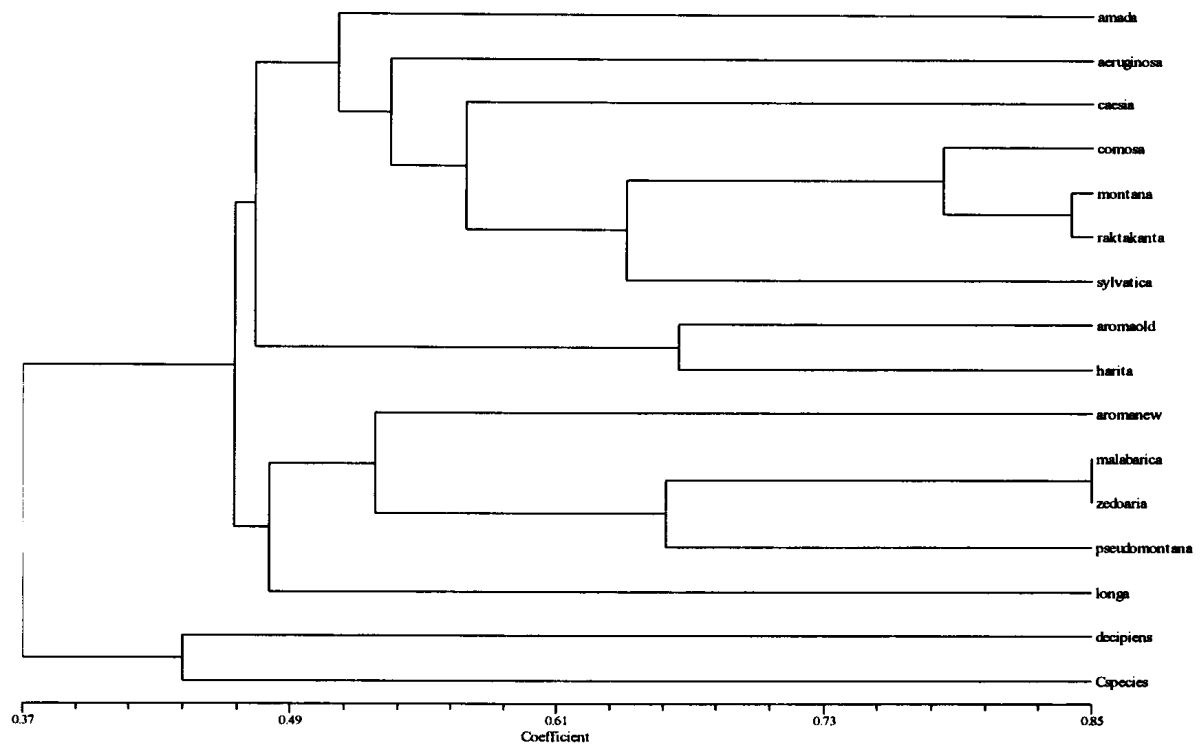


Fig. 31. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on ISSR analysis using Jaccard's similarity coefficients

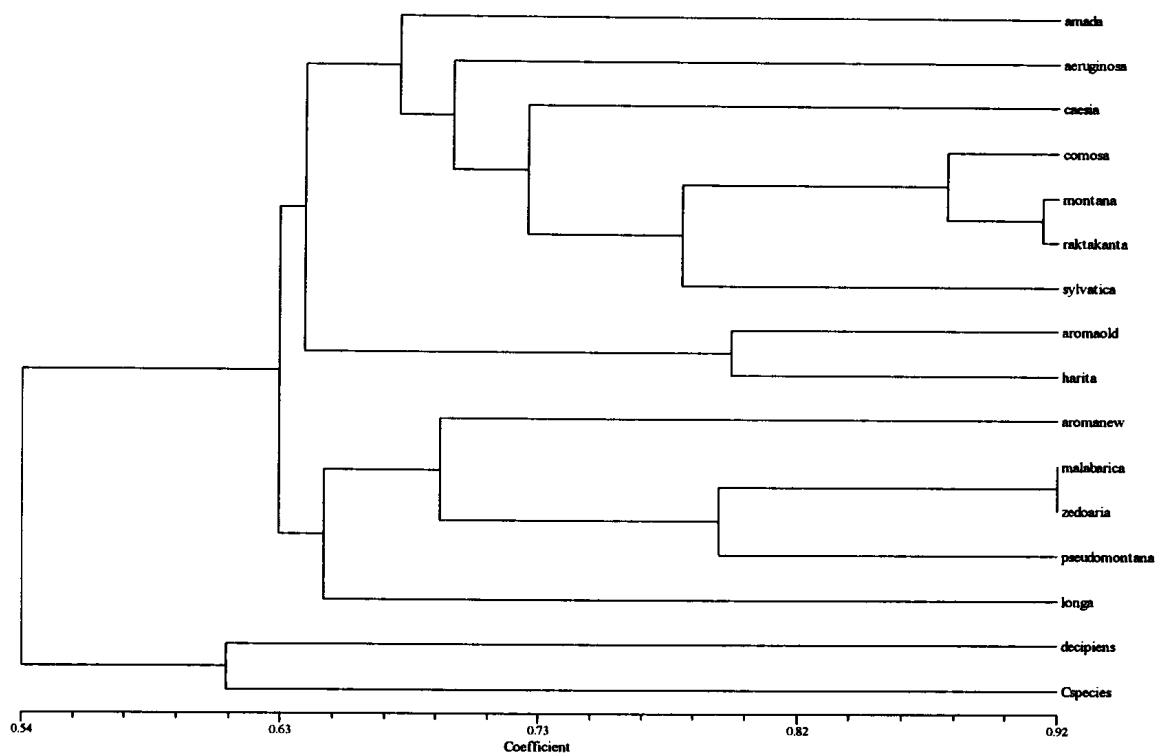


Fig.32. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on ISSR analysis using Sorensen - Dice similarity coefficients

C. aromatica^b, the member of group III in the dendrogram generated using Jaccard and Sorensen- Dice similarity coefficients grouped with the *C. haritha* and *C. aromatica*^a to form the fourth group of the dendrogram constructed using simple matching similarity coefficients. This was the only difference among the three coefficients studied.

- Group I - *C. amada*, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. montana*, *C. raktakanta* and *C. sylvatica*.
- Group II - *C. malabarica*, *C. zedoaria* and *C. pseudomontana*
- Group III - *C. longa*
- Group IV - *C. aromatica*^a, *C. haritha* and *C. aromatica*^b
- Group V - *C. decipiens*
- Group VI - *Curcuma* species

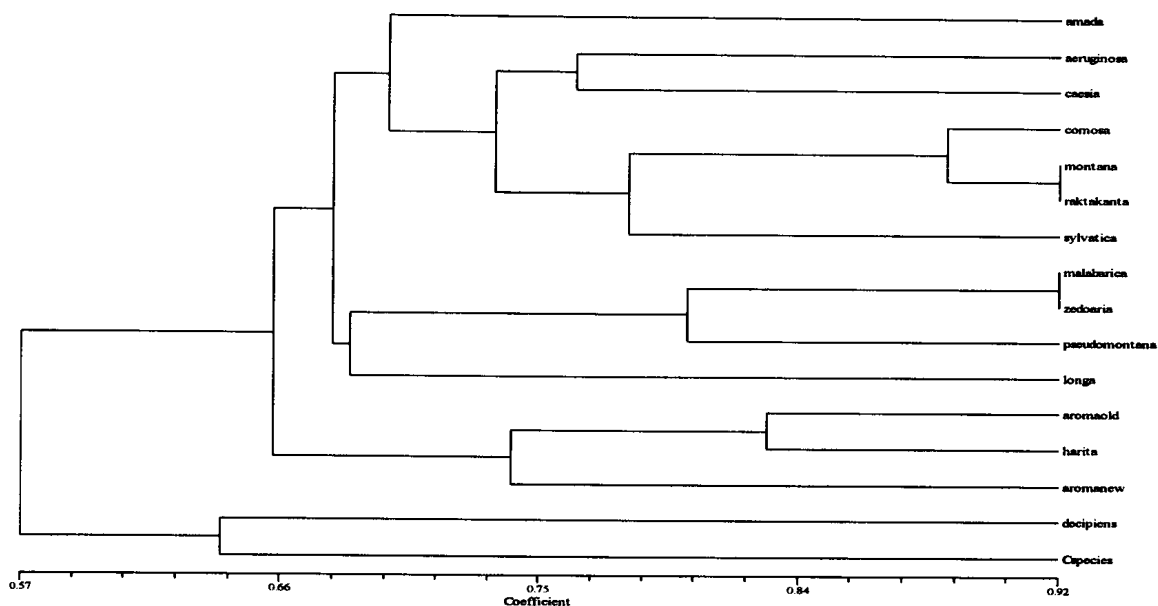


Fig. 33. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on ISSR analysis using Simple Matching similarity coefficients.

Highest and lowest similarities between different *Curcuma* species in the different groups of the dendrogram produced by three similarity coefficients using ISSR marker data are given in Table 46.

Table 46. Highest and lowest similarities between different *Curcuma* species in the different groups of the dendrogram produced by three similarity coefficients using ISSR marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	<i>C. montana</i> & <i>C. raktakanta</i> (0.64)	<i>C. montana</i> & <i>C. raktakanta</i> (0.92)	<i>C. montana</i> & <i>C. raktakanta</i> (0.92)
	Lowest	<i>C. amada</i> & <i>C. montana</i> (0.48)	<i>C. amada</i> & <i>C. Montana</i> (0.64)	<i>C. amada</i> & <i>C. montana</i> (0.66)
II	Highest	<i>C. aromatica</i> ^b & <i>C. haritha</i> (0.67)	<i>C. aromatica</i> ^b & <i>C. haritha</i> (0.80)	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.92)
	Lowest	-	-	<i>C. malabarica</i> & <i>C. pseudomontana</i> (0.80)
III	Highest	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.85)	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.92)	<i>C. longa</i>
	Lowest	<i>C. aromatica</i> ^b & <i>C. malabarica</i> (0.52)	<i>C. aromatica</i> ^b & <i>C. malabarica</i> (0.69)	-
IV	Highest	<i>C. longa</i>	<i>C. longa</i>	<i>C. aromatica</i> ^a & <i>C. haritha</i> (0.83)
	Lowest	-	-	<i>C. aromatica</i> ^b & <i>C. haritha</i> (0.72)
V	Highest	<i>C. decipiens</i>	<i>C. decipiens</i>	<i>C. decipiens</i>
VI	Lowest	-	-	-
	Highest	<i>Curcuma</i> species	<i>Curcuma</i> species	<i>Curcuma</i> species
	Lowest	-	-	-

4.2.5. Characterization of *Curcuma* species using combined RAPD and ISSR markers

Fourty eight RAPD and eight ISSR primers together produced a total of five hundred and fourty markers among the fifteen *Curcuma* species studied, out of which four hundred and ninety six (91.85%) were polymorphic.

4.2.5.1. Cluster analysis

The combined dendrograms of RAPD and ISSR markers were developed using UPGMA method from matrices of genetic similarity obtained from Jaccard's, Sorensen- Dice coefficients and Simple Matching,. The similarity matrices obtained are represented in Tables 47 –49. In all the similarity matrices produced by the two markers together, *C. raktakanta* and *C. montana* showed maximum similarity (0.83, 0.91 and 0.91 in Jaccard's, Sorensen - Dice and Simple Matching, respectively. The least similarity was showed by *C. montana* and *C. decipiens* (0.33) in the Jaccard's and Simple Matching (0.50) similarity matrices while *C. raktakanta* and *C. decipiens* (0.50) showed the least similarity in Sorensen – Dice similarity matrix..

Table 47. Similarity matrix of different species of *Curcuma* based on combined RAPD and ISSR data generated using Jaccard's similarity coefficients

Row/Col	amada	aromachl	aromanew	aruginosa	caesia	comosa	decipiens	Crepens	kurita	malabarica	montana	pseudomontana	raktakanta	rybratica	mdouzia	longa
amada	1.0000000															
aromachl	0.5217391	1.0000000														
aromanew	0.4771242	0.7261905	1.0000000													
aruginosa	0.4633333	0.4410774	0.3888889	1.0000000												
caesia	0.5328947	0.4253968	0.3597561	0.5296167	1.0000000											
comosa	0.3934426	0.4565217	0.4207317	0.4388715	0.5635179	1.0000000										
decipiens	0.3462687	0.3690852	0.3385093	0.3291536	0.3484848	0.3636364	1.0000000									
Crepens	0.4295082	0.4107744	0.3774834	0.3771044	0.3870968	0.3761468	0.4510490	1.0000000								
kurita	0.4585987	0.6641509	0.6343284	0.4224422	0.3993808	0.4216867	0.3699060	0.3745928	1.0000000							
malabarica	0.5000000	0.4638158	0.4684385	0.4948454	0.4662379	0.4736842	0.3611111	0.3741935	0.4177215	1.0000000						
montana	0.5537459	0.4603175	0.4236760	0.4376997	0.5047923	0.7535714	0.3255814	0.3695652	0.4290123	0.4825397	1.0000000					
pseudomontana	0.5032258	0.4673203	0.4768212	0.5457746	0.4935065	0.4723926	0.3819876	0.3782051	0.4303797	0.5724138	0.4718750	1.0000000				
raktakanta	0.5457516	0.4294671	0.4024768	0.4527687	0.5361842	0.7709091	0.3372781	0.3829114	0.4254658	0.4468750	0.8314176	0.4503106	1.0000000			
rybratica	0.5157233	0.5048544	0.4713376	0.5166667	0.4736842	0.5261538	0.3802395	0.4025157	0.4952077	0.5629139	0.5460317	0.5404531	0.5530547	1.0000000		
mdouzia	0.4410774	0.4027304	0.4216028	0.4653036	0.3927393	0.4166667	0.3566667	0.3470790	0.4178082	0.6015326	0.4337748	0.5401460	0.4300000	0.5208333	1.0000000	
longa	0.4580645	0.4309211	0.4594395	0.3798701	0.4249201	0.4654088	0.3948220	0.3774834	0.4038462	0.4684385	0.4694534	0.4622951	0.4660194	0.4666667	0.3877551	1.0000000

Table 48. Similarity matrix of different species of *Curcuma* based on combined RAPD and ISSR data generated using Sorensen - Dice similarity coefficients

Row/Col	amada	aromacold	aromanew	aeruginosa	caesia	comosa	decipiens	Cppecies	harita	malabarica	montana	pseudomontana	raktakanta	syriatica	indonesia	longa
amada	1.0000000															
aromacold	0.6857143	1.0000000														
aromanew	0.6460177	0.8413793	1.0000000													
aeruginosa	0.6516854	0.6121495	0.5600000	1.0000000												
caesia	0.6952790	0.5968820	0.5291480	0.6924829	1.0000000											
comosa	0.7448560	0.6268657	0.5922747	0.6100218	0.7208333	1.0000000										
decipiens	0.5144124	0.5391705	0.5058005	0.4952830	0.5168539	0.5333333	1.0000000									
Cppecies	0.6009174	0.5823389	0.5480769	0.5476773	0.5581395	0.5466667	0.6216867	1.0000000								
harita	0.6286210	0.7981859	0.7762557	0.5939675	0.5707965	0.5932203	0.5400458	0.5450237	1.0000000							
malabarica	0.6666667	0.6337079	0.6380090	0.6620690	0.6339649	0.6428571	0.5306122	0.5446009	0.5892857	1.0000000						
montana	0.7127883	0.6304348	0.5951860	0.6088889	0.6709130	0.8394705	0.4912281	0.5396825	0.6004320	0.6509636	1.0000000					
pseudomontana	0.6695279	0.6369710	0.6457399	0.7061503	0.6608696	0.6416667	0.5528090	0.5488372	0.6017699	0.7280702	0.6411890	1.0000000				
raktakanta	0.7061311	0.6008772	0.5739514	0.6233184	0.6980728	0.8706366	0.5044248	0.5537757	0.5969499	0.6177106	0.9079498	0.6209830	1.0000000			
syriatica	0.6804979	0.6709677	0.6406926	0.6813187	0.6428571	0.6895161	0.5309761	0.5739910	0.6623932	0.7203390	0.7063655	0.7016807	0.7122153	1.0000000		
indonesia	0.6121495	0.5742092	0.5931373	0.6334165	0.5639810	0.5882353	0.5257985	0.5153061	0.5893720	0.7511962	0.6050808	0.7014218	0.6013986	0.6849315	1.0000000	
longa	0.6283186	0.6022989	0.6296296	0.5505882	0.5964126	0.6351931	0.5661253	0.5480769	0.5753425	0.6380090	0.6389497	0.6322870	0.6357616	0.6363636	0.5388235	1.0000000

Table 49. Similarity matrix of different species of *Curcuma* based on combined RAPD and ISSR data generated using Simple Matching similarity coefficients

Row/Col	amada	aromacold	aromanew	aeruginosa	caesia	comosa	decipiens	Cppecies	harita	malabarica	montana	pseudomontana	raktakanta	syriatica	indonesia	longa
amada	1.0000000															
aromacold	0.6944444	1.0000000														
aromanew	0.6581197	0.8525641	1.0000000													
aeruginosa	0.6688034	0.6452991	0.6004274	1.0000000												
caesia	0.6965812	0.6132479	0.5512821	0.7115385	1.0000000											
comosa	0.7350427	0.6260684	0.5940171	0.6175214	0.7136752	1.0000000										
decipiens	0.5320513	0.5726496	0.5448718	0.5427350	0.5405983	0.5363248	1.0000000									
scalcarata	0.6282051	0.6260684	0.5982906	0.6047009	0.5940171	0.5641026	0.6645299	1.0000000								
harita	0.6367521	0.8098291	0.7905983	0.6260684	0.5854701	0.5897436	0.5705128	0.5897436	1.0000000							
malabarica	0.6709402	0.6517094	0.6581197	0.6858974	0.6452991	0.6367521	0.5576923	0.5854701	0.6068376	1.0000000						
montana	0.7072650	0.6367521	0.6047009	0.6239316	0.6688034	0.8525641	0.5042735	0.5662393	0.6047009	0.6517094	1.0000000					
pseudomontana	0.6709402	0.6517094	0.6623932	0.7243590	0.6666667	0.6324786	0.5747863	0.5854701	0.6153846	0.7350427	0.6388889	1.0000000				
raktakanta	0.7029915	0.6111111	0.5876068	0.6410256	0.6987179	0.8653846	0.5213675	0.5833333	0.6047009	0.6217949	0.9059829	0.6217949	1.0000000			
syriatica	0.6709402	0.6730769	0.6452991	0.6901709	0.6367521	0.6709402	0.5576923	0.5940171	0.6623932	0.7179487	0.6944444	0.6963812	0.7029915	1.0000000		
indonesia	0.6452991	0.6260684	0.6452991	0.6858974	0.6068376	0.6111111	0.5876068	0.5940171	0.6367521	0.7777778	0.6346154	0.7307692	0.6346154	0.7051282	1.0000000	
longa	0.6410256	0.6303419	0.6581197	0.5918803	0.6153846	0.6367521	0.6004274	0.5982906	0.6025641	0.6581197	0.6474359	0.6495726	0.6474359	0.6410256	0.6153846	1.0000000

The dendrograms constructed using UPGMA method from matrices of genetic distances obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on combined RAPD and ISSR markers are given in Figs. 34 – 36. The dendrograms generated using Jaccard's similarity coefficient and Sorensen - Dice similarity coefficients were alike while the clustering pattern in the dendrogram generated using Simple Matching coefficients exhibited a slight variation (*C. caesia*, the member of group I clustered with *C. aeruginosa* in dendrograms generated using Simple Matching coefficients).

The dendrograms constructed using Jaccard's and Sorensen - Dice similarity coefficients were splits at coefficients 0.56 and 0.69 produced seven groups each as below.

- Group I - *C. amada*, *C. comosa*, *C. montana*, and *C. raktakanta* and *C. caesia*
- Group II - *C. aeruginosa*
- Group III - *C. malabarica*, *C. zedoaria*, *C. pseudomontana* and *C. sylvatica*
- Group IV - *C. longa*
- Group V - *C. aromatica*^a, *C. aromatica*^b and *C. haritha*
- Group VI - *C. decipiens*
- Group VII - *Curcuma* species

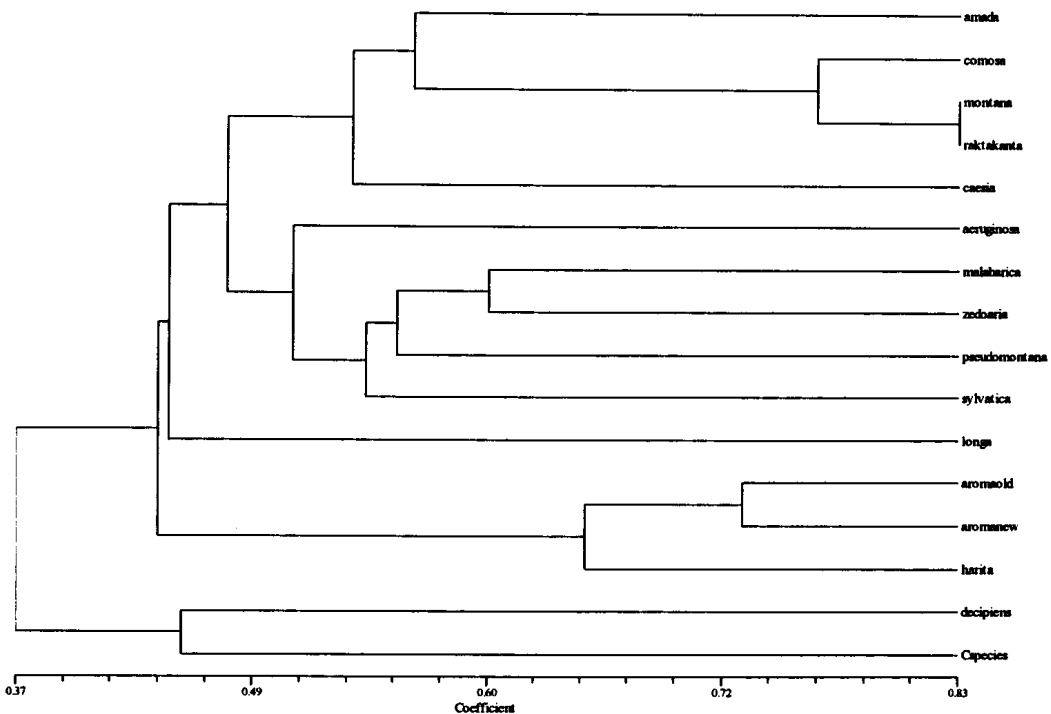


Fig. 34. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on combined RAPD and ISSR markers using Jaccard's similarity coefficients.

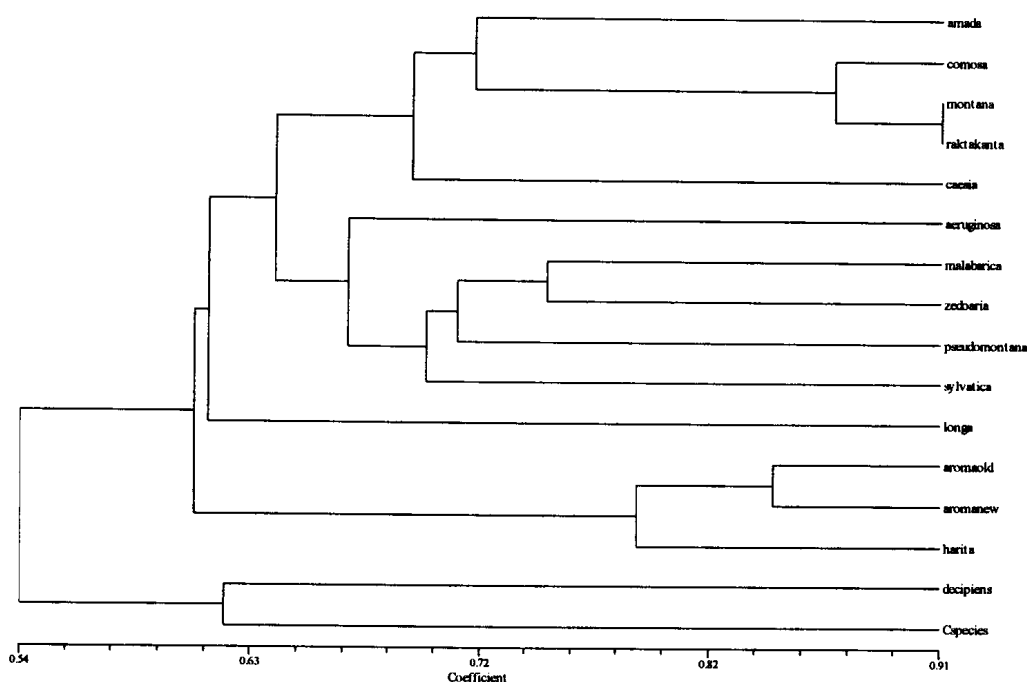


Fig. 35. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on combined RAPD and ISSR markers using Sorensen - Dice similarity coefficients.

The dendrogram of fifteen *Curcuma* species using Simple matching coefficients splits at coefficient 0.68 produced seven groups as given below.

- Group I - *C. amada*, *C. comosa*, *C. montana*, and *C. raktakanta*
- Group II - *C. aeruginosa* and *C. caesia*
- Group III - *C. malabarica*, *C. zedoaria*, *C. pseudomontana* and *C. sylvatica*
- Group IV - *C. longa*
- Group V - *C. aromatica*^a, *C. aromatica*^b and *C. haritha*
- Group VI - *C. decipiens*
- Group VII - *Curcuma* species

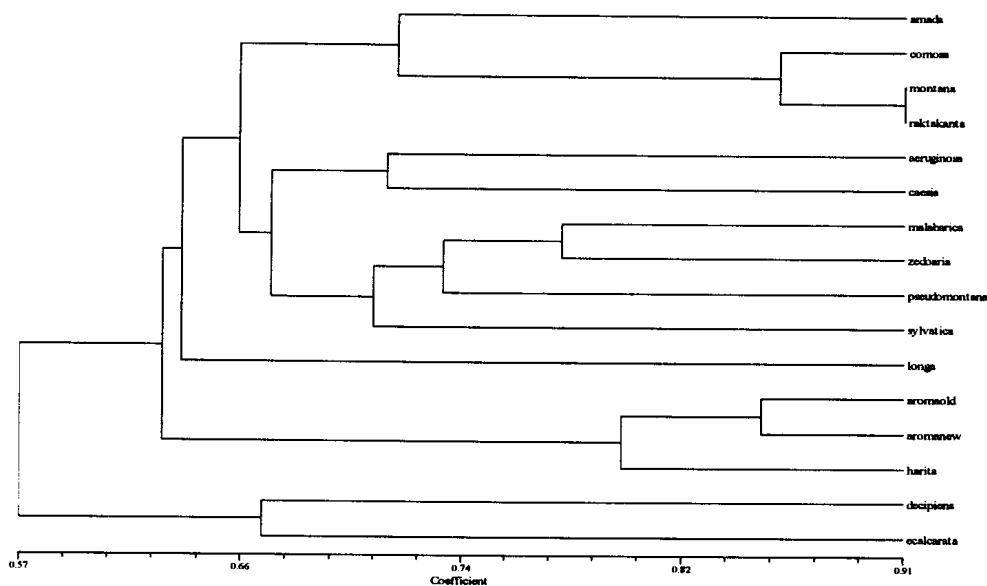


Fig. 36. Dendrogram of genetic similarity between 15 *Curcuma* spp. based on RAPD and ISSR markers using Simple Matching similarity coefficients.

Highest and lowest similarities between different *Curcuma* species in the different groups of the dendrogram produced by three similarity coefficients using combined RAPD/ISSR marker data is shown in Table 50.

Table 50. Highest and lowest similarities between the varieties and cultivars in the different groups of the dendrogram produced by three similarity coefficients using combined RAPD-ISSR marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	<i>C. montana</i> & <i>C. raktakanta</i> (0.83)	<i>C. montana</i> & <i>C. raktakanta</i> (0.91)	<i>C. montana</i> & <i>C. raktakanta</i> (0.91)
	Lowest	<i>C. caesia</i> & <i>C. montana</i> (0.55)	<i>C. caesia</i> & <i>C. montana</i> (0.67)	<i>C. amada</i> & <i>C. montana</i> (0.71)
II	Highest	<i>C. aeruginosa</i>	<i>C. aeruginosa</i>	<i>C. aeruginosa</i> & <i>C. caesia</i> (0.71)
III	Lowest	-	-	-
	Highest	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.51)	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.75)	<i>C. malabarica</i> & <i>C. zedoaria</i> (0.78)
IV	Lowest	<i>C. sylvatica</i> & <i>C. zedoaria</i> (0.52)	<i>C. sylvatica</i> & <i>C. zedoaria</i> (0.68)	<i>C. sylvatica</i> & <i>C. zedoaria</i> (0.68)
	Highest	<i>C. longa</i>	<i>C. longa</i>	<i>C. longa</i>
V	Lowest	-	-	-
	Highest	<i>C. aromatica</i> ^b & <i>aromatica</i> ^a (0.73)	<i>C. aromatica</i> ^b & <i>aromatica</i> ^a (0.84)	<i>C. aromatica</i> ^b & <i>aromatica</i> ^a (0.85)
VI	Lowest	<i>C. aromatica</i> ^b & <i>haritha</i> (0.63)	<i>C. aromatica</i> ^b & <i>haritha</i> (0.78)	<i>C. aromatica</i> ^b & <i>haritha</i> (0.79)
	Highest	<i>C. decipiens</i>	<i>C. decipiens</i>	<i>C. decipiens</i>
VII	Lowest	-	-	-
	Highest	<i>Curcuma</i> species	<i>Curcuma</i> species	<i>Curcuma</i> species

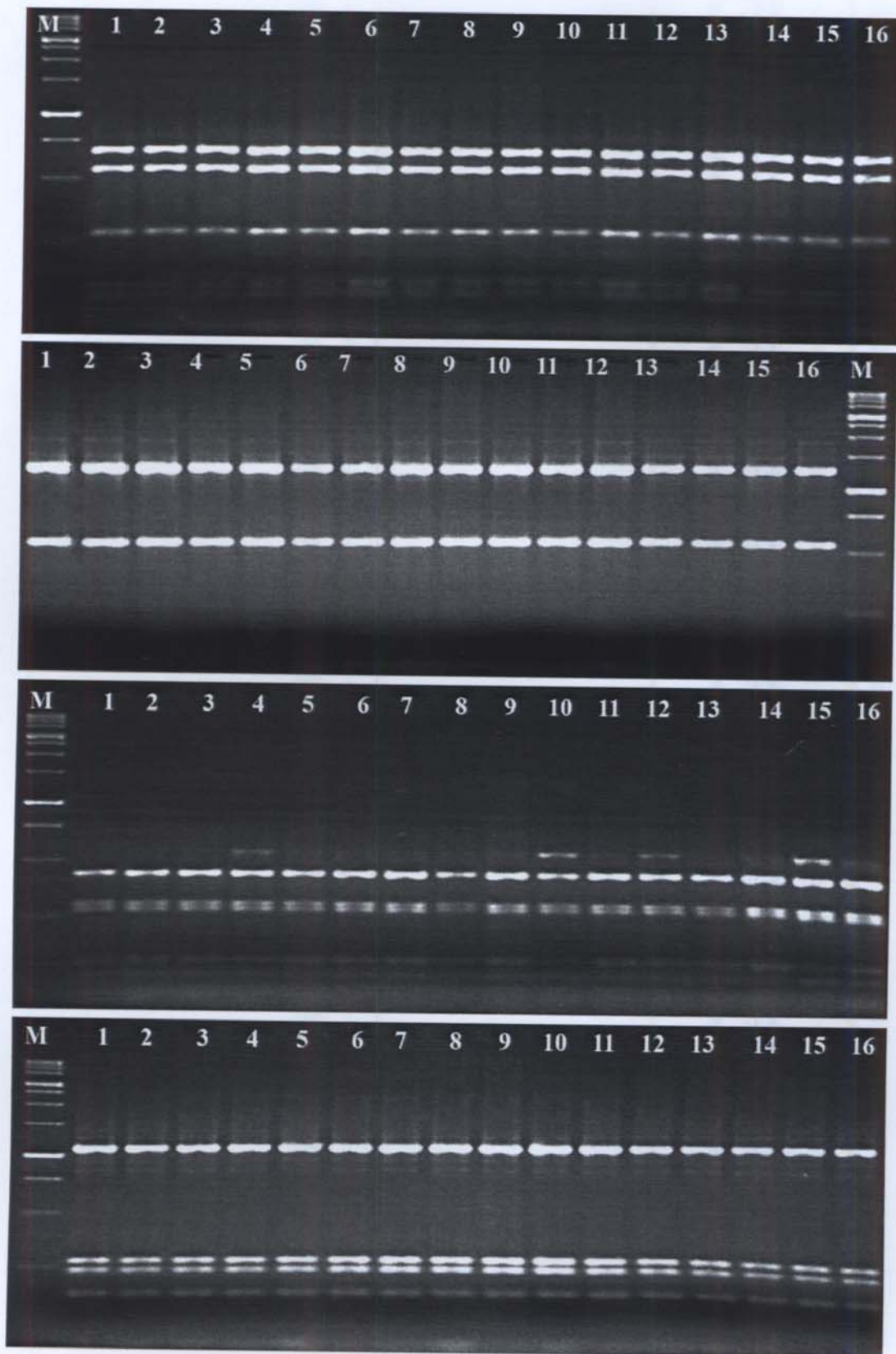


Fig. 38. PCR RFLP analysis of 18S rRNA gene using Msp I, Bam HI, Hae III and Rsa restriction enzymes. Lane 1 - 12 are 1). *C. aeruginosa*, 2). *C. amada*, 3). *C. aromatica*^a, 4). *C. aromatica*^b, 5) *C. caesia*, 6). *C. comosa*, 7). *C. decipiens*, 8) *Curcuma* species, 9) *C. haritha*, 10). *C. longa*, 11). *C. malabarica*, 12). *C. montana*, 13). *C. pseudomontana*, 14). *C. raktakanta*, 15). *C. sylvatica* and 16). *C. zedoaria*, respectively. M- Marker 1 Kb ladder (Biogene, USA).

4.2.6. 18S rRNA analysis

4.2.6.1. 18S rRNA amplification

A 1800 bp product was amplified in all the 15 *Curcuma* species using the specific primers (Cao *et al.*, 2001) (Fig. 37).



Fig. 37. *Curcuma* species DNA amplified using 18S rRNA primers. M- 1 Kb ladder, (Biogene, USA), Lane 1 – 15 are *C. amada*, *C. aromatica*, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species., *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica* and *C. zedoaria*, respectively.

4.2.6.2. 18S rRNA PCR RFLP

Restriction analysis of 18S rRNA products was done using four different restriction enzymes. The restriction pattern placed the fifteen *Curcuma* species in two groups (Fig. 38)

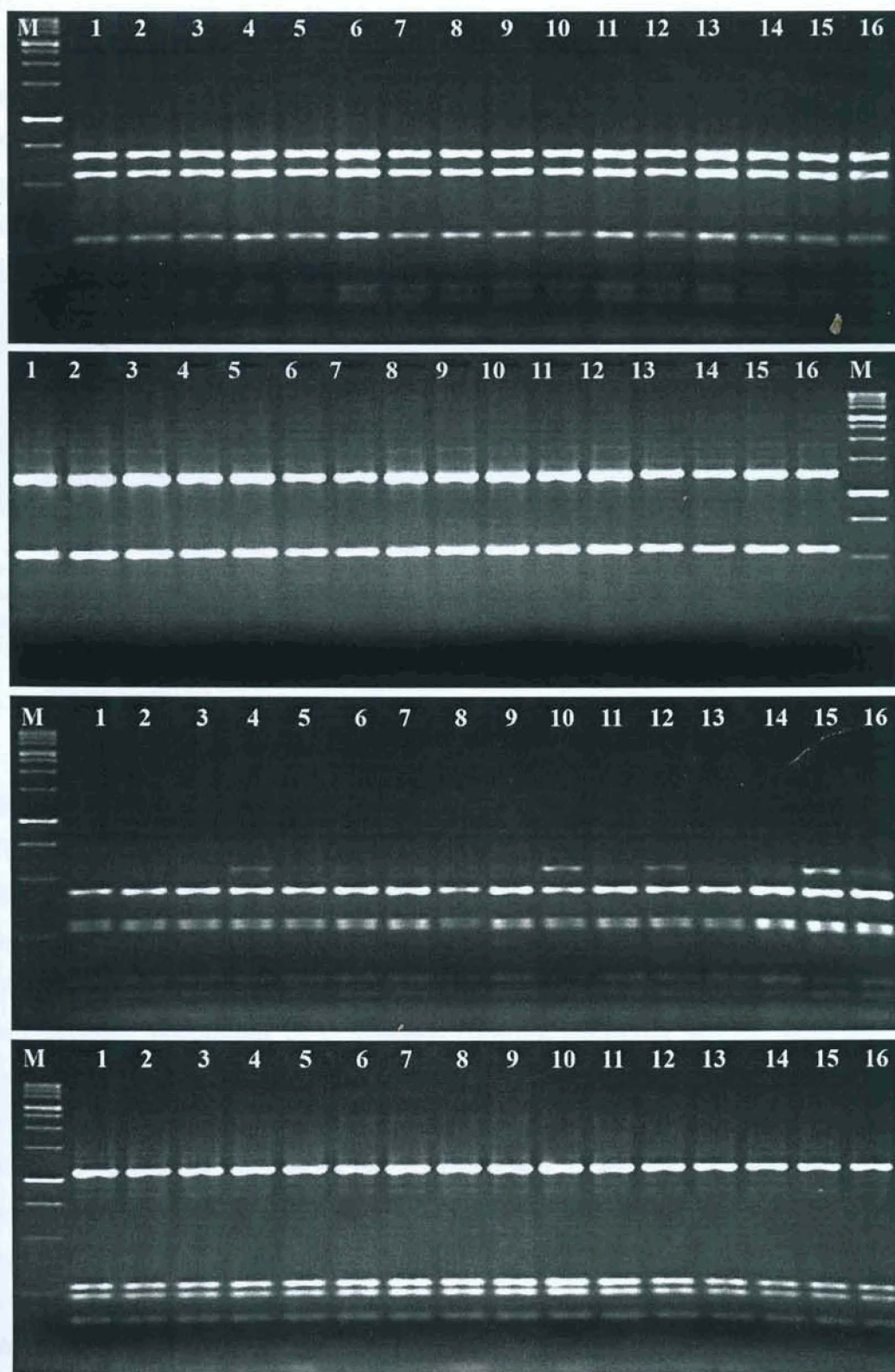


Fig. 38. PCR RFLP analysis of 18S rRNA gene using Msp I, Bam HI, Hae III and Rsa restriction enzymes. Lane 1 - 12 are 1). *C. aeruginosa*, 2). *C. amada*, 3). *C. aromatica*^a, 4). *C. aromatica*^b, 5) *C. caesia*, 6). *C. comosa*, 7). *C. decipiens*, 8) *Curcuma* species, 9) *C. haritha*, 10). *C. longa*, 11). *C. malabarica*, 12). *C. montana*, 13). *C. pseudomontana*, 14). *C. raktakanta*, 15). *C. sylvatica* and 16). *C. zedoaria*, respectively. M- Marker 1 Kb ladder (Biogene, USA).

4.2.6.3. Amplification of 18s rRNA gene using the designed primers

The 18S rRNA sequences were amplified using three sets (forward & reverse) of primers. The reliability of the sequence was confirmed by sequencing in both orientations. The size of the amplified product for all the three fragments was approximately 650 bp length (Fig. 39). The overlapping sequences were edited and assembled to yield the complete 18S rRNA sequence.



Fig. 39. 18S rRNA internal sequences of different *Curcuma* species amplified by using the specific primers designed. M – Marker (pTZ57 plasmid (100ng)), L – 100 bp ladder, Lane 1 – 45 are the internal sequences (three sequences for each species) of *C. aeruginosa*, *C. amada*, *C. aromatica*, *C. caesia*, *C. comosa*, *C. decipiens*, *Curcuma* species, *C. haritha*, *C. longa*, *C. malabarica*, *C. montana*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica*, and *C. zedoaria*, respectively

4.2.6.4. 18S rRNA sequence analysis

18S rRNA sequence was compared with the nucleotide sequences of 18S rRNA gene from the different species of *Curcuma* deposited at the NCBI GenBank. The single nucleotide polymorphism at the 234th position of partial 18S rRNA gene of *C. aromatica*, *C. longa* and *C. aeruginosa* agreed with the already reported sequences of these species from China (cytosine residue at 234th position of 18S rRNA gene), while the sequence of Indian *C. zedoaria* species showed the similarity with Japanese populations of *C. zedoaria* (thymine nucleotide at 234th position) rather than Chinese population. (Fig. 40). The Indian *C. zedoaria* showed similarity with the *C. xanthorrhiza* reported from China. The rest of the species studied are the first time report and the species viz, *C. raktakanta*, *C. sylvatica*, *C. montana*, *C. haritha* and *C. comosa* showed cytosine as the nucleotide at 234th position while *C. malabarica* and *C. amada* showed thymine as the nucleotide at 234th position. In addition to the SNP at 234th position (thymine) *C. decipiens* and *Curcuma* species showed three transitions at 223, 238 and 1490 (C – T) and a transversion (T – A) at 1500 position. *C. caesia* also showed a base transition at 526th position (A – G) in the 18S rRNA gene (Fig.40).

C. amada-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.pseudomontana-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.malabarica-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.zedoaria-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB261849.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.raktakanta-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.sylvatica-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.montana-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.longa-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.hariltha-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.comosa-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.aromatica-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.aeruginosa-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.caesia-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047710.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047718.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047720.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047723.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047726.2 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047707.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047708.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047711.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047712.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047713.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047714.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047715.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047721.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047722.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047725.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047717.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047727.2 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB047724.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
AB261848.1 Curcuma	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.decipiens-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240
C.species-IISR	180	AA	CCCCGACCTTTGGGAGGGGATGCA	TTTATTAGATAAAAAGT	CGATCGGGGCTTGC	CG	240

C. amada-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. pseudomontana-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. malabarica-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. zedoaria-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB261849.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. raktakanta-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. sylvatica-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. montana-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. longa-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. haritha-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. comosa-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. aromatica-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. aeruginosa-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. caesia-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047710.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047718.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047720.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047723.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047726.2 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047707.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047708.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047711.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047712.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047713.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047714.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047715.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047721.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047722.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047725.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047717.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047727.2 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB047724.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
AB261848.1 Curcuma	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. decipiens-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540
C. species-IISR	480	AATAACAATATCGGGCTCTTTGAGTTTGGTAATTGGAAATGACTACAACTCTAAATCCCTTA	540

C.amada-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.pseudomontana-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.malabarica-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.zedaria-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB261849.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.rakrakanta-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.sylvatica-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.montana-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.longa-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.harltha-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.comosa-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.aromatica-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.aeruginosa-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.caesia-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047710.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047718.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047720.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047723.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047726.2 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047707.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047708.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047711.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047712.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047713.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047714.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047715.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047721.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047722.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047725.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047717.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047727.2 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB047724.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
AB261848.1 Curcuma	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.decipiens-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500
C.species-IISR	1440	TGTGATGCCCTTAGATGTTCTGGGCGCAGCGGCGCTACACTGATGTATCCAAAGAGTCT	1500

Fig. 40. Single nucleotide polymorphism in *Curcuma* species.

The bootstrap analysis placed the Indian *Curcuma* species studied in five different groups (Fig. 41)

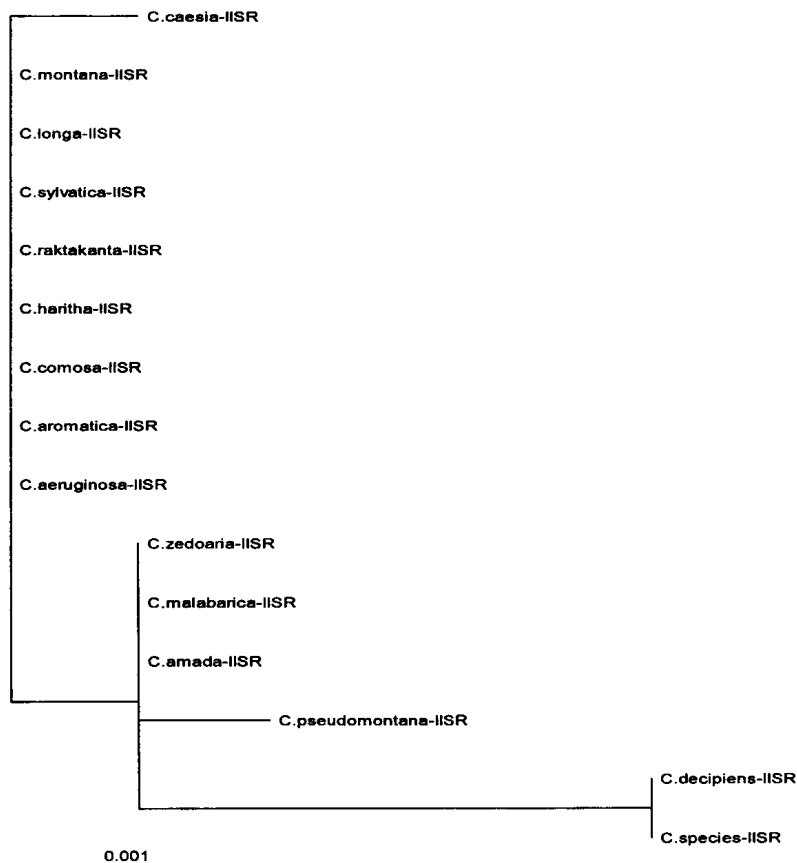


Fig. 41. Dendrograms showing the clustering of different *Curcuma* species based on the 18S rRNA sequence analysis

4.2.7. Characterisation of turmeric varieties/cultivars using RAPD markers – infraspecific variation

Forty one random decamer primers produced a total of 498 scorable bands in the 36 cultivars/varieties studied out of which 394 (79.1%) were polymorphic. The percentage of polymorphism ranged from a maximum of 95 percent showed by primer OPB 08 to a minimum of 54 percent showed by primer OPJ 10. The sequence of the RAPD primers used for the molecular genetic fingerprinting and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table 51 and Fig. 42 – 51.

Table 51. Sequence of RAPD primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in different turmeric varieties/ cultivars

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	OPA 03	AGTCAGCCAC	13	05	38.5
2	OPA 05	AGGGGTCTTG	13	11	84.6
3	OPA 07	GAAACGGGTG	17	13	76.5
4	OPA 11	CAATCGCCGT	13	12	92.3
5	OPA 12	TCGGCGATAG	10	07	70.0
6	OPA 14	TCTGTGCTGG	10	06	60.0
7	OPA 15	TTCCGAACCC	08	05	62.5
8	OPA 17	GACCGCTTGT	18	15	83.3
9	OPA 18	AGGTGACCGT	10	08	80.0
10	OPA 19	CAAACGTCGG	16	15	93.7
11	OPB 07	GGTGACGCAC	14	13	92.8
12	OPB 08	GTCCACACGG	22	21	95.5
13	OPB 09	TGGGGGACTC	10	06	60.0
14	OPB 10	CTGCTGGGAC	11	08	72.7
15	OPB 11	GTAGACCCGT	14	09	64.3
16	OPB 12	CCTTGACGCA	15	14	93.3
17	OPB 16	TTTGCCCGGA	11	08	72.7
18	OPB 18	CCACAGCAGT	09	08	88.8
19	OPB 19	ACCCCCGAAG	04	03	75.0
20	OPC 07	GTCCCGACGA	12	11	91.6
21	OPC 08	TGGACCGGTG	16	15	93.7
22	OPC 09	CTCACCGTCC	14	12	85.7
23	OPC 10	TGTCTGGGTG	14	12	85.7
24	OPC 11	AAAGCTGCGG	11	09	81.8
25	OPC 12	TGTCATCCCC	13	12	92.3
26	OPC 14	TGCGTGCTTG	18	16	88.8
27	OPC 18	TGAGTGGGTG	07	05	71.4
28	OPD 01	ACCGCGAAGG	12	11	91.6
29	OPD 04	TCTGGTGAGG	12	09	75.0
30	OPD 05	TGAGCGGACA	10	06	60.0
31	OPD 07	TTGGCACGGG	09	06	66.6
32	OPD 08	GTGTGCCCCA	12	08	66.6
33	OPD 13	GGGGTGACGA	13	10	76.9
34	OPD 14	CTTCCCCAAG	11	09	81.8
35	OPD 15	CATCCGTGCT	10	08	80.0
36	OPD 16	AGGGCGTAAG	16	12	75.0
37	OPJ 05	CTCCATGGGG	07	06	85.7
38	OPJ 09	TGAGCCTCAC	10	06	60.0
39	OPJ 10	AAGCCCGAGG	11	06	54.5
40	OPJ 17	ACGCCAGTTC	12	11	91.6
41	OPJ 18	TGGTCGCAGA	10	07	70.0
	Total		498	394	79.1

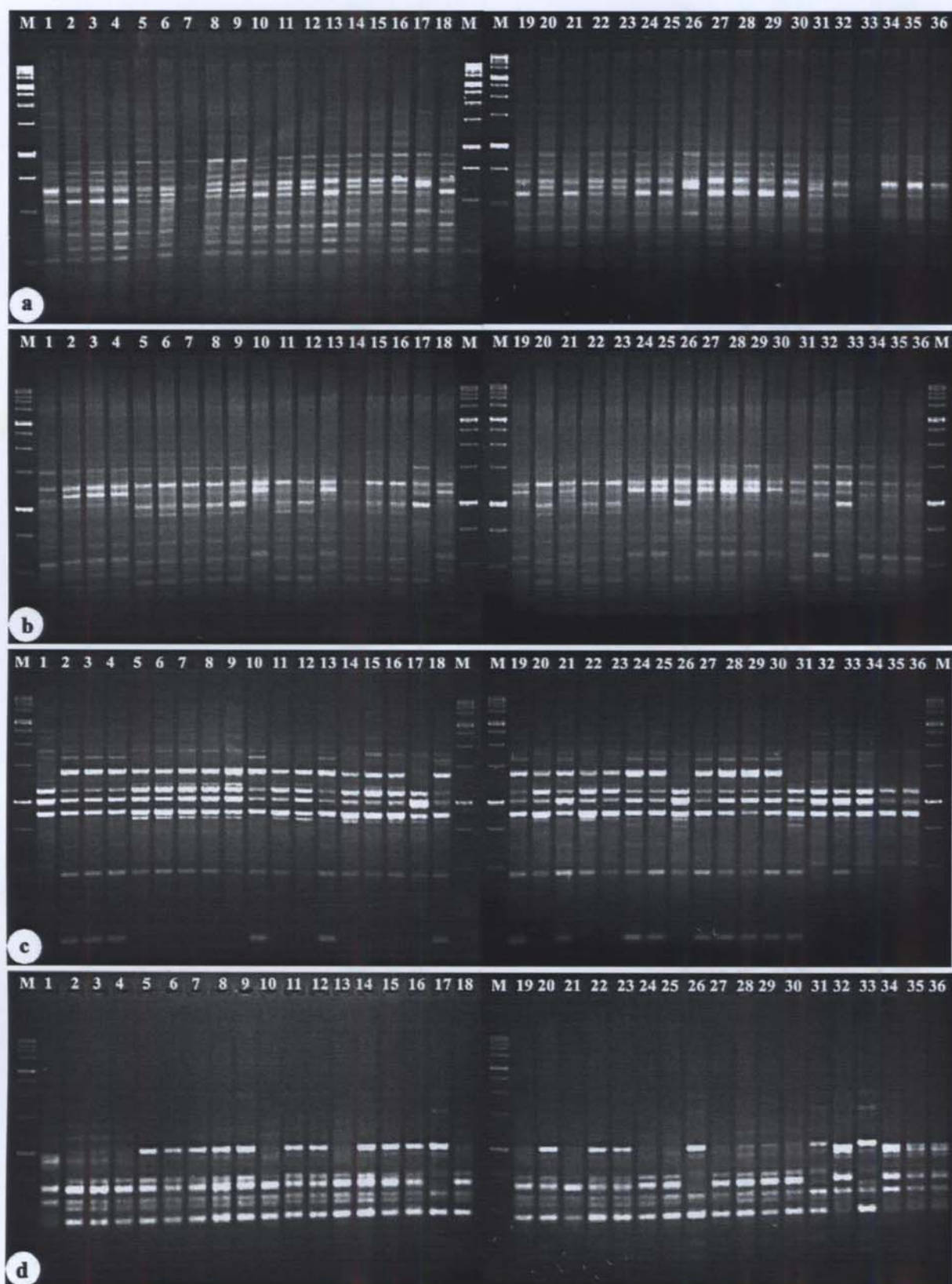


Fig. 42. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPA 03, OPA 05, OPA 07 and OPA 11. Lane 1-36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aiswal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prat-hibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

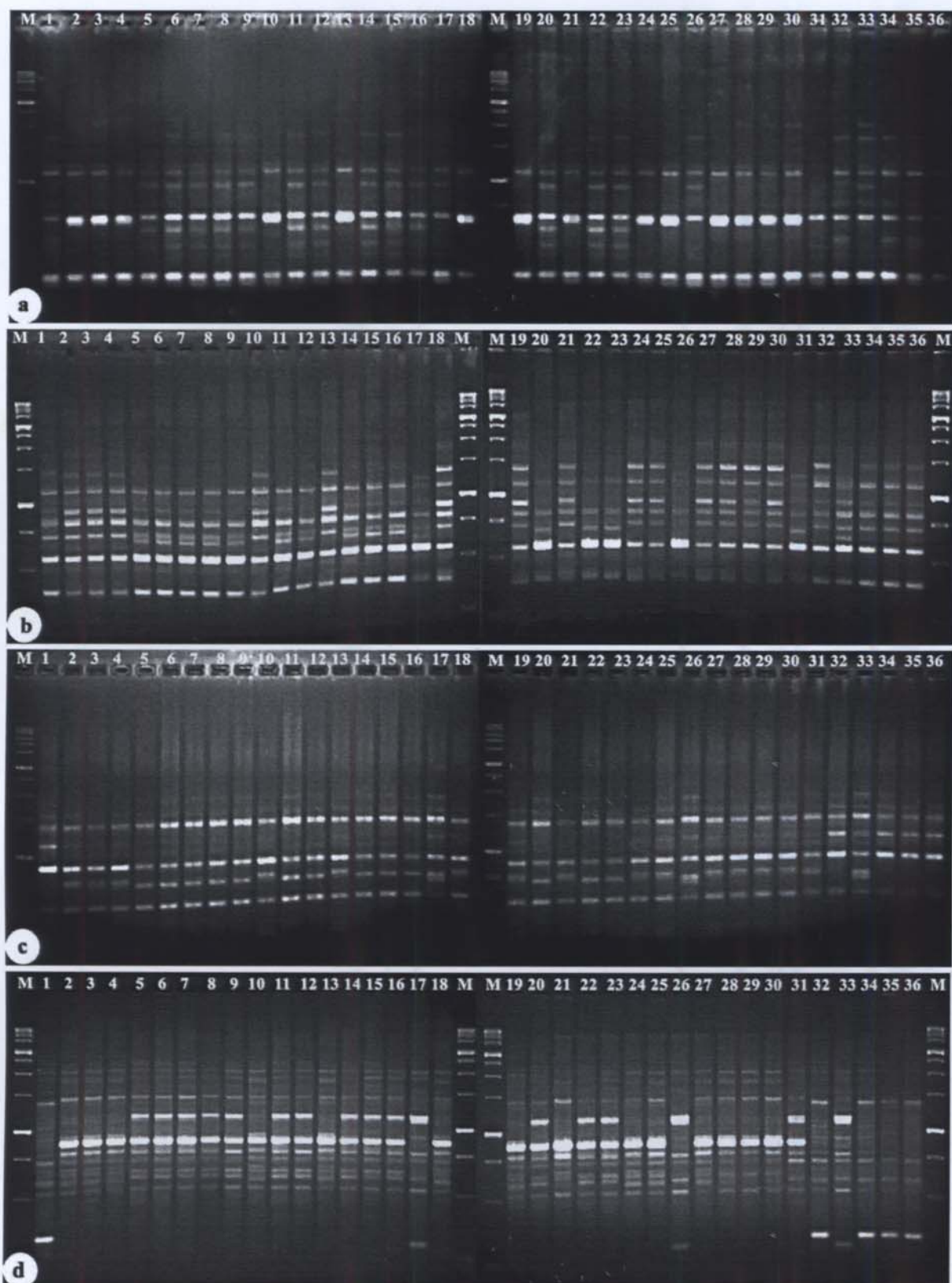


Fig. 43. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPA 12, OPA 14, OPA 15 and OPA 17. Lane 1-36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aiswal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA)).

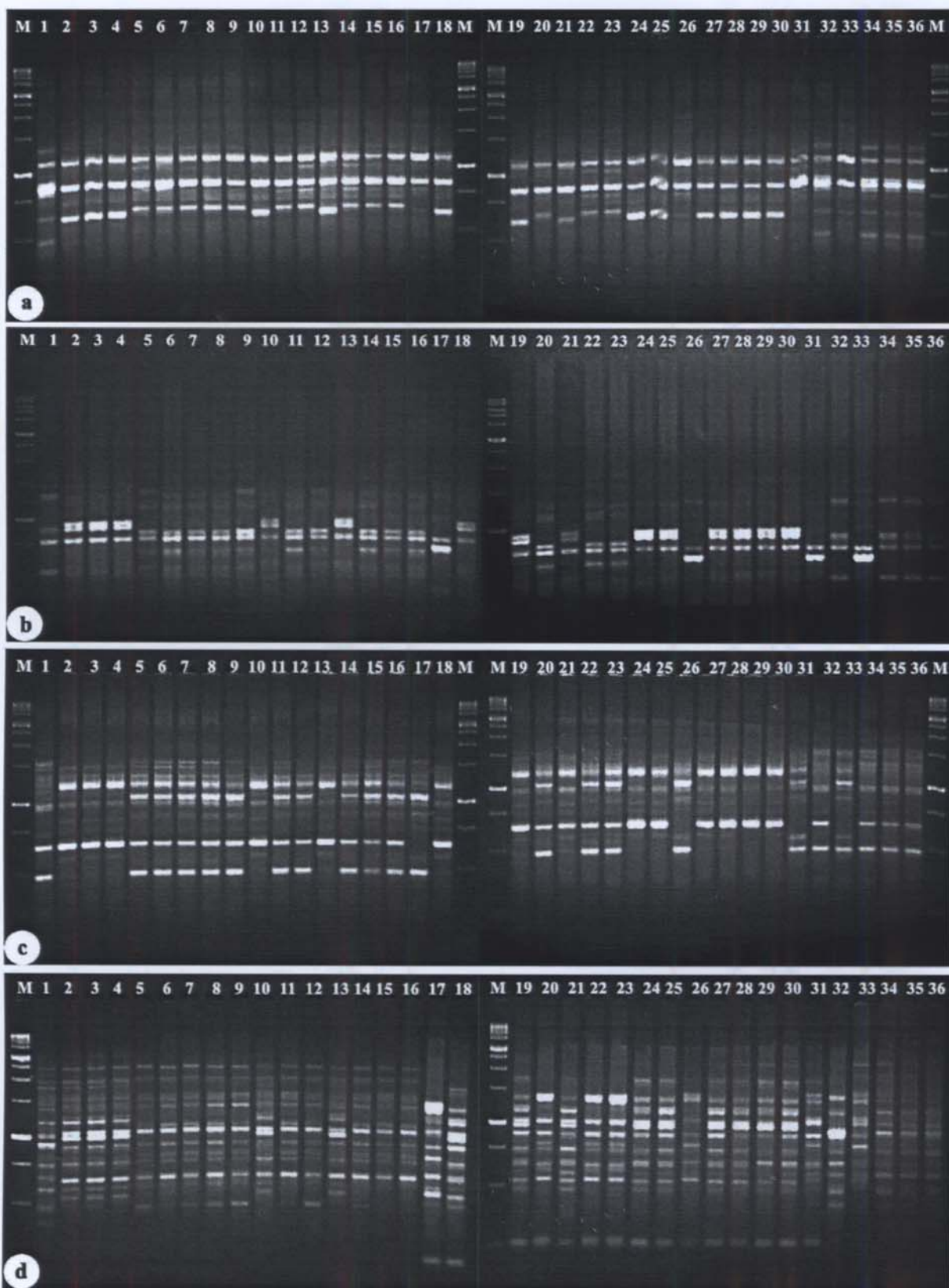


Fig. 44. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPA 18, OPA 19, OPB 07 and OPB 08. Lane 1-36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aiswal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Pratihbha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

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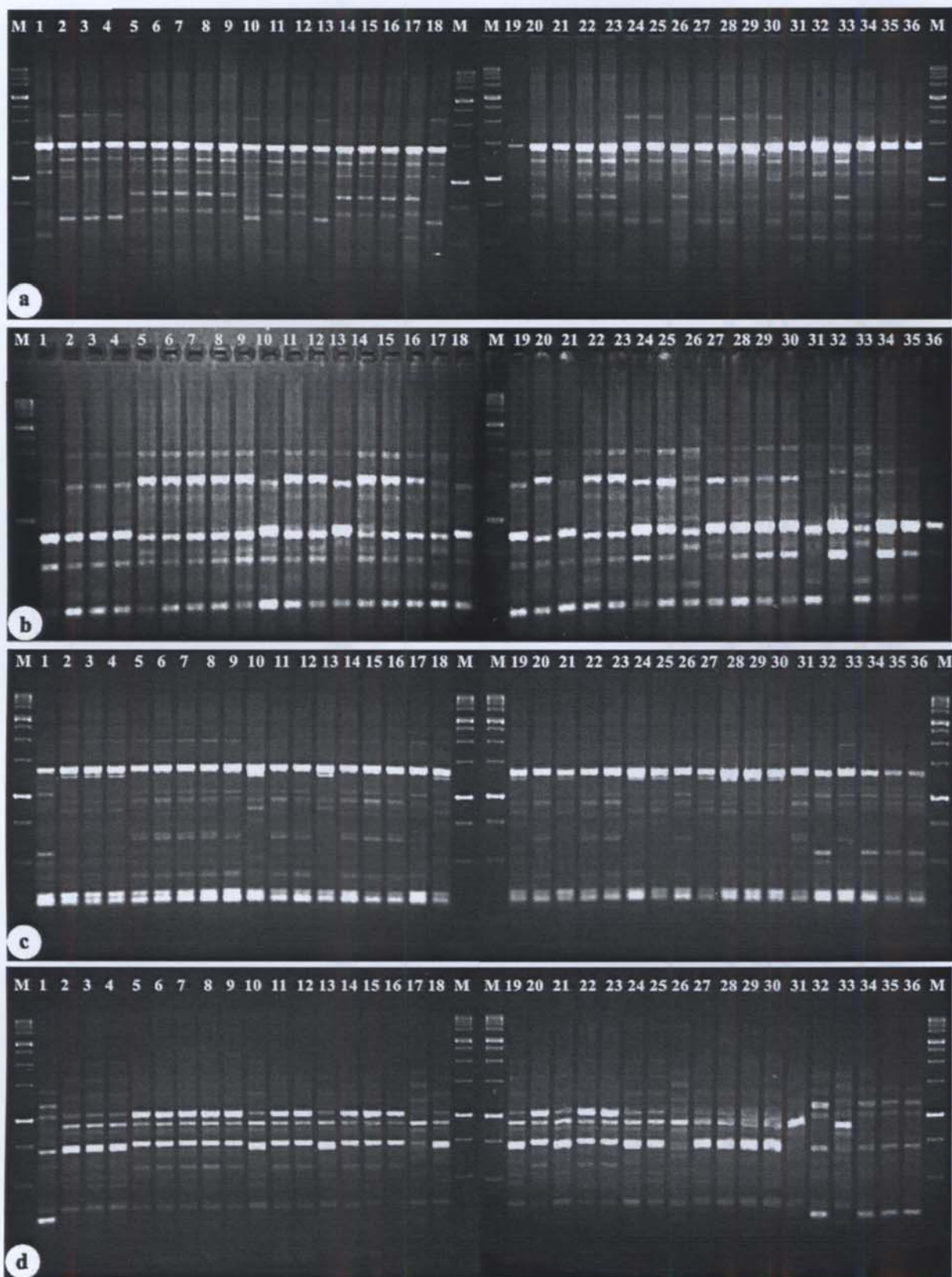


Fig. 45. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPB 09, OPB 10, OPB 11 and OPB 12. Lane 1-36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aiswal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

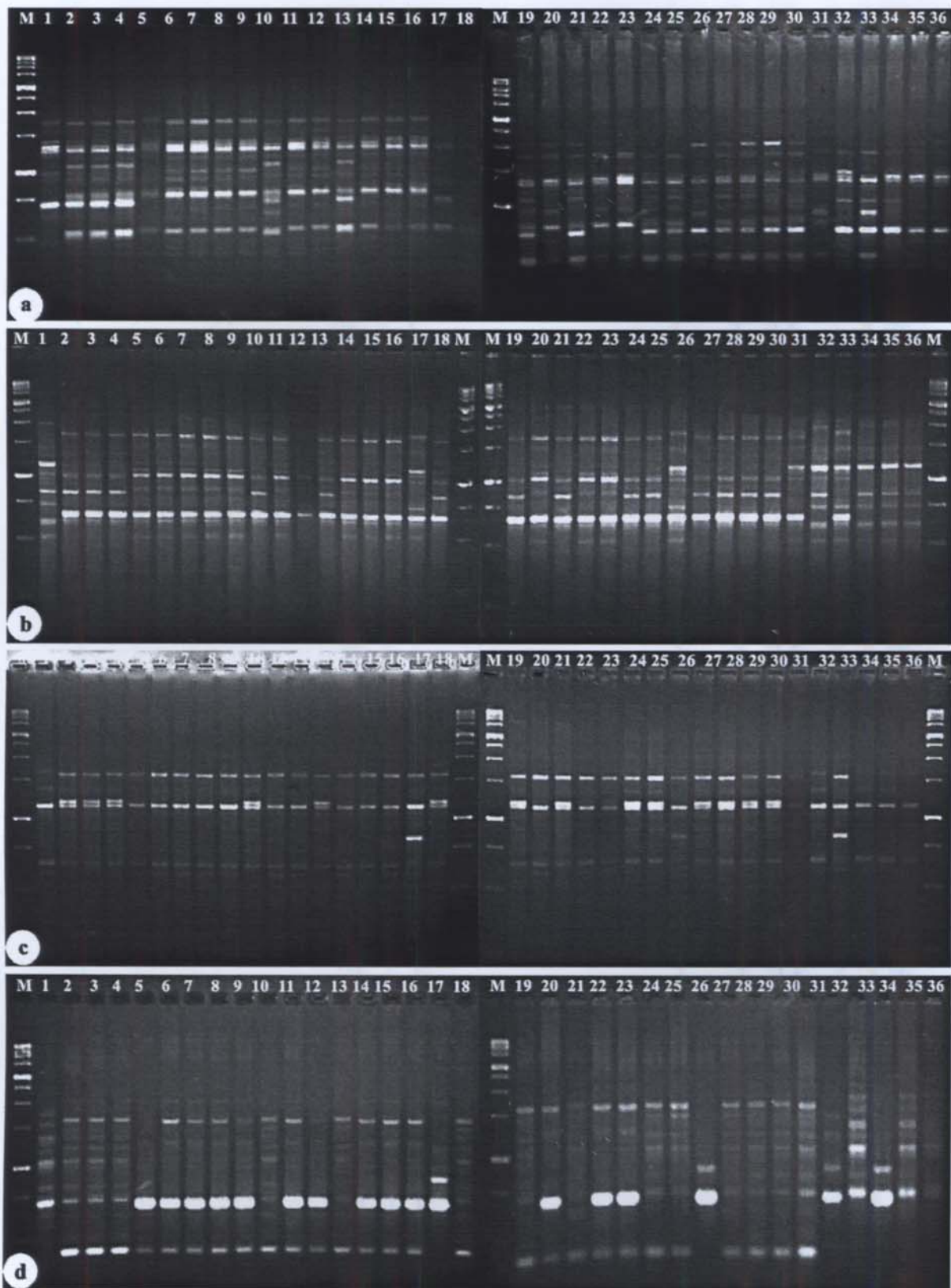


Fig. 46. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPB 16, OPB 18, OPB 19 and OPC 07. Lane 1-36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aiswal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

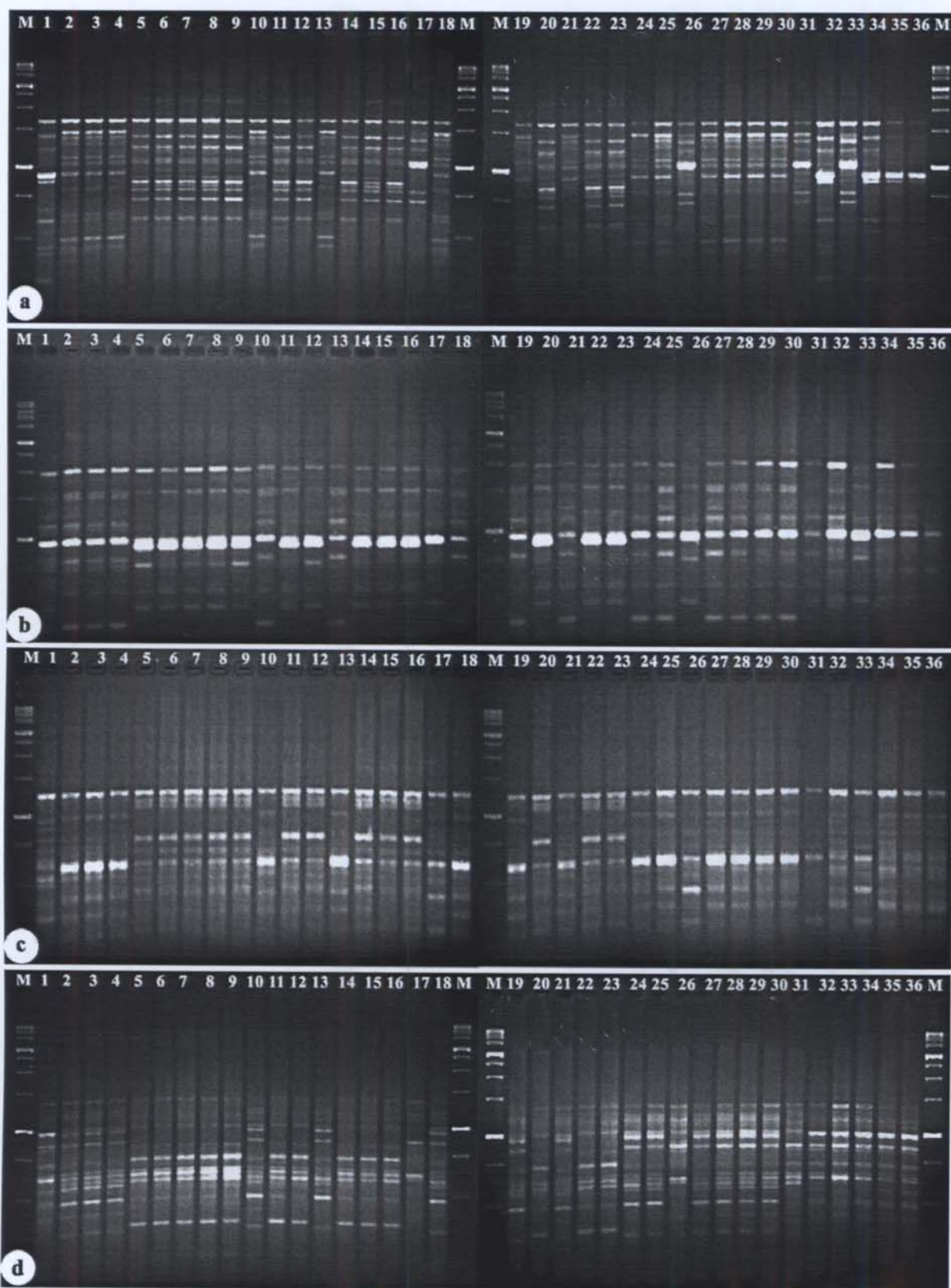


Fig. 47. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPC 08, OPC 09, OPC 10 and OPC 11. Lane 1 - 36 are 1) 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aizwal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Sunrem', respectively. M- Marker (1 Kb ladder (Biogene, USA).

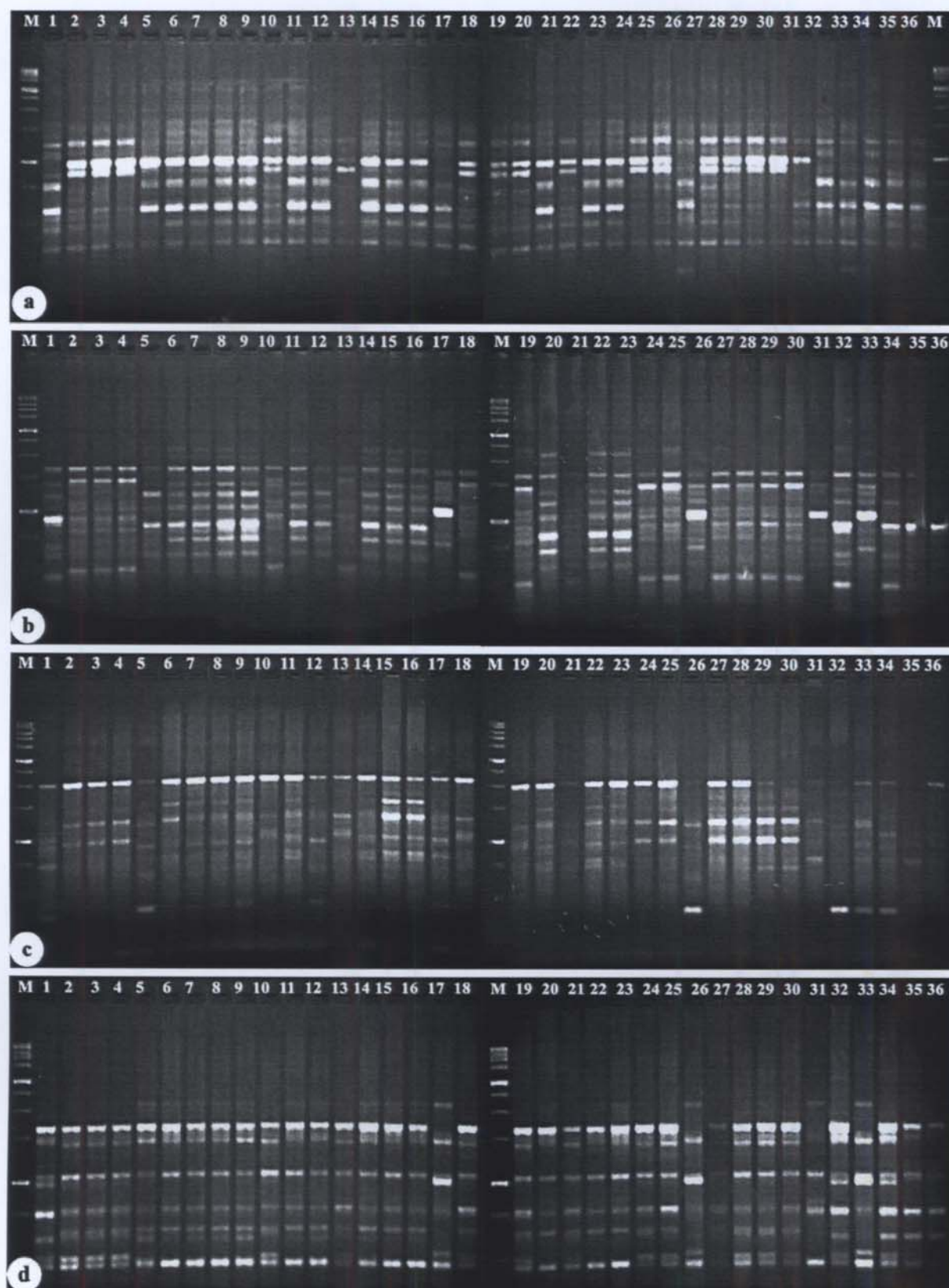


Fig. 48. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPC 12, OPC 14, OPC 18 and OPD 01. Lane 1 - 36 are 1) 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aizwal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

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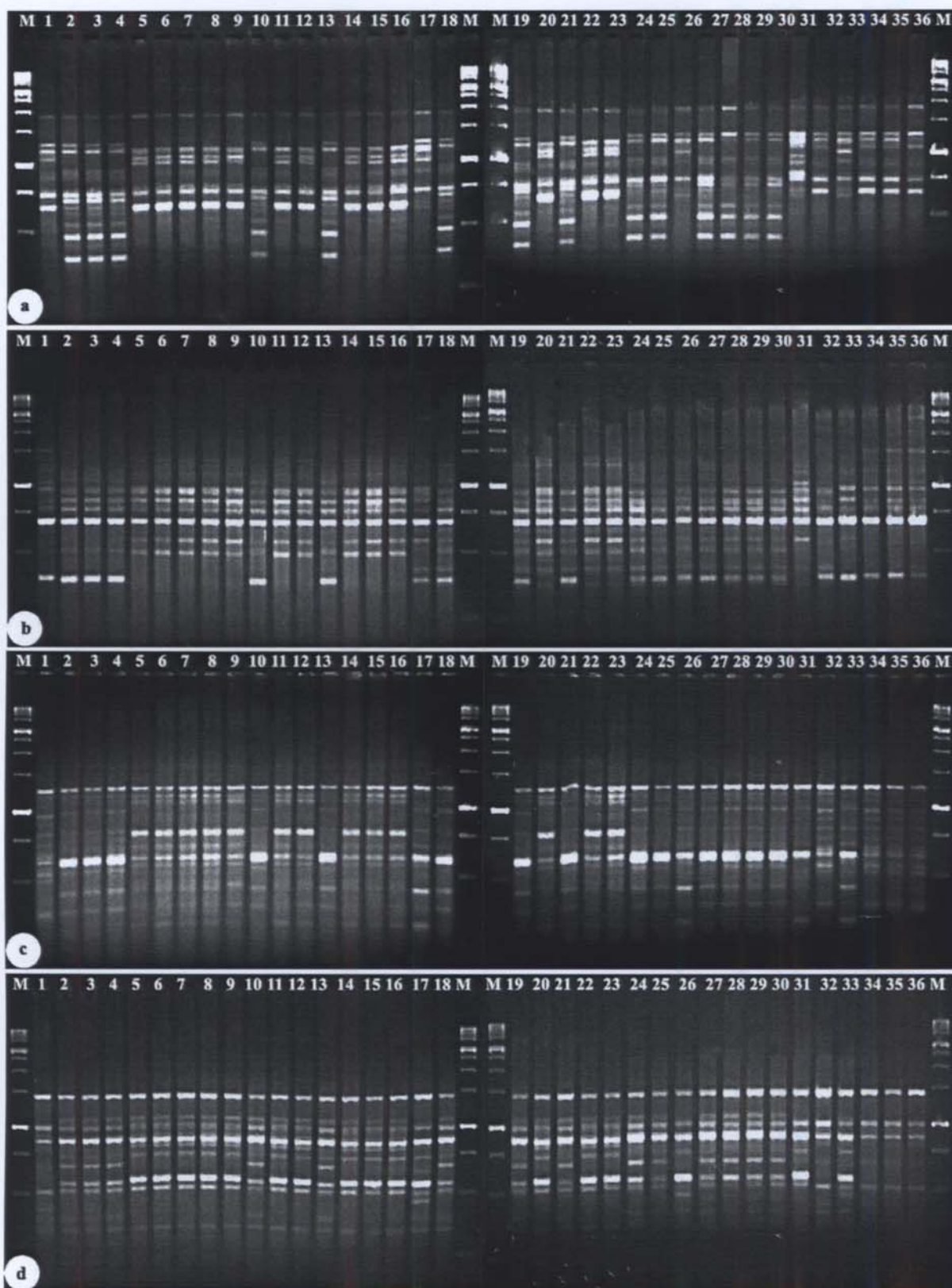


Fig. 49. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPD 04, OPD 05, OPD 07 and OPD 08. Lane 1 - 36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aizwal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA)).

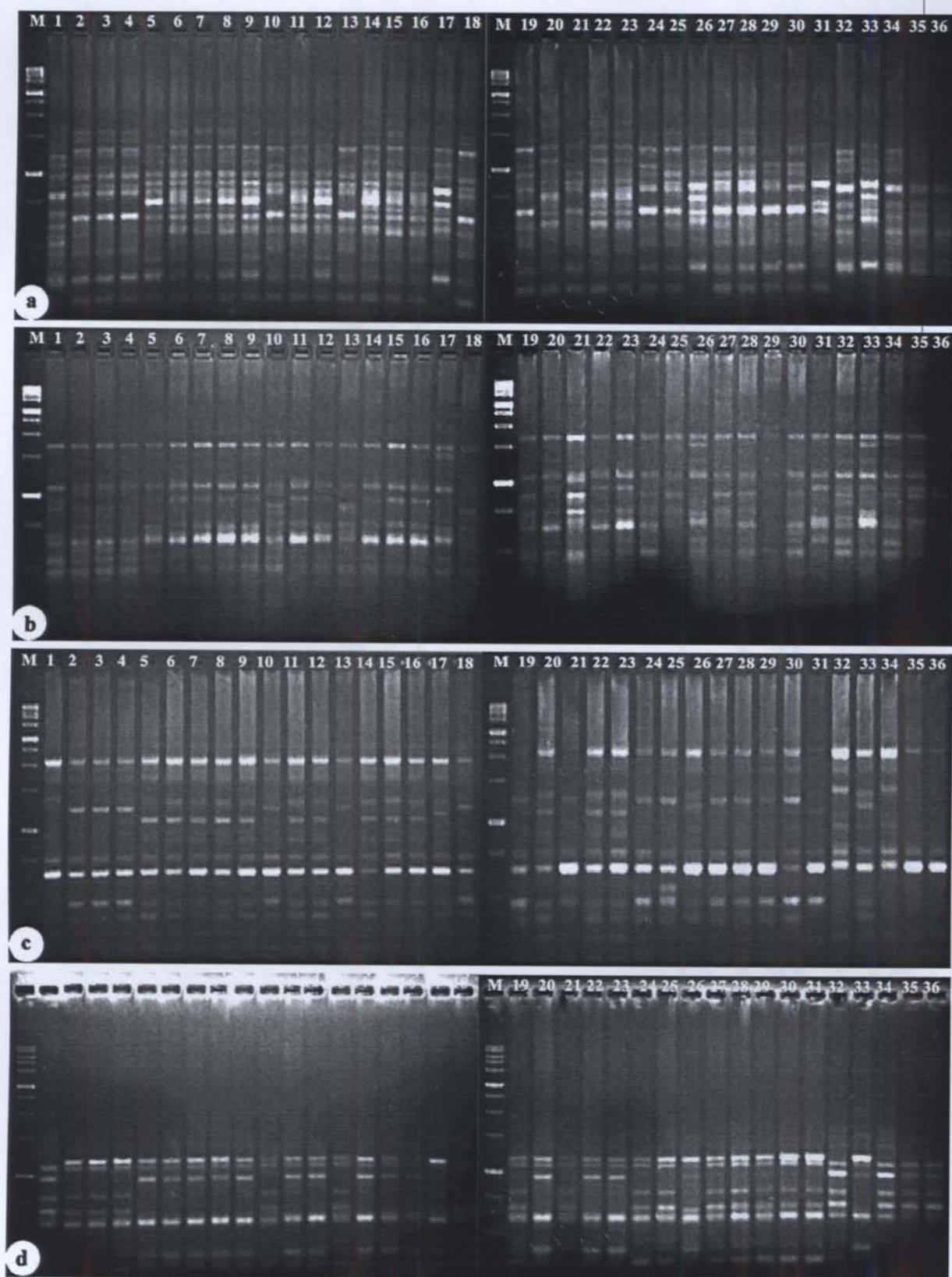


Fig. 50. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPC 08, OPC 09, OPC 10 and OPC 11. Lane 1 - 36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aizwal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvarna', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Prathibha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

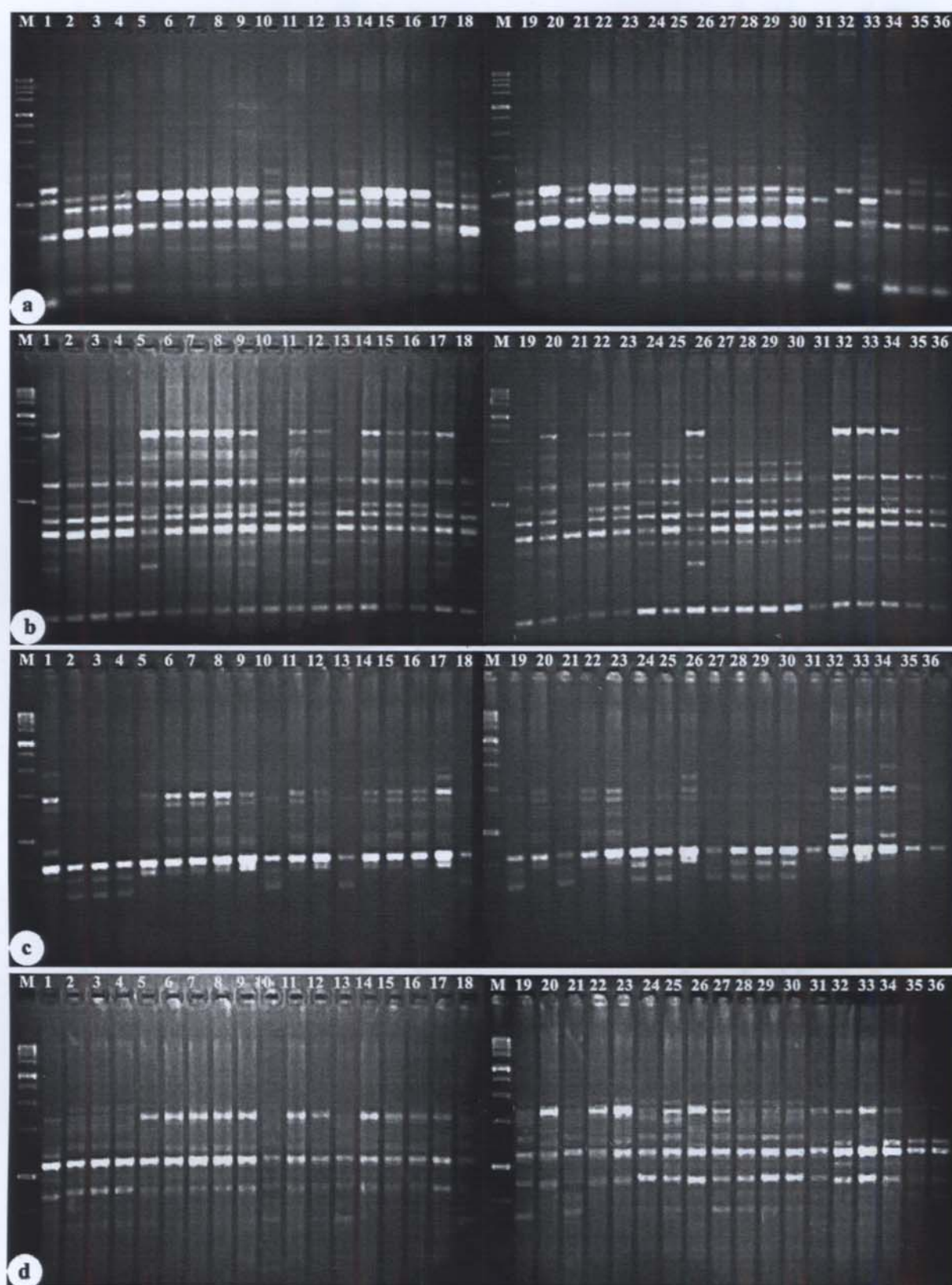


Fig. 51. a - d are RAPD profiles of 36 turemeric varieties/cultivars using primers OPJ 09, OPJ 10, OPJ 17 and OPJ 18. Lane 1 - 36 are 1). 'Alleppey', 2) 'Amalapuram', 3) 'Amrithapani', 4) 'Armoor', 5) 'Jabedi', 6) 'Dhagi', 7) 'Katergia', 8) 'Chayapasupu', 9) 'Avanigadda', 10) 'Dindigam', 11) 'Ethamkula', 12) 'GL Puram', 13) 'Karhadi local', 14) 'Kasturi', 15) 'Kodur', 16) 'Kuchipudi', 17) 'Mamomthody', 18) 'Muvatupuzha', 19) 'Nandyal type', 20) 'Rajapuri', 21) 'Sugandham', 22) 'Vondimitta', 23) 'Wynad local', 24) 'Aizwal', 25) 'Ayur', 26) 'Basar Along', 27) 'Gaspani', 28) 'Hajo', 29) 'Jorhat', 30) 'Suvana', 31) 'Sudarsana', 32) 'Kedaram', 33) 'Suguna', 34) 'Pratihbha', 35) 'Prabha' and 36) 'Alleppey Supreme', respectively. M- Marker (1 Kb ladder (Biogene, USA).

4.2.7. 1. Cluster analysis

Similarity coefficients were compared among the 36 cultivars/varieties of turmeric obtained from RAPD markers. In all the three similarity matrices the cultivar 'Armoor' and 'Amrithapani' showed maximum similarity between them (1.00); these two cultivars were unable to be separated by the 41 random decamer primers studied. The similarity matrix obtained using Jaccard's similarity coefficients showed the least similarity between cultivars 'Basar Along' and 'GL Puram' (0.43) while the similarity matrices obtained using Sorensen – Dice and Simple Matching showed the least similarity between cultivars 'Basar Along' and 'Dindigam' (0.60 and 0.53 respectively). Similarity matrix obtained from RAPD data using three similarity coefficients are given in Tables 52 – 54.

Table 52. Similarity matrix obtained from RAPD data using Jaccard's similarity coefficients in turmeric varieties/cultivars

Row/Cult	Alleppey	Anasapuzha	Anantapuzha	Armoor	Johadi	Dhaji	Kataga	Chayapa	Aravind	Dandagan	Ethamkulam	Glynnam	Kathadi	Katturi	Kodur	Kuchipudi	Mammoth	Morappuzha	Nandyl	Rajapur	Seppanadu	Vendim	Wynad	Aizawl	Ayur	Banashankar	Gurupani	Hijo	Jorhat	Servana	Solanam	Kodanam	Supna	Prakha	Prathib	Alleppey	
Alleppey	1.000000																																				
Anasapuzha	0.5205128	1.000000																																			
Anantapuzha	0.5179487	0.9967213	1.000000																																		
Armoor	0.5179487	0.9967213	1.000000	1.000000																																	
Johadi	0.4654731	0.4750000	0.4761905	0.4761905	1.000000																																
Dhaji	0.4747475	0.4913151	0.4888337	0.4888337	0.9174917	1.000000																															
Kataga	0.4823232	0.4876847	0.4852217	0.4852217	0.9210526	0.989666	1.000000																														
Chayapa	0.4836272	0.4926108	0.4901478	0.4901478	0.9132327	0.9833887	0.9967550	1.000000																													
Aravind	0.4688279	0.4889435	0.4864865	0.4864865	0.9150327	0.9573770	0.9607843	0.9675203	1.000000																												
Dandagan	0.5101523	0.8830769	0.8800000	0.8800000	0.4474328	0.4635922	0.4602410	0.4650602	0.4615385	1.000000																											
Ethamkulam	0.4835443	0.4925743	0.4900990	0.4900990	0.8626198	0.9285714	0.9530388	0.9198718	0.8955696	0.4613527	1.000000																										
Glynnam	0.4860759	0.4803922	0.4779412	0.4779412	0.9210526	0.9070513	0.9166667	0.9108280	0.9169329	0.4567308	0.9196141	1.000000																									
Kathadi	0.4910941	0.4949693	0.4946628	0.4946628	0.4538653	0.4630542	0.4632353	0.4681373	0.4645477	0.9025157	0.4679803	0.4632353	1.000000																								
Katturi	0.4836272	0.4962963	0.4938272	0.4938272	0.8662420	0.9134615	0.9169329	0.9050633	0.8871473	0.4650602	0.9575163	0.9169329	0.4717445	1.000000																							
Kodur	0.4748744	0.4950495	0.4925743	0.4925743	0.8598726	0.9318182	0.9290323	0.9280769	0.8987342	0.4744526	0.9530388	0.8864353	0.4813896	0.9480519	1.000000																						
Kuchipudi	0.4846939	0.4937656	0.4912718	0.4912718	0.8617363	0.9342652	0.9315961	0.9255663	0.9009585	0.4694377	0.9473684	0.9067524	0.4763092	0.9444444	0.9635762	1.000000																					
Mammoth	0.5248042	0.4766585	0.4742015	0.4742015	0.5618280	0.5702918	0.5820493	0.5835544	0.5877660	0.4671533	0.5840000	0.5951743	0.4666667	0.5928000	0.5782493	0.5860215	1.000000																				
Morappuzha	0.5126904	0.4863855	0.4843725	0.4843725	0.4641975	0.4731707	0.4733010	0.4781553	0.4745763	0.8524096	0.4780488	0.4697337	0.8646154	0.4710145	0.4941076	0.4827586	0.4950495	1.000000																			
Nandyl	0.4987469	0.4864668	0.4849376	0.4849376	0.4691258	0.4780488	0.4781553	0.4830097	0.4794189	0.8473054	0.4829268	0.4639423	0.8424242	0.4794189	0.4853659	0.4803922	0.4889435	0.9074074	1.000000																		
Rajapur	0.4328338	0.4788300	0.4800000	0.4800000	0.7906230	0.8364780	0.8401254	0.8291925	0.8291925	0.4512195	0.8603175	0.8286604	0.4696717	0.8390442	0.8401254	0.8394249	0.5741240	0.4679803	0.4950000	1.000000																	
Seppanadu	0.4652956	0.7841945	0.7811530	0.7811530	0.4651163	0.4744898	0.4746193	0.4796954	0.4796954	0.8067485	0.4646465	0.4634761	0.7901225	0.4648241	0.4647193	0.4693878	0.4744898	0.8098160	0.8328172	0.4652956	1.000000																
Vendim	0.4430693	0.4814815	0.4826733	0.4826733	0.8012422	0.8526646	0.8562500	0.8452012	0.8452012	0.4506024	0.8763823	0.8333333	0.4678218	0.8683386	0.8562500	0.8390442	0.5760000	0.4707317	0.5012407	0.9433333	0.4644670	1.000000															
Wynad	0.4579208	0.4852941	0.4828431	0.4828431	0.8142415	0.8656250	0.8691589	0.8380247	0.8380247	0.8203336	0.8777429	0.8518519	0.4717445	0.8695652	0.8691589	0.8710692	0.5920000	0.4781553	0.4902439	0.9183668	0.4722222	0.9477124	1.000000														
Aizawl	0.4961637	0.8297872	0.8323171	0.8323171	0.4686970	0.4751244	0.4752475	0.4801980	0.4836710	0.8247734	0.4727723	0.4789082	0.7973831	0.4729064	0.4643733	0.4738155	0.5012658	0.8113772	0.8065476	0.4735516	0.8099750	0.4726368	0.4912718	1.000000													
Ayur	0.4836272	0.8475610	0.8501529	0.8501529	0.4469136	0.4490291	0.4492754	0.4541063	0.4576271	0.8393272	0.4574209	0.4692754	0.8374223	0.4541063	0.4527845	0.4512195	0.4850746	0.8398792	0.8628049	0.4651741	0.8218750	0.4679803	0.4647202	0.8864353	1.000000												
Banashankar	0.4869792	0.4518519	0.4529703	0.4529703	0.5690608	0.5564516	0.5635836	0.5783784	0.4320388	0.5618280	0.5772358	0.4275184	0.5698925	0.5561497	0.5594395	0.7706422	0.4520885	0.4714640	0.5861111	0.4413265	0.5835616	0.5826558	0.4572864	0.4600000	1.000000												
Gurupani	0.4858612	0.8251534	0.8276923	0.8276923	0.4329152	0.4434398	0.4436275	0.4485294	0.4449878	0.8256881	0.4447174	0.4330900	0.8148148	0.4414634	0.4507389	0.4455446	0.4760705	0.4657940	0.8575851	0.4670051	0.8216561	0.4588529	0.4628713	0.8117284	0.8468750	0.4435249	1.000000										
Hijo	0.4967406	0.8018624	0.8628049	0.8628049	0.4581281	0.4633922	0.4637681	0.4685990	0.4721550	0.8549849	0.4613527	0.4567308	0.8333333	0.4580936	0.4602410	0.4552038	0.4852217	0.8524096	0.8610976	0.4800995	0.8463930	0.4718826	0.4793187	0.8641975	0.8881988	0.4640199	0.9196141	1.000000									
Jorhat	0.5022510	0.8389587	0.8538282	0.8538282	0.470705	0.4813896	0.4814815	0.4864198	0.4900990	0.8313253	0.4681373	0.4703882	0.8096677	0.4682927	0.4703882	0.4691358	0.4830746	0.4834373	0.8459215	0.4798995	0.8105590	0.4718049	0.4827386	0.8629283	0.8637771	0.4710327	0.8584906	0.9363057	1.000000								
Servana	0.5076531	0.8393272	0.8562691	0.8562691	0.4773869	0.4826733	0.4827386	0.4876847	0.4913580	0.8263473	0.4730292	0.4718826	0.7994012	0.4697864	0.4754902	0.4740741	0.4790123	0.8293413	0.8352393	0.4738155	0.7889908	0.4765432	0.4840295	0.8573851	0.8527607	0.4723618	0.8359133	0.9122257	0.9483871	1.000000							
Solanam	0.4465241	0.4645669	0.4657895	0.4657895	0.5639535	0.5525408	0.5593220	0.5605634	0.5649718	0.4698163	0.5564972	0.5593220	0.4772118	0.5605634	0.5549296	0.5628571	0.6003831	0.4880637	0.4933687	0.5590778	0.4902307	0.5657143	0.5649718	0.4824798	0.4932615	0.6291793	0.4838066	0.4775726	0.4852547	0.4787234	1.000000						
Kodanam	0.7906230	0.4786967	0.4761905	0.4761905	0.4805195	0.4859335	0.4936061	0.4948980	0.4910941	0.4800000	0.4910486	0.4936061	0.4611529	0.4910941	0.4822335	0.4961240	0.5249344	0.4788030	0.4800995	0.4580153	0.4804178	0.4536341	0.4722618	0.4884910	0.4722618	0.4947368	0.4743590	0.5025381	0.5064267	0.5193798	0.4540541	1.000000					
Kodanam	0.7906230	0.4786967	0.4761905	0.4761905	0.4805195	0.4859335	0.4936061	0.4948980	0.4910941	0.4800000	0.4910486	0.4936061	0.4611529	0.4910941	0.4822335	0.4961240	0.5249344	0.4788030	0.4800995	0.4580153	0.4804178	0.4536341	0.4722618	0.4884910	0.4722618	0.4947368	0.4743590	0.5025381	0.5064267	0.5193798	0.4540541	1.000000					
Supna	0.5255602	0.4645477	0.4656863	0.4656863	0.5906593	0.5817604	0.5898123	0.5903091	0.6037736	0.4447115	0.5871314	0.5940880	0.4439024	0.5951743	0.5855615	0.5849057	0.7611940	0.4611630	0.4660194	0.5803217	0.4436080	0.5664894	0.5740741	0.4701493	0.4691358	0.7379518	0.4455446	0.4587379	0.4691358	0.4704453	0.6377246	0.5277845	1.000000				
Prakha	0.7988254	0.4648241	0.4659950	0.4659950	0.4776903	0.4753784	0.4832905	0.4846154	0.4808184	0.4699492	0.4845361	0.4871134	0.4382278	0.4808184	0.4794872	0.4857143	0.5105283	0.4797980	0.4736842	0.4438776	0.4659886	0.4386985	0.4547729	0.4666367	0.4584383	0.4724409	0.4713026	0.4810127	0.4732824	0.4821429	0.4547945	0.8892617	0.5417790	1.000000			
Prathib	0.7435897	0.4438763	0.4470284	0.4470284	0.4582210	0.4603175	0.4682540	0.4619423	0.4581152	0.4661438	0.4734043	0.4682540																									

Table 53. Similarity matrix obtained from RAPD data using Sorensen - Dice similarity coefficients in turmeric varieties/cultivars

Row\Col	Alleppey	Anamizh	Anantyan	Armoor	Johdi	Dhaji	Katanga	Chayapa	Aravangal	Dandapan	Ethambal	GLysam	Kachadi	Karim	Kode	Kuchel	Mannathol	Miriyapp	Mudyal	Rappu	Sagundha	Vandam	Wynad	Aimal	Ayer	Banankon	Gurpani	Hijo	Jochat	Sorana	Solan	Kodaran	Sugna	Prakha	Prathib	Alleppey	
Alleppey	1.000000																																				
Anamizh	0.684543	1.000000																																			
Anantyan	0.6824324	0.998380	1.000000																																		
Armoor	0.6824324	0.998380	1.000000	1.000000																																	
Johdi	0.6352531	0.6440678	0.6451613	0.6451613	1.000000																																
Dhaji	0.6438336	0.6389018	0.6366667	0.6366667	0.9569707	1.000000																															
Katanga	0.6307666	0.6356291	0.6339997	0.6339997	0.938041	0.9949580	1.000000																														
Chayapa	0.6319525	0.6600660	0.6378512	0.6378512	0.9556314	0.9916248	0.9933333	1.000000																													
Aravangal	0.6383701	0.6367637	0.6345455	0.6345455	0.9556314	0.9782245	0.9800000	0.9833887	1.000000																												
Dandapan	0.6756303	0.9379085	0.9361702	0.9361702	0.6182432	0.6334992	0.6300630	0.6348684	0.6313789	1.000000																											
Ethambal	0.6318771	0.6600332	0.6378073	0.6378073	0.9262436	0.9629630	0.9648241	0.9382638	0.9449082	0.6314030	1.000000																										
GLysam	0.6341738	0.6490066	0.6467662	0.6467662	0.9389041	0.9512605	0.9365217	0.9533333	0.9566667	0.6270627	0.9381240	1.000000																									
Kachadi	0.6387031	0.9187396	0.9189435	0.9189435	0.6243568	0.6329966	0.6331638	0.6377295	0.6343907	0.9487803	0.6373839	0.6331638	1.000000																								
Karim	0.6319525	0.6633663	0.6611570	0.6611570	0.9283276	0.9547739	0.9566667	0.9301661	0.9401993	0.6348684	0.9782972	0.9566667	0.6410684	1.000000																							
Kode	0.6439523	0.6622517	0.6600332	0.6600332	0.9246575	0.9647039	0.9632107	0.9600000	0.9466667	0.6435644	0.9648241	0.9387993	0.6499162	0.9733333	1.000000																						
Kuchel	0.6329210	0.6611018	0.6388629	0.6388629	0.9257340	0.9661017	0.9643888	0.9613445	0.9478992	0.6389351	0.9728730	0.9510961	0.6452703	0.9714286	0.9814503	1.000000																					
Mannathol	0.6883562	0.6453907	0.6433333	0.6433333	0.7194492	0.7263514	0.7327731	0.7370184	0.7403885	0.6368159	0.7373737	0.7462185	0.6363636	0.7437186	0.7327731	0.7389831	1.000000																				
Miriyapp	0.6778523	0.9188026	0.9130327	0.9130327	0.6340641	0.6423841	0.6423041	0.6489622	0.6436782	0.9203252	0.6488647	0.6392092	0.9272927	0.6403941	0.6323888	0.6311628	0.6422517	1.000000																			
Mudyal	0.6655518	0.9170732	0.9185668	0.9185668	0.6385555	0.6468647	0.6468622	0.6513912	0.6481178	0.9173420	0.6513158	0.6338239	0.9144737	0.6481178	0.6353304	0.6490066	0.6367637	0.9314563	1.000000																		
Rappu	0.6041667	0.6475548	0.6486486	0.6486486	0.8830716	0.9109389	0.9131175	0.9066214	0.9066214	0.6218487	0.9249147	0.9065032	0.6382253	0.9235993	0.9131175	0.9243986	0.7294521	0.6375839	0.6622074	1.000000																	
Sagundha	0.6350877	0.8790460	0.8771331	0.8771331	0.6349206	0.6433996	0.6437177	0.6483705	0.6483705	0.8903090	0.6344828	0.6333907	0.8827586	0.6346484	0.6437177	0.6388889	0.6433996	0.8949133	0.9087838	0.6350877	1.000000																
Vandam	0.6140652	0.6300000	0.6310851	0.6310851	0.8896532	0.9204738	0.9223589	0.9161074	0.9161074	0.6212625	0.9342327	0.9089099	0.6374368	0.9295382	0.9223589	0.9235993	0.7309645	0.6401327	0.6677686	0.9708405	0.6343154	1.000000															
Wynad	0.6281834	0.6334653	0.6312397	0.6312397	0.8976109	0.9279732	0.9300000	0.9233880	0.9233880	0.6282895	0.9348915	0.9200000	0.6410684	0.9302326	0.9300000	0.9310924	0.7437186	0.6468622	0.6379378	0.9575552	0.6413394	0.9731544	1.000000														
Aimal	0.6632479	0.9069767	0.9084839	0.9084839	0.6391753	0.6441821	0.6442953	0.6488294	0.6521739	0.9039735	0.6420168	0.6476510	0.8873950	0.6421405	0.6342282	0.6429780	0.6677909	0.8928678	0.8929160	0.6427350	0.8946439	0.6418919	0.6388629	1.000000													
Ayer	0.6319525	0.9174917	0.9190083	0.9190083	0.6177474	0.6197653	0.6200000	0.6243847	0.6279070	0.9243421	0.6277129	0.6200000	0.9115192	0.6243847	0.6233333	0.6218487	0.6332663	0.9129721	0.9263302	0.6349745	0.9022288	0.6373839	0.6345515	0.9397993	1.000000												
Banankon	0.6549912	0.6224490	0.6235094	0.6235094	0.7235321	0.7150239	0.7182131	0.7226027	0.7328767	0.6033898	0.7194492	0.7319388	0.989673	0.7260274	0.7147766	0.7173043	0.8704663	0.6267394	0.6400894	0.7395543	0.6122894	0.7370242	0.7363014	0.6273862	0.6301370	1.000000											
Gurpani	0.6339792	0.9042017	0.9057229	0.9057229	0.4052174	0.6143345	0.6144010	0.6192893	0.6159052	0.9045226	0.6156463	0.6044143	0.8979392	0.6125212	0.6123922	0.6164384	0.6430512	0.9103880	0.9233333	0.6366782	0.9022979	0.6280398	0.6328237	0.8960818	0.9170897	0.6143106	1.000000										
Hijo	0.6655462	0.9240366	0.9263502	0.9263502	0.6283784	0.6234992	0.6336634	0.6381579	0.6414474	0.9218241	0.6314050	0.6270627	0.9089099	0.6282895	0.6336330	0.6256400	0.6333997	0.9203252	0.9367908	0.6487395	0.9168081	0.6411980	0.6480263	0.9271523	0.9407895	0.6338983	0.9381240	1.000000									
Jochat	0.6689304	0.9240824	0.9223140	0.9223140	0.6450512	0.64499162	0.6300000	0.6344830	0.6378073	0.9078947	0.6377295	0.6400000	0.8948247	0.6378738	0.6400000	0.6396555	0.6332663	0.9089880	0.9165303	0.6485569	0.8953688	0.6409396	0.6311628	0.9264214	0.9269103	0.6404110	0.9228579	0.9671053	1.000000								
Sorana	0.6734349	0.9243421	0.9227500	0.9227500	0.6462385	0.6310851	0.6511628	0.6356291	0.6389404	0.9049180	0.6246259	0.6411960	0.8881591	0.6390728	0.6451813	0.6432161	0.6477462	0.9067103	0.9102773	0.6429780	0.8820513	0.6454849	0.6523179	0.9223333	0.9205298	0.6416382	0.9106239	0.9548084	0.9735899	1.000000							
Solan	0.6173752	0.6344086	0.6353476	0.6353476	0.721896	0.7140255	0.7173913	0.7184116	0.7228217	0.6392837	0.7150635	0.7173913	0.6460980	0.7184116	0.7137881	0.7202925	0.7304554	0.6359715	0.6877460	0.7171904	0.6794399	0.7226277	0.7220217	0.6389091	0.6606498	0.7723881	0.6319237	0.6464286	0.6334296	0.6474820	1.000000						
Kodaran	0.8830716	0.6474576	0.6451613	0.6451613	0.6491228	0.6340448	0.6609389	0.6621160	0.6387031	0.6486486	0.6386621	0.6609389	0.6312178	0.6387031	0.6308489	0.6632124	0.6884882	0.6475548	0.6487395	0.6282723	0.6490300	0.6241379	0.6416382	0.6563574	0.6416382	0.6619718	0.6434783	0.6689189	0.6722549	0.6836735	0.6245353	1.000000					
Kodaran	0.8830716	0.6474576	0.6451613	0.6451613	0.6491228	0.6340448	0.6609389	0.6621160	0.6387031	0.6486486	0.6386621	0.6609389	0.6312178	0.6387031	0.6308489	0.6632124	0.6884882	0.6475548	0.6487395	0.6282723	0.6490300	0.6241379	0.6416382	0.6563574	0.6416382	0.6619718	0.6434783	0.6689189	0.6722549	0.6836735	0.6245353	1.000000					
Sugna	0.8872852	0.6349307	0.6354515	0.6354515	0.7426398	0.7353932	0.7419899	0.7428571	0.7529412	0.6156406	0.7398469	0.7436206	0.6148649	0.7462185	0.7386172	0.7380953	0.8644088	0.6312292	0.6357616	0.7182131	0.6143833	0.7232598	0.7294118	0.6393939	0.6386555	0.8492201	0.6164384	0.6289517	0.6386555	0.6398660	0.7787934	0.6908463	1.000000				
Prakha	0.8869258	0.6346484	0.6357388	0.6357388	0.6463364	0.6445993	0.6314644	0.6328497	0.6493955	0.6393162	0.6527778	0.6551127	0.6284722	0.6493955	0.6481802	0.6330462	0.6759382	0.6484420	0.6428571	0.6149410	0.6357143	0.6108202	0.6252159	0.6365217	0.6286701	0.6417112	0.6408451	0.6495726	0.6424870	0.6350824	0.6252354	0.9413854	0.7027972	1.000000			
Prathib	0.8529412	0.6167538	0.6178571	0.6178571	0.6284638	0.6304348	0.6378378	0.6319569	0.6283662	0.6328792	0.6425993	0.6378078	0.6245487																								

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The UPGMA dendrogram constructed using the genetic similarity obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on RAPD are given in Figs. 52 – 54. In all the three dendrograms, the clustering pattern was similar and produced four different groups by splitting at coefficients 0.60, 0.75 and 0.70, respectively. The different members in the groups are

- Group I - 'Alleppey', 'Kedaram', 'Prabha', 'Prathibha', and 'Alleppey' Supreme
- Group II - 'Jabedi', 'GL Puram', 'Dhagi', 'Katergia', 'Chayapasupu', 'Avanigadda', 'Ethamkula', 'Kasturi', 'Kodur', 'Kuchipudi', 'Rajapuri', 'Vondimita' and 'Wynad local'
- Group III - 'Mamomthody', 'Basar Along', 'Suguna' and 'Sudarsana'
- Group IV - 'Amalapuram', 'Amrithapani', 'Armoor', 'Dindigam', 'Karhadi local', 'Muvatupuzha', 'Nandyal', 'Aizwal', 'Ayur', 'Gaspani', 'Hajo', 'Jorhat', 'Suvarna' and 'Sugandham',

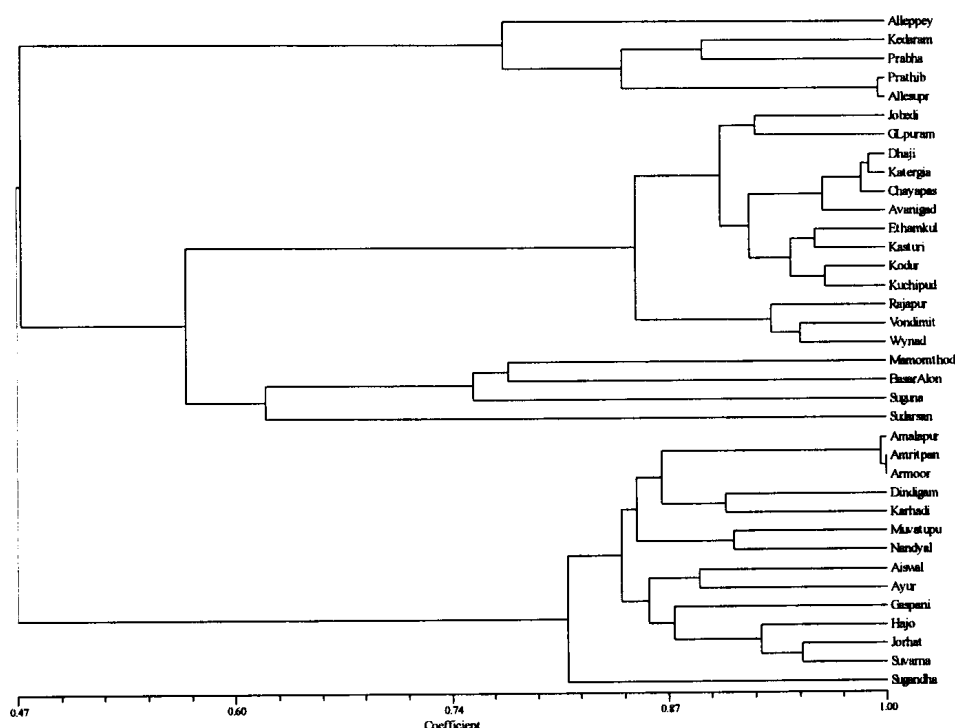


Fig. 52. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD markers using Jaccard's similarity coefficients.

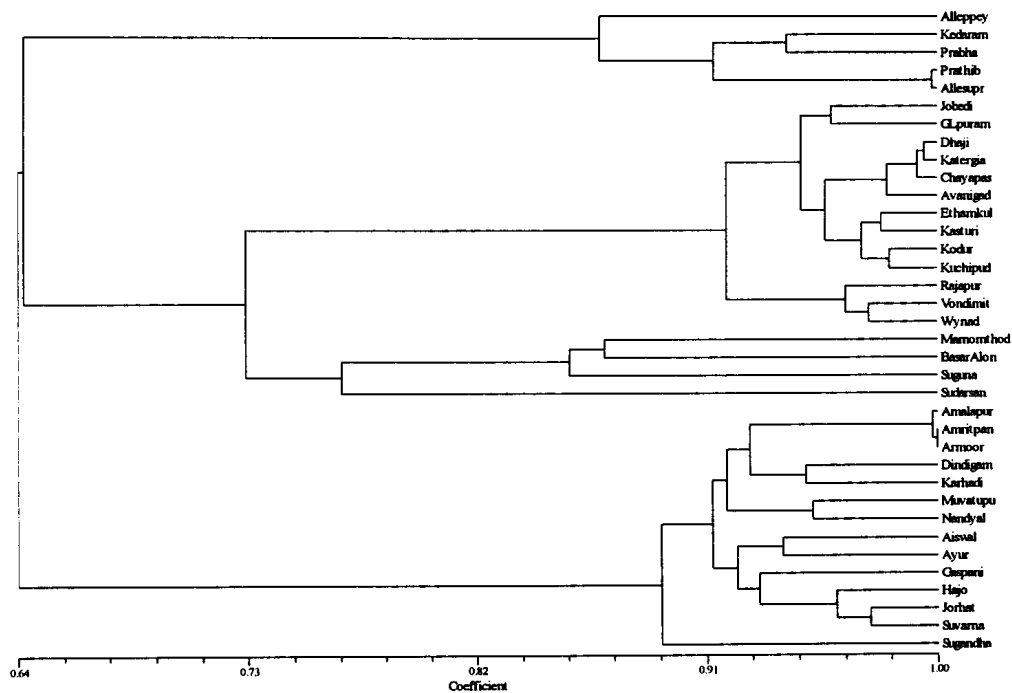


Fig. 53. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD markers using Sorensen - Dice similarity coefficients.

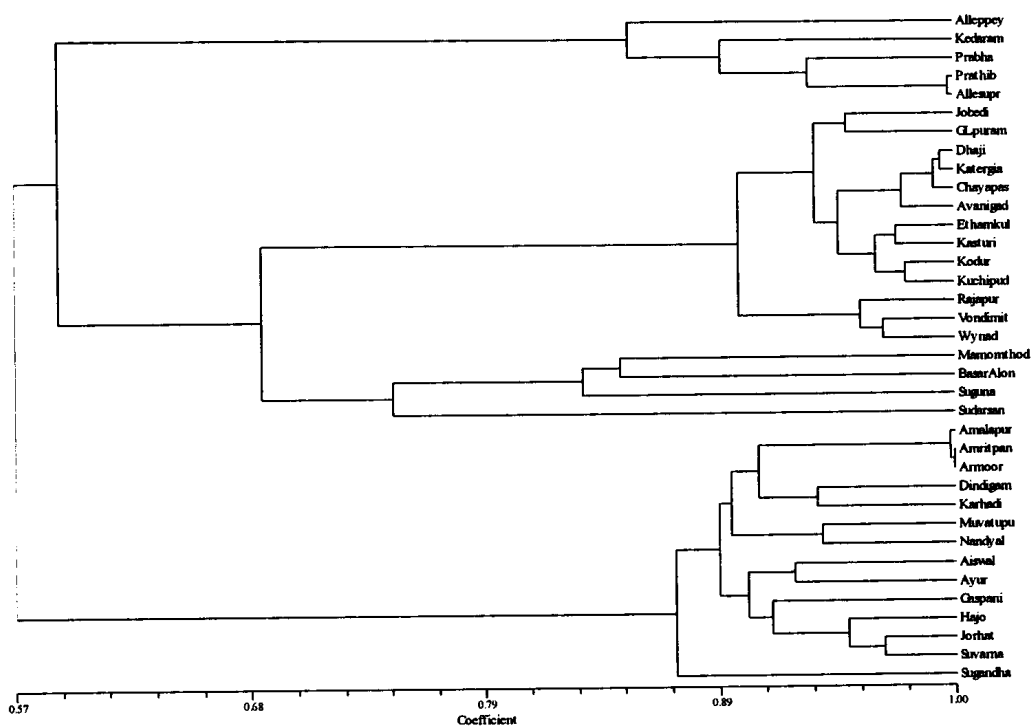


Fig. 54. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD markers using Simple Matching similarity coefficients.

Eventhough, the different similarity coefficients produced a similar clustering pattern in the turmeric varieties/cultivars studied, the degree of similarity of the accessions in each group varied in the dendrograms. The different groups in

the dendrograms produced using Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients from RAPD marker data and the highest and least similarities in each group are given in Table 55.

Table 55. Highest and lowest similarities between the varieties and cultivars in the different groups of the dendrogram produced by three similarity coefficients using RAPD marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	'Prathibha' & 'Alleppey' Supreme (0.88)	'Prathibha' & 'Alleppey' Supreme (0.99)	'Prathibha' & 'Alleppey' Supreme (0.99)
	Lowest	'Alleppey' & 'Alleppey Supreme' (0.74)	'Alleppey' & 'Alleppey Supreme' (0.85)	'Alleppey' & 'Alleppey Supreme' (0.84)
II	Highest	'Dhagi' & 'Katergia' (0.98)	'Dhagi' & 'Katergia' (0.99)	'Dhagi' & 'Katergia' (0.99)
	Lowest	'Rajapuri' & 'Jabedi' (0.79)	'Rajapuri' & 'Jabedi' (0.88)	'Rajapuri' & 'Jabedi' (0.86)
III	Highest	'Mamomthody' & 'Basar Along' (0.77)	'Mamomthody' & 'Basar Along' (0.87)	'Mamomthody' & 'Basar Along' (0.85)
	Lowest	'Sudarsana' & 'Mamomthody' (0.60)	'Sudarsana' & 'Mamomthody' (0.75)	'Sudarsana' & 'Mamomthody' (0.72)
IV	Highest	'Amrithapani' & 'Armoor' (1.00)	'Amrithapani' & 'Armoor' (1.00)	'Amrithapani' & 'Armoor' (1.00)
	Lowest	'Sugandham' & 'Amrithapani' & 'Armoor' (0.78)	'Sugandham' & 'Amrithapani' & 'Armoor' (0.88)	'Sugandham' & 'Amrithapani' & 'Armoor' (0.85)

4.2. 8. Characterisation of turmeric varieties/cultivars using ISSR markers

Fifteen selected ISSR primers were used for characterizing the cultivars/varieties. The sequence of the ISSR primers used for the molecular genetic fingerprinting and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table 56 (Figs. 55 – 58). In case of the fifteen ISSR primers, out of the 160 scorable bands, 118 were polymorphic constituting 73.8 % of the total markers produced. No single primer yielded 100% polymorphic bands. Maximum percentage of polymorphic bands was produced by the ISSR primer ISSR 16 (85.7 %) and a minimum of 50% by ISSR 15.

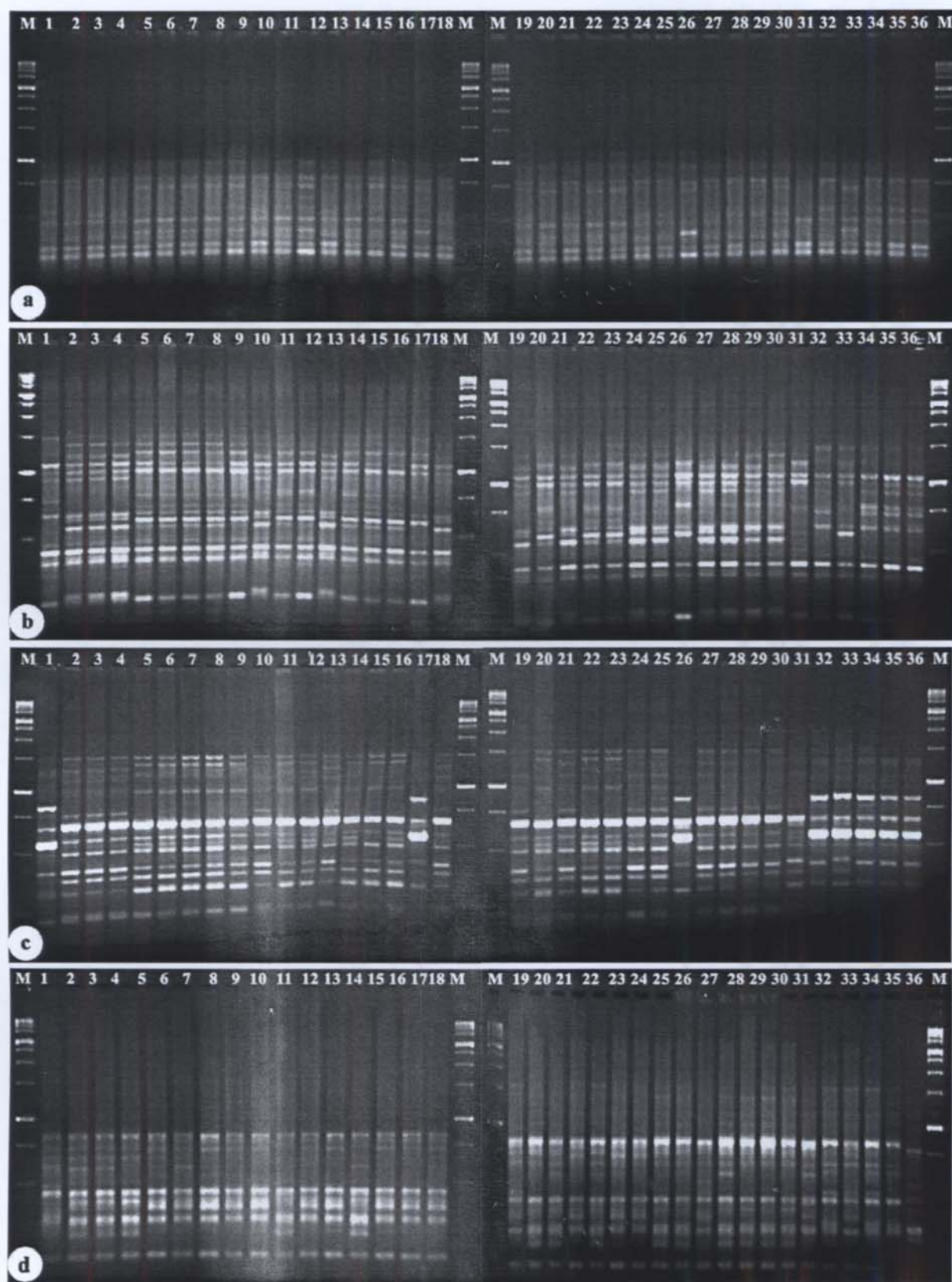


Fig. 55. a-d are the ISSR profiles of 36 turemeric varieties/cultivars using primers ISSR 04, ISSR 05, ISSR 06 and ISSR 07. Lane 1-36 are 1) Alleppey, 2) Amalapuram, 3) Amrithapani, 4) Armoor, 5) Jabedi, 6) Dhagi, 7) Katergia, 8) Chayapasupu, 9) Avanigadda, 10) Dindigam, 11) Ethamkula, 12) GL Puram, 13) Karhadi local, 14) Kasturi, 15) Kodur, 16) Kuchipudi, 17) Mamomthody, 18) Muvatupuzha, 19) Nandyal type, 20) Rajapuri, 21) Sugandham, 22) Vondimitta, 23) Wynad local, 24) Aizwal, 25) Ayur, 26) Basar Along, 27) Gaspani, 28) Hajo, 29) Jorhat, 30) Suvarna, 31) Sudarsana, 32) Kedaram, 33) Suguna, 34) Prathibha, 35) Prabha and 36) Alleppey Supreme. M- Marker 1 KB ladder (Biogene, USA).

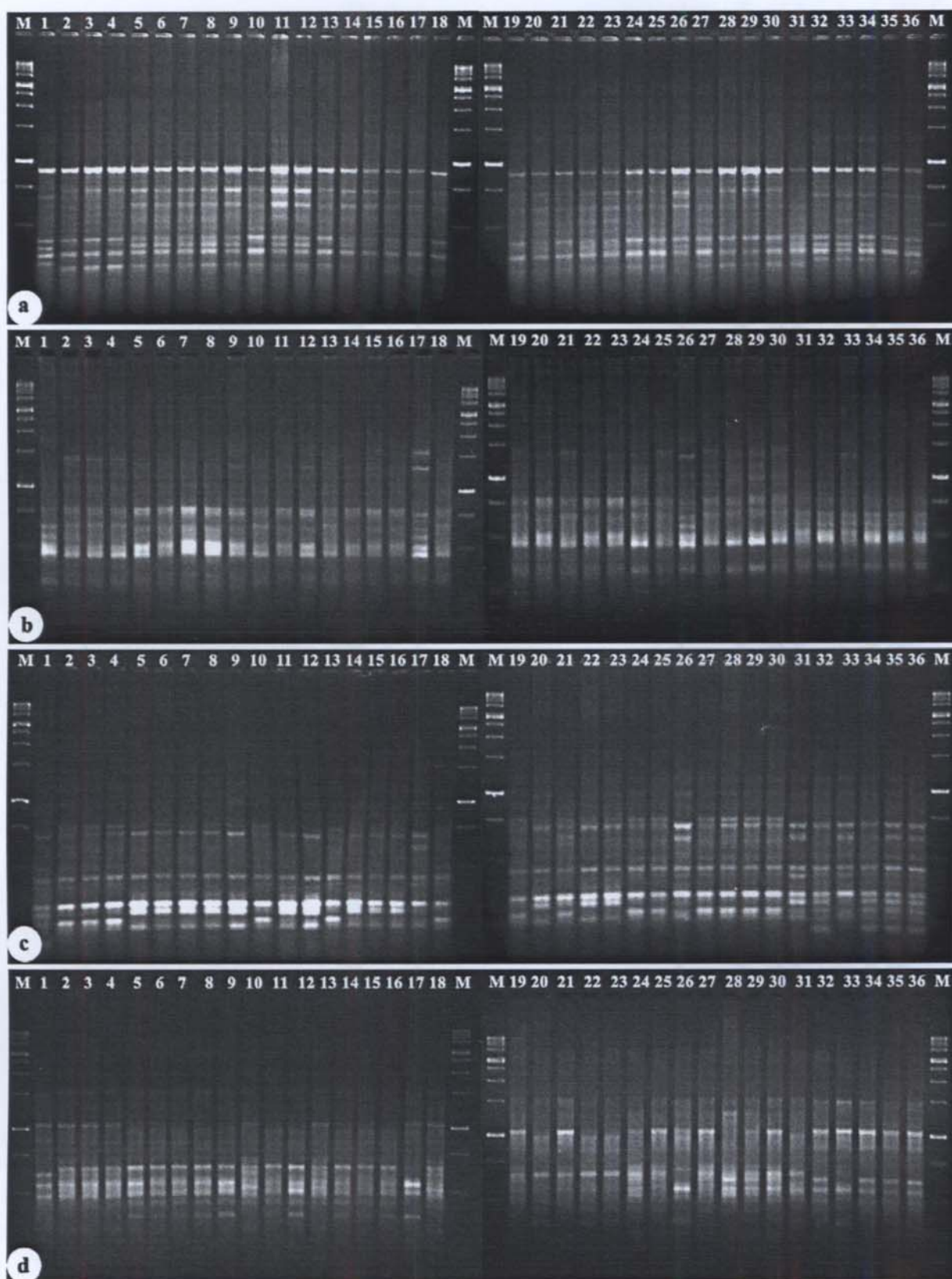


Fig. 56. a-d are the ISSR profiles of 36 turemeric varieties/cultivars using primers ISSR 08, ISSR 09, ISSR 10 and ISSR 11. Lane 1-36 are 1). Alleppey, 2) Amalapuram, 3) Amrithapani, 4) Armour, 5) Jabedi, 6) Dhagi, 7) Katergia, 8) Chayapasupu, 9) Avanigadda, 10) Dindigam, 11) Ethamkula, 12) GL Puram, 13) Karhadi local, 14) Kasturi, 15) Kodur, 16) Kuchipudi, 17) Mamomthody, 18) Muvatupuzha, 19) Nandyal type, 20) Rajapuri, 21) Sugandham, 22) Vondimitta, 23) Wynad local, 24) Alizwal, 25) Ayur, 26) Basar Along, 27) Gaspani, 28) Hajo, 29) Jorhat, 30) Suvarna, 31) Sudarsana, 32) Kedaram, 33) Suguna, 34) Prathibha, 35) Prabha and 36) Alleppey Supreme, respectively. M- Marker 1 Kb ladder (Biogene, USA).

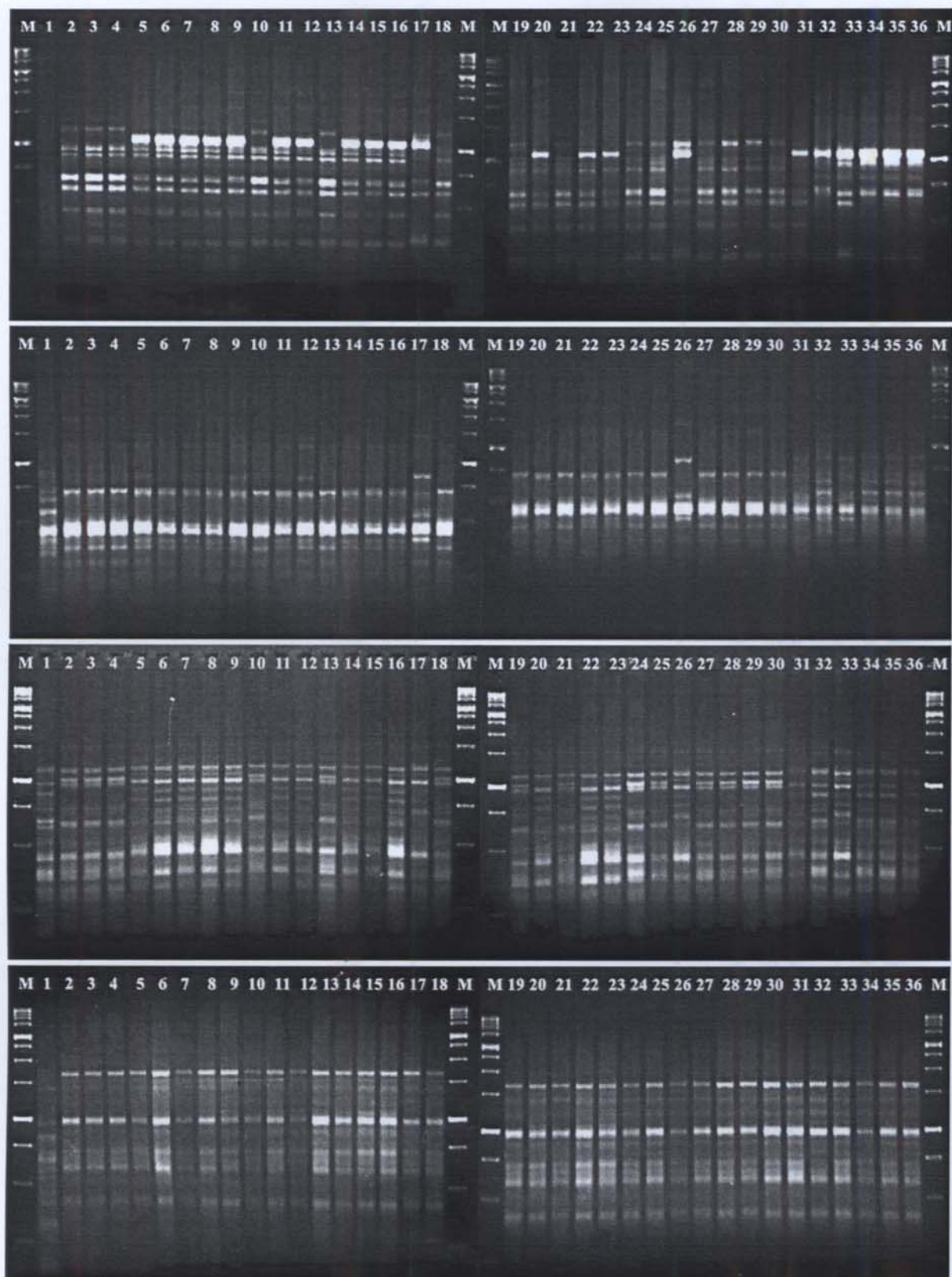


Fig. 57. a-d are the ISSR profiles of 36 turemeric varieties/cultivars using primers ISSR 12, ISSR 13, ISSR 14 and ISSR 15. Lane 1-36 are 1). Alleppey, 2) Amalapuram, 3) Amrithapani, 4) Armoor, 5) Jabedi, 6) Dhagi, 7) Katergia, 8) Chayapasupu, 9) Avanigadda, 10) Dindigam, 11) Ethamkula, 12) GL Puram, 13) Karhadi local, 14) Kasturi, 15) Kodur, 16) Kuchipudi, 17) Mamomthody, 18) Muvatupuzha, 19) Nandyal type, 20) Rajapuri, 21) Sugandham, 22) Vondimitta, 23) Wynad local, 24) Aizwal, 25) Ayur, 26) Basar Along, 27) Gaspani, 28) Hajo, 29) Jorhat, 30) Suvarna, 31) Sudarsana, 32) Kedaram, 33) Suguna, 34) Prathibha, 35) Prabha and 36) Alleppey Supreme. M-Marker 1 KB ladder (Biogene, USA).

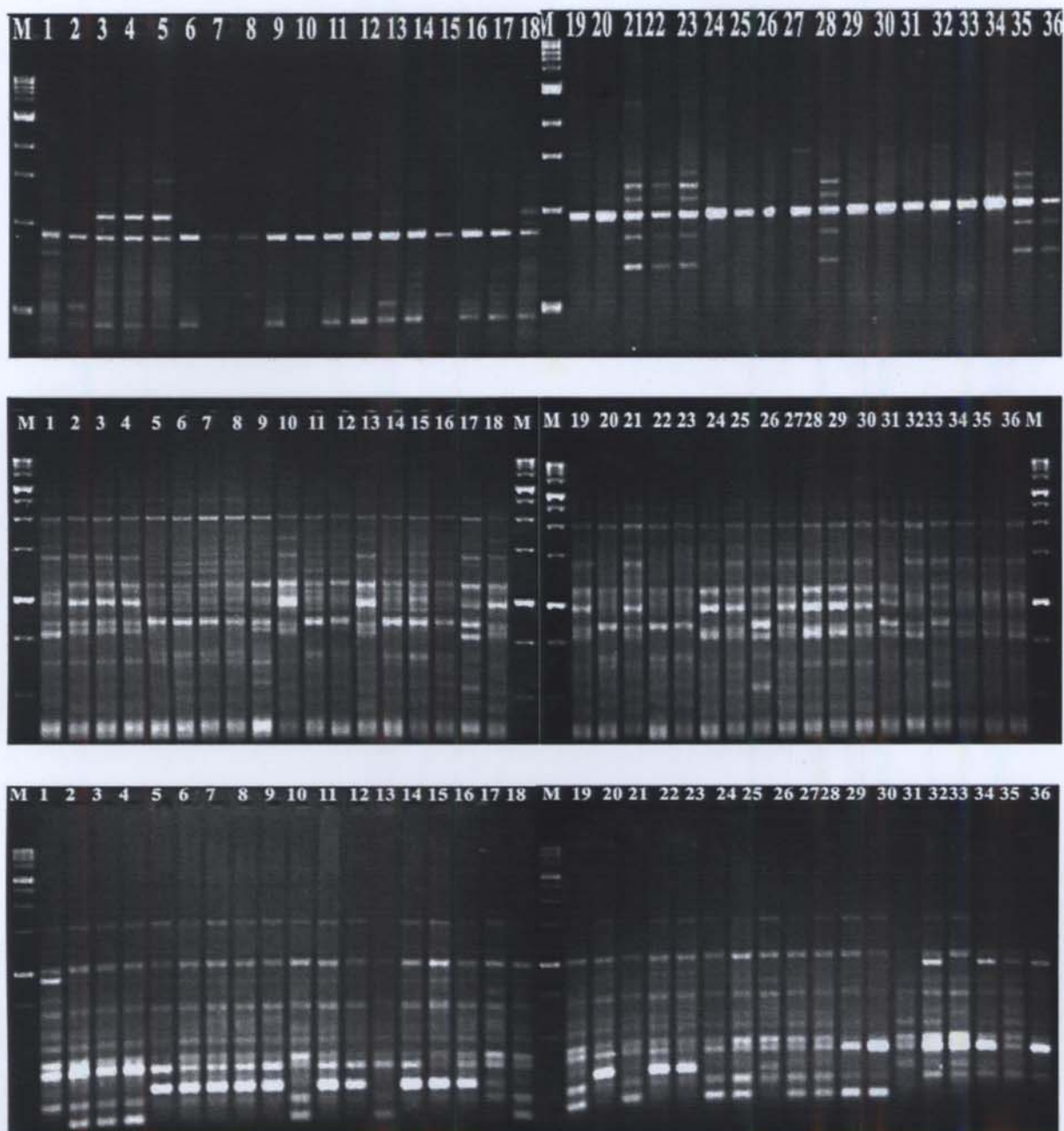


Fig. 58. a- c are the ISSR profiles of 36 turemeric varieties/cultivars using primers ISSR 16, ISSR 17 and ISSR 18. Lane 1-36 are 1). Alleppey, 2) Amalapuram, 3) Amrithapani, 4) Armoor, 5) Jabedi, 6) Dhagi, 7) Katergia, 8) Chayapasupu, 9) Avanigadda, 10) Dindigam, 11) Ethamkula, 12) GL Puram, 13) Karhadi local, 14) Kasturi, 15) Kodur, 16) Kuchipudi, 17) Mamomthody, 18) Muvatupuzha, 19) Nandyal type, 20) Rajapuri, 21) Sugandham, 22) Vondimitta, 23) Wynad local, 24) Aizwal, 25) Ayur, 26) Basar Along, 27) Gaspani, 28) Hajo, 29) Jorhat, 30) Suvarna, 31) Sudarsana, 32) Kedaram, 33) Suguna, 34) Prathibha, 35) Prabha and 36) Alleppey Supreme. M- Marker 1 KB ladder (Biogene, USA).

Table 56. Sequence of ISSR primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in different turmeric varieties/ cultivars

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	ISSR 4	(AGC) ₄ GT	08	06	75.0
2	ISSR 5	(CAC) ₃ GC	18	14	77.7
3	ISSR 6	(CTC) ₃ GC	18	14	77.7
4	ISSR 7	(GACA) ₃	12	08	66.6
5	ISSR 8	(GACA) ₃ GC	08	05	62.5
6	ISSR 9	(TC) ₇ G	10	08	80.0
7	ISSR 10	(CT) ₇ TG	10	06	60.0
8	ISSR 11	(AGTG) ₃ TT	10	07	70.0
9	ISSR 12	(CCCT) ₄	12	10	83.3
10	ISSR 13	(AGTG) ₃	09	07	77.7
11	ISSR 14	(AG) ₈ T	11	09	81.8
12	ISSR 15	(TC) ₈ C	08	04	50.0
13	ISSR 16	(AC) ₇ T	07	06	85.7
14	ISSR 17	(GA) ₇ A	09	06	66.6
15	ISSR 18	(GA) ₈ T	10	08	80.0
		Total	160	118	72.9

4.2.8. 1. Cluster analysis

Similarity matrix of thirty six cultivars/varieties of turmeric based on ISSR markers using three different coefficients are given in Table 57- 59. In all the three similarity matrices, the cultivars 'Amalapuram' and 'Amrithapani' showed maximum similarity between them (1.00); these two cultivars were unable to be separated by the 15 ISSR primers studied. The similarity matrix obtained using Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients showed the least similarity between the variety 'Suguna' and the cultivar 'Nandyal' (0.47, 0.64 and 0.54 respectively).

Table 57. Similarity matrix of different turmeric varieties/cultivars based on ISSR data using Jaccard's similarity coefficient

Row/Col	Alhey	Amalper	Amalpa	Amoor	Johi	Dhaji	Kataga	Chapung	Aravalli	Dudigan	Dhambal	Glaxun	Kahali	Katui	Kohr	Kuchipudi	Mamontchod	Murugan	Kandyl	Rajputi	Saguntham	Vandita	Wynah	Aival	Ayer	Bansalang	Gupani	Epo	Jahat	Serum	Selasma	Kadum	Sigma	Prabha	Prabhla	Alisqum		
Alhey	1.000000																																					
Amalper	0.5703125	1.000000																																				
Amalpa	0.5703125	1.000000	1.000000																																			
Amoor	0.562500	0.9904762	0.9904762	1.000000																																		
Johi	0.5153846	0.5725191	0.5725191	0.5789231	1.000000																																	
Dhaji	0.5343511	0.5908091	0.5908091	0.5833333	0.9433962	1.000000																																
Kataga	0.5267176	0.5833333	0.5833333	0.5877863	0.9522810	0.9904762	1.000000																															
Chapung	0.4962406	0.5522388	0.5522388	0.5563910	0.9065421	0.9439252	0.9528302	1.000000																														
Aravalli	0.5461538	0.5789474	0.5789474	0.5833333	0.9433962	0.9444444	0.9532710	0.9082569	1.000000																													
Dudigan	0.5230769	0.8648649	0.8648649	0.8727273	0.5736434	0.5801527	0.5846154	0.5530303	0.5801527	1.000000																												
Dhambal	0.500000	0.5563910	0.5563910	0.5606061	0.9333333	0.9528302	0.9619048	0.9158879	0.9166667	0.8065992	1.000000																											
Glaxun	0.5227273	0.5671642	0.5671642	0.5597015	0.9252336	0.9090909	0.9000000	0.8571429	0.9090909	0.5801527	0.9166667	1.000000																										
Kahali	0.5419847	0.9003009	0.9003009	0.9090909	0.5881818	0.5746269	0.5789474	0.5397015	0.5746269	0.9082569	0.5522388	0.5514706	1.000000																									
Katui	0.4962406	0.5522388	0.5522388	0.5563910	0.9245283	0.9439252	0.9528302	0.9619048	0.9082569	0.5789231	0.9522810	0.8908091	0.5481481	1.000000																								
Kohr	0.5075758	0.5639098	0.5639098	0.5681818	0.8715396	0.8990806	0.9074074	0.8571429	0.6015625	0.8981481	0.8245614	0.5833333	0.9074074	1.000000																								
Kuchipudi	0.4885496	0.5572519	0.5572519	0.5615385	0.9047619	0.9245283	0.9333333	0.9423077	0.9065421	0.5826772	0.9326923	0.8715396	0.5530303	0.9423077	0.9056604	1.000000																						
Mamontchod	0.6000000	0.5255474	0.5255474	0.5182482	0.6532238	0.6387302	0.6507937	0.6580000	0.6854839	0.5147039	0.6480000	0.6587302	0.5000000	0.6895548	0.6560000	0.6776880	1.000000																					
Murugan	0.5038168	0.8230088	0.8230088	0.8303571	0.5300330	0.5373134	0.5413534	0.5454545	0.5373134	0.8454545	0.5378788	0.5147039	0.8482143	0.5454545	0.5692308	0.5748031	0.5185185	1.000000																				
Kandyl	0.5230769	0.8157895	0.8157895	0.8070175	0.5496183	0.5681818	0.5606061	0.5488355	0.5563910	0.7586207	0.5338346	0.5220588	0.8086957	0.5648855	0.5789231	0.5703125	0.5488722	0.8454545	1.000000																			
Rajputi	0.4923077	0.5839375	0.5839375	0.5781230	0.8090909	0.8288288	0.8198198	0.7946429	0.7807018	0.5748031	0.8348624	0.8125000	0.5454545	0.8272727	0.7946429	0.8073394	0.6422764	0.5307692	0.5748031	1.000000																		
Saguntham	0.5348837	0.8157895	0.8157895	0.8230088	0.5615385	0.5681818	0.5725191	0.5530303	0.5681818	0.7739130	0.5454545	0.5220588	0.8245614	0.5530303	0.5789231	0.5381395	0.5373134	0.7964682	0.9065421	0.5748031	1.000000																	
Vandita	0.4961240	0.5905512	0.5905512	0.5952381	0.8000000	0.8198198	0.8272727	0.8018018	0.7876106	0.5799351	0.8256881	0.7876106	0.5615385	0.8181818	0.7857143	0.8148148	0.6209677	0.5468750	0.5793651	0.9688970	0.5920000	1.000000																
Wynah	0.4883721	0.5826772	0.5826772	0.5873916	0.8073394	0.8272727	0.8348624	0.7927928	0.7946429	0.5840000	0.8165138	0.7786111	0.5638915	0.7927928	0.7927928	0.8055556	0.6128032	0.5390625	0.5714286	0.9207921	0.5940000	0.9494949	1.000000															
Aival	0.5267176	0.8495575	0.8495575	0.8571429	0.5891473	0.5954198	0.6000000	0.5681818	0.5954198	0.8070175	0.5725191	0.5397015	0.8240870	0.5681818	0.5923077	0.5736434	0.5182482	0.7962456	0.8538539	0.5781230	0.8772727	0.5952381	0.6128032	1.000000														
Ayer	0.5488730	0.8648649	0.8648649	0.8538539	0.5615385	0.5801527	0.5725191	0.5413534	0.5681818	0.8214286	0.5454545	0.5447761	0.8245614	0.5413534	0.5648855	0.5461538	0.5147039	0.7964682	0.8715396	0.5748031	0.8715396	0.5793651	0.5967742	0.9433962	1.000000													
Bansalang	0.5780000	0.5488722	0.5488722	0.5530303	0.6422764	0.6549206	0.6400000	0.6062992	0.6612903	0.5615385	0.6504065	0.6480000	0.5220588	0.6320000	0.6190476	0.6000000	0.7083333	0.5300330	0.5037037	0.6383333	0.5149254	0.6500000	0.6416667	0.5530303	0.5378788	1.000000												
Gupani	0.5419847	0.8672566	0.8672566	0.8730000	0.5923077	0.5984848	0.6030334	0.5714286	0.5984848	0.8086957	0.5757576	0.5626430	0.8434783	0.5714286	0.5833333	0.5789231	0.5328467	0.8518584	0.8908091	0.5937300	0.8908091	0.6111111	0.8031746	0.9266255	0.9238239	0.5447761	1.000000											
Epo	0.5303030	0.8508772	0.8508772	0.8594071	0.5681818	0.5746269	0.5789474	0.5481481	0.5746269	0.8086957	0.5639098	0.5514706	0.8275862	0.5397015	0.5397015	0.5530303	0.5000000	0.8157895	0.8571429	0.5692308	0.8571429	0.5839375	0.5638915	0.9080909	0.8908091	0.5333333	0.9272727	1.000000										
Jahat	0.5384615	0.8495575	0.8495575	0.8407080	0.5789231	0.5833333	0.5757576	0.5447761	0.5714286	0.7913043	0.5606061	0.5714286	0.8280870	0.5563910	0.5363910	0.5496183	0.5077464	0.7962456	0.8538539	0.5781230	0.8392857	0.5826772	0.5625000	0.8908091	0.8899083	0.5288307	0.9080909	0.9444444	1.000000									
Serum	0.5426537	0.8245614	0.8245614	0.8318584	0.5813953	0.5877863	0.5923077	0.5606061	0.5877863	0.7962456	0.5789231	0.5639098	0.8333333	0.5725191	0.5725191	0.5658915	0.5109489	0.7894737	0.8468468	0.5703125	0.8468468	0.5873016	0.5687291	0.8818182	0.8636364	0.5338346	0.9000000	0.9351852	0.9528302	1.000000								
Selasma	0.5284533	0.5153846	0.5153846	0.5193798	0.6353932	0.6416667	0.6470388	0.6115702	0.6554622	0.5275591	0.6381197	0.6354622	0.5287869	0.6386555	0.6115702	0.6039420	0.6063574	0.5317440	0.5275591	0.6964286	0.5275591	0.7027027	0.6783714	0.5312500	0.5156250	0.6782609	0.5468750	0.5483071	0.5600000	1.000000								
Kadum	0.7635551	0.5967742	0.5967742	0.6016880	0.5156250	0.5230769	0.5271318	0.4961832	0.5468750	0.5600000	0.5000000	0.5230769	0.5793651	0.4961832	0.5078923	0.4883721	0.5634921	0.5275591	0.5116279	0.5158730	0.5354331	0.5322881	0.5041935	0.5511811	0.5354331	0.6302521	0.5307892	0.5426357	0.5390625	0.5555556	0.6228070	1.000000						
Sigma	0.6837807	0.5239259	0.5239259	0.5288307	0.8031746	0.6093750	0.6141732	0.5813953	0.6349206	0.5149254	0.6111111	0.6220472	0.5109489	0.5937300	0.5937300	0.5748031	0.6803279	0.4852841	0.47110145	0.5920000	0.5037037	0.5840000	0.5760000	0.4748201	0.4817518	0.7565217	0.6892086	0.6892086	0.6963304	0.5000000	0.7079446	0.7477477	1.000000					
Prabha	0.7636364	0.5615385	0.5615385	0.5638915	0.5307692	0.5378788	0.5419847	0.5112782	0.5615385	0.5302876	0.5267176	0.5496183	0.5772519	0.5227273	0.5028168	0.5781230	0.5075758	0.4925373	0.5193798	0.5267176	0.5354331	0.5156250	0.5187970	0.5037594	0.6311475	0.5111111	0.5222881	0.5187970	0.5343511	0.6101495	0.9100000	0.7456140	1.000000					
Prabhla	0.684286	0.5307692	0.5307692	0.5388337	0.5000000	0.4962406	0.5000000	0.5271318	0.5190840	0.5078923	0.4804848	0.5075758	0.5384615	0.5153846	0.5028168	0.4961240	0.5394551	0.5234575	0.5078923	0.5000000	0.5193798	0.4843730	0.4887218	0.4736842	0.5853639	0.4814815	0.4925373	0.4887218	0.4924242	0.5762712	0.8333333	0.6956522	0.9200000	1.000000				
Alisqum	0.7079446	0.5338462	0.5338462	0.5381395	0.5348837	0.5303030	0.5343511	0.5307394	0.5338462	0.5426357	0.5190840	0.5419847	0.5496183	0.5153515	0.533739																							

Table 58. Similarity matrix of different turmeric varieties/cultivars based on ISSR data using Sorensen - Dice similarity coefficient

Var/Cult	Albany	Amalgam	Amethya	Amoor	Johi	Dhag	Kataga	Chayapang	Aravindh	Dindigul	Ethambal	GLparam	Katsh	Katni	Ker	Kachipul	Masconchod	Moragunth	Mandyl	Rajperi	Sugantham	Vondimla	Wynad	Aimal	Ayer	Basambag	Gurani	Ego	Johat	Servana	Sahasana	Kalam	Sugam	Pukha	Prathibha	Alldayam	
Albany	1.000000																																				
Amalgam	0.726382	1.000000																																			
Amethya	0.726382	1.000000	1.000000																																		
Amoor	0.720000	0.995215	0.995215	1.000000																																	
Johi	0.690203	0.728153	0.728153	0.731703	1.000000																																
Dhag	0.696517	0.742857	0.742857	0.736842	0.970873	1.000000																															
Kataga	0.690000	0.736842	0.736842	0.740384	0.975808	0.995215	1.000000																														
Chayapang	0.663316	0.711535	0.711535	0.714978	0.950804	0.971158	0.975845	1.000000																													
Aravindh	0.706467	0.733333	0.733333	0.736842	0.970873	0.971428	0.976076	0.951923	1.000000																												
Dindigul	0.696887	0.927536	0.927536	0.930388	0.729640	0.734295	0.737864	0.712195	0.734295	1.000000																											
Ethambal	0.666667	0.714978	0.714978	0.718466	0.963317	0.973845	0.980382	0.958896	0.956521	0.754902	1.000000																										
GLparam	0.685472	0.723895	0.723895	0.717703	0.961163	0.952381	0.947368	0.923078	0.952381	0.734295	0.956521	1.000000																									
Katsh	0.702970	0.947867	0.947867	0.952810	0.724637	0.729878	0.733333	0.717703	0.729878	0.951923	0.711535	0.710905	1.000000																								
Katni	0.683316	0.711535	0.711535	0.714978	0.960784	0.971158	0.975845	0.980382	0.951923	0.731703	0.975808	0.942307	0.708134	1.000000																							
Ker	0.673348	0.721158	0.721158	0.724637	0.931372	0.942307	0.946839	0.951456	0.923078	0.751219	0.946341	0.903846	0.736842	0.951456	1.000000																						
Kachipul	0.656103	0.715883	0.715883	0.719218	0.950000	0.960784	0.965517	0.970270	0.950804	0.736318	0.965174	0.931372	0.712195	0.970270	0.950493	1.000000																					
Masconchod	0.730000	0.688952	0.688952	0.682892	0.790249	0.794284	0.788415	0.792270	0.813397	0.679617	0.786407	0.794284	0.666667	0.801952	0.792270	0.807818	1.000000																				
Moragunth	0.670000	0.902912	0.902912	0.907317	0.693083	0.699029	0.702430	0.705824	0.699029	0.916256	0.699074	0.679617	0.917874	0.705824	0.725490	0.730000	0.682928	1.000000																			
Mandyl	0.688887	0.885507	0.885507	0.893039	0.709336	0.724637	0.718466	0.721951	0.714978	0.862745	0.680784	0.683993	0.894208	0.721951	0.731703	0.726382	0.708739	0.916256	1.000000																		
Rajperi	0.639798	0.738918	0.738918	0.732673	0.894774	0.906409	0.900901	0.885572	0.678847	0.730000	0.910000	0.896551	0.703884	0.905476	0.885572	0.893401	0.782172	0.693467	0.730000	1.000000																	
Sugantham	0.688887	0.885507	0.885507	0.902912	0.719218	0.724637	0.728153	0.712195	0.724637	0.872549	0.703884	0.683993	0.903846	0.712195	0.731703	0.716119	0.699029	0.886895	0.950804	0.730000	1.000000																
Vondimla	0.663214	0.742574	0.742574	0.746287	0.888889	0.900901	0.905476	0.890000	0.881188	0.733683	0.904526	0.881188	0.719218	0.900000	0.880000	0.897932	0.766182	0.707070	0.733683	0.984614	0.743718	1.000000															
Wynad	0.656200	0.736318	0.736318	0.740000	0.893401	0.905476	0.910000	0.884221	0.885572	0.737737	0.888889	0.875621	0.722772	0.884221	0.884221	0.892307	0.760000	0.700376	0.727272	0.958742	0.737737	0.974933	1.000000														
Aimal	0.680000	0.918683	0.918683	0.923078	0.741463	0.746415	0.750000	0.724637	0.746415	0.893209	0.728153	0.717703	0.904761	0.724637	0.743861	0.729640	0.682892	0.887804	0.922301	0.732673	0.932088	0.746387	0.760000	1.000000													
Ayer	0.707070	0.927536	0.927536	0.922301	0.719218	0.734295	0.728153	0.702430	0.724637	0.901968	0.703884	0.705340	0.903846	0.702430	0.721951	0.706467	0.679617	0.886895	0.951372	0.730000	0.931372	0.733683	0.747474	0.970873	1.000000												
Basambag	0.730945	0.708739	0.708739	0.712195	0.782172	0.766990	0.780478	0.754902	0.761165	0.719218	0.788177	0.786407	0.683993	0.774508	0.764709	0.750000	0.829283	0.693083	0.669307	0.793898	0.679830	0.787888	0.781259	0.712195	0.699074	1.000000											
Gurani	0.902970	0.928910	0.928910	0.933333	0.743861	0.748815	0.752810	0.727272	0.727272	0.894208	0.707892	0.720791	0.915843	0.717703	0.736842	0.731703	0.695281	0.908216	0.942307	0.745080	0.942307	0.738627	0.752672	0.961908	0.961538	0.705340	1.000000										
Ego	0.693083	0.919433	0.919433	0.923895	0.724637	0.729878	0.733333	0.708134	0.729878	0.894208	0.721158	0.710905	0.903604	0.717703	0.717703	0.712195	0.666667	0.898507	0.923078	0.725490	0.923078	0.738918	0.722772	0.952810	0.942307	0.695622	0.962242	1.000000									
Johat	0.700000	0.918683	0.918683	0.913461	0.731703	0.736842	0.730789	0.705340	0.727272	0.883495	0.718466	0.727272	0.904761	0.714978	0.714978	0.709536	0.673078	0.887804	0.922301	0.732673	0.912621	0.736318	0.730000	0.942307	0.941746	0.693629	0.952810	0.971428	1.000000								
Servana	0.703176	0.903846	0.903846	0.908216	0.735291	0.740384	0.743861	0.718466	0.740384	0.887804	0.731703	0.721158	0.908009	0.728153	0.728153	0.722772	0.676382	0.882329	0.917072	0.726382	0.917072	0.740000	0.726181	0.937198	0.928829	0.690784	0.947368	0.966372	0.975845	1.000000							
Sahasana	0.691894	0.680000	0.680000	0.683673	0.777202	0.781729	0.787143	0.738974	0.791872	0.680721	0.793814	0.791872	0.680887	0.779472	0.738974	0.753926	0.755100	0.694305	0.680721	0.821056	0.690721	0.825398	0.808510	0.693876	0.680414	0.808282	0.707070	0.707070	0.704081	0.717948	1.000000						
Kalam	0.667749	0.747474	0.747474	0.751280	0.680414	0.680887	0.690353	0.663263	0.707070	0.717948	0.666667	0.680887	0.733683	0.673408	0.656200	0.720812	0.680721	0.676923	0.680583	0.697439	0.694738	0.687837	0.710659	0.697439	0.773199	0.693467	0.703517	0.700976	0.714285	0.767576	1.000000						
Sugam	0.812187	0.689304	0.689304	0.699204	0.752472	0.757281	0.760976	0.735291	0.766990	0.798207	0.786993	0.767328	0.745080	0.745080	0.730000	0.809751	0.653463	0.640394	0.743718	0.669307	0.737737	0.730945	0.643904	0.630243	0.861381	0.657008	0.657008	0.663416	0.666667	0.820155	0.855670	1.000000					
Pukha	0.863974	0.719218	0.719218	0.722772	0.693467	0.699307	0.702970	0.676618	0.719218	0.710000	0.680000	0.709236	0.715883	0.685472	0.676616																						

Table 59. Similarity matrix of different turmeric varieties/cultivars based on ISSR data using Simple Matching similarity coefficients

Row/Col	Alleppey	Annapur	Anuthiya	Anzoor	Johel	Dhag	Kataga	Chayapam	Aravagudi	Dandagan	Elakshel	Glupam	Kashel	Kartai	Koh	Kuchipudi	Mammoth	Mervagudi	Nandyl	Rajguzi	Sugantham	Vandima	Wyndan	Aival	Ayer	Baanabag	Gupam	Hajo	Johel	Sorana	Suleasa	Kalam	Sigma	Prabha	Prabhika	Alleppey	
Alleppey	1.000000																																				
Annapur	0.6540881	1.000000																																			
Anuthiya	0.6540881	1.000000	1.000000																																		
Anzoor	0.6477987	0.9937107	0.9937107	1.000000																																	
Johel	0.6037736	0.6477987	0.6477987	0.6540881	1.000000																																
Dhag	0.6163522	0.6603774	0.6603774	0.6540881	0.9622642	1.000000																															
Kataga	0.6100629	0.6540881	0.6540881	0.6603774	0.9685335	0.9937107	1.000000																														
Chayapam	0.5786164	0.6226415	0.6226415	0.6289308	0.9371069	0.9622642	0.9685335	1.000000																													
Aravagudi	0.6289308	0.6477987	0.6477987	0.6540881	0.9622642	0.9622642	0.9685335	0.9371069	1.000000																												
Dandagan	0.6100629	0.9056604	0.9056604	0.9119497	0.6540881	0.6603774	0.6289308	0.6540881	1.000000																												
Elakshel	0.5849057	0.6289308	0.6289308	0.6352201	0.9539748	0.9685335	0.9740428	0.9433962	0.6853346	1.000000																											
Glupam	0.6037736	0.6352201	0.6352201	0.6289308	0.9496855	0.9371069	0.9308176	0.8937111	0.9371069	0.6540881	0.9433962	1.000000																									
Kashel	0.6226415	0.9308176	0.9308176	0.9371069	0.6413094	0.6413094	0.6477987	0.6289308	0.6413094	0.9371069	0.6226415	0.6163522	1.000000																								
Kartai	0.5786164	0.6226415	0.6226415	0.6289308	0.9496855	0.9622642	0.9685335	0.9740428	0.9371069	0.6540881	0.9685335	0.9245283	0.6163522	1.000000																							
Koh	0.5911930	0.6352201	0.6352201	0.6413094	0.9119497	0.9245283	0.9308176	0.9371069	0.8937111	0.6792453	0.9308176	0.8742138	0.6540881	0.9371069	1.000000																						
Kuchipudi	0.5786164	0.6352201	0.6352201	0.6413094	0.9371069	0.9496855	0.9539748	0.9622642	0.9371069	0.6666667	0.9539748	0.9119497	0.6289308	0.9622642	0.9371069	1.000000																					
Mammoth	0.6853346	0.5911930	0.5911930	0.5849057	0.7295397	0.7295397	0.7232704	0.7295397	0.7547170	0.5849057	0.7232704	0.7295397	0.5397484	0.7421384	0.7295397	0.7547170	1.000000																				
Mervagudi	0.5911930	0.8742138	0.8742138	0.8803031	0.6100629	0.6100629	0.6163522	0.6226415	0.6100629	0.8930818	0.6163522	0.5849057	0.8930818	0.6226415	0.6477987	0.6603774	0.5911930	1.000000																			
Nandyl	0.6100629	0.8679245	0.8679245	0.8616352	0.6289308	0.6413094	0.6352201	0.6413094	0.6289308	0.8238994	0.6100629	0.5911930	0.8616352	0.6413094	0.6540881	0.6540881	0.6226415	0.8930818	1.000000																		
Rajguzi	0.5849057	0.6666667	0.6666667	0.6603774	0.8679245	0.8803031	0.8742138	0.8534539	0.8427673	0.6603774	0.8679245	0.8679245	0.6226415	0.8803031	0.8534539	0.8679245	0.7232704	0.6163522	0.6603774	1.000000																	
Sugantham	0.6226415	0.8679245	0.8679245	0.8742138	0.6413094	0.6413094	0.6477987	0.6289308	0.6413094	0.8364780	0.6226415	0.5911930	0.8742138	0.6289308	0.6540881	0.6413094	0.6100629	0.8534539	0.9371069	0.6603774	1.000000																
Vandima	0.5911930	0.6729560	0.6729560	0.6792453	0.8616352	0.8742138	0.8803031	0.8616352	0.8490566	0.6666667	0.8803031	0.8490566	0.6413094	0.8742138	0.8490566	0.8742138	0.7044025	0.6352201	0.6666667	0.9811321	0.6792453	1.000000															
Wyndan	0.5849057	0.6666667	0.6666667	0.6729560	0.8679245	0.8803031	0.8679245	0.8534539	0.8534539	0.6729560	0.8742138	0.8427673	0.6477987	0.8534539	0.8534539	0.8679245	0.6911132	0.6289308	0.6603774	0.9496855	0.6729560	0.9685335	1.000000														
Aival	0.6100629	0.8930818	0.8930818	0.8993711	0.6666667	0.6666667	0.6729560	0.6413094	0.6666667	0.8616352	0.6477987	0.6289308	0.8742138	0.6413094	0.6666667	0.6540881	0.5849057	0.8534539	0.8937111	0.6603774	0.9119497	0.6792453	0.6911321	1.000000													
Ayer	0.6352201	0.9056604	0.9056604	0.8993711	0.6413094	0.6540881	0.6477987	0.6163522	0.6413094	0.8742138	0.6226415	0.6163522	0.8742138	0.6163522	0.6413094	0.6289308	0.5849057	0.8534539	0.9119497	0.6603774	0.9119497	0.6666667	0.6853346	0.9622642	1.000000												
Baanabag	0.6666667	0.6226415	0.6226415	0.6289308	0.7232704	0.7106918	0.7169811	0.6853346	0.7338491	0.6413094	0.7295397	0.7232704	0.5911930	0.7106918	0.6981132	0.6853346	0.7798742	0.6100629	0.5786164	0.7421384	0.5911930	0.7338491	0.7295397	0.6289308	0.6163522	1.000000											
Gupam	0.6226415	0.9056604	0.9056604	0.9119497	0.6666667	0.6666667	0.6729560	0.6413094	0.6666667	0.8616352	0.6477987	0.6289308	0.8679245	0.6413094	0.6540881	0.6540881	0.3974843	0.8803031	0.9245283	0.6729560	0.9245283	0.6918239	0.6853346	0.9496855	0.9496855	0.6163522	1.000000										
Hajo	0.6100629	0.8930818	0.8930818	0.8993711	0.6413094	0.6413094	0.6477987	0.6163522	0.6413094	0.8616352	0.6352201	0.6163522	0.8742138	0.6289308	0.6289308	0.6289308	0.3974843	0.8679245	0.8937111	0.6477987	0.8993711	0.6666667	0.6477987	0.9371069	0.9245283	0.86037736	0.9496855	1.000000									
Johel	0.6226415	0.8930818	0.8930818	0.8679245	0.6540881	0.6477987	0.6163522	0.6413094	0.8490566	0.6352201	0.6413094	0.8742138	0.6289308	0.6289308	0.6289308	0.5723270	0.8534539	0.8937111	0.6603774	0.8679245	0.6666667	0.6477987	0.9245283	0.9245283	0.86037736	0.9371069	0.9622642	1.000000									
Sorana	0.6289308	0.8742138	0.8742138	0.8803031	0.6603774	0.6603774	0.6666667	0.6352201	0.6603774	0.8534539	0.6540881	0.6352201	0.8803031	0.6477987	0.6477987	0.6477987	0.5786164	0.8490566	0.8930818	0.6540881	0.8930818	0.6729560	0.6540881	0.9182390	0.9056604	0.6100629	0.9308176	0.9539748	0.9685335	1.000000							
Suleasa	0.6352201	0.8037736	0.8037736	0.6100629	0.7295397	0.7295397	0.7338491	0.7044025	0.7421384	0.6226415	0.7484277	0.7421384	0.6100629	0.7295397	0.7044025	0.7044025	0.6981132	0.6289308	0.6226415	0.7861635	0.6226415	0.7924538	0.7733849	0.6226415	0.6100629	0.7672956	0.6352201	0.6352201	0.6540881	1.000000							
Kalam	0.8427673	0.6853346	0.6853346	0.6918239	0.6100629	0.6100629	0.6163522	0.5849057	0.6352201	0.6540881	0.5911930	0.6100629	0.6666667	0.5849057	0.5974843	0.5849057	0.6540881	0.6226415	0.6037736	0.6163522	0.6289308	0.6352201	0.6289308	0.6413094	0.6289308	0.7232704	0.6163522	0.6289308	0.6289308	0.6477987	0.7295397	1.000000					
Sigma	0.7672956	0.3974843	0.3974843	0.8037736	0.6853346	0.6853346	0.6918239	0.6603774	0.7106918	0.5911930	0.6918239	0.6981132	0.5786164	0.6729560	0.6729560	0.6603774	0.7547170	0.5397484	0.5408805	0.6792453	0.5786164	0.6729560	0.6666667	0.5408805	0.5534591	0.8238994	0.5534591	0.5534591	0.5603777	0.5723270	0.7924538	0.8238994	1.000000				
Prabha	0.8264780	0.6413094	0.6413094	0.6477987	0.6163522	0.6163522	0.6226415	0.5911930	0.6413094	0.6352201	0.6100629	0.6289308	0.6352201	0.8037736	0.5911930	0.5911930	0.6603774	0.5911930	0.5723270	0.6100629	0.6100629	0.6289308	0.6100629	0.5974843	0.5849057	0.7169811	0.5849057	0.5974843	0.5974843	0.6163522	0.7106918	0.9433962	0.8176101	1.000000			
Prabhika	0.7861635	0.6163522	0.6163522	0.6226415	0.5911930	0.5786164	0.5849057	0.6163522	0.8037736	0.5974843	0.5723270	0.5911930	0.6226415	0.8037736	0.5911930	0.5911930	0.6477987	0.6163522	0.3974843	0.5974843	0.6100629	0.6163522	0.5849057	0.5723270	0.5974843	0.6792453	0.5397484	0.5723270	0.5723270	0.5786164	0.6853346	0.8930818	0.7798742	0.9496855	1.000000		
Alleppey	0.7924538	0.6352201	0.6352201	0.6413094	0.6226415	0.6100629	0.6163522	0.5849057	0.6352201	0.6289308	0.6037736	0.6226415	0.6289308	0.5974843	0.5849057	0.5849057	0.6289308	0.6100629	0.5911930	0.5911930	0.6289308	0.6100629	0.6100629	0.6037736	0.												

The UPGMA dendrogram constructed using the genetic similarity obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on ISSR markers are given in Figs. 59 – 61. In all the three dendrograms the clustering pattern was similar and produced four different groups. The denrograms splits at coefficients 0.65, 0.79 and 0.73, respectively in the three similarity coefficient studied and the different members in the groups are:

- Group I - 'Alleppey', 'Kedaram', 'Prabha', 'Alleppey Supreme' and 'Prathibha'
- Group II - 'Amalapuram', 'Amrithapani', 'Armoor', 'Dindigam', 'Karhadi local', 'Muvatupuzha', 'Nandyal', 'Sugandham', 'Aizwal', 'Ayur', 'Gaspani', 'Hajo', 'Jorhat' and 'Suvama'
- Group III - 'Jabedi', 'Avanigadda', 'Dhagi', 'Katergia', 'Ethamkula', 'Chayapasupu', 'Kasturi', 'Kuchipudi', 'GL Puram', 'Kodur', 'Rajapuri', 'Vondimita' and 'Wynadlocal'
- Group IV - 'Mamomthody', 'Basar Along', 'Suguna' and 'Sudarsana'

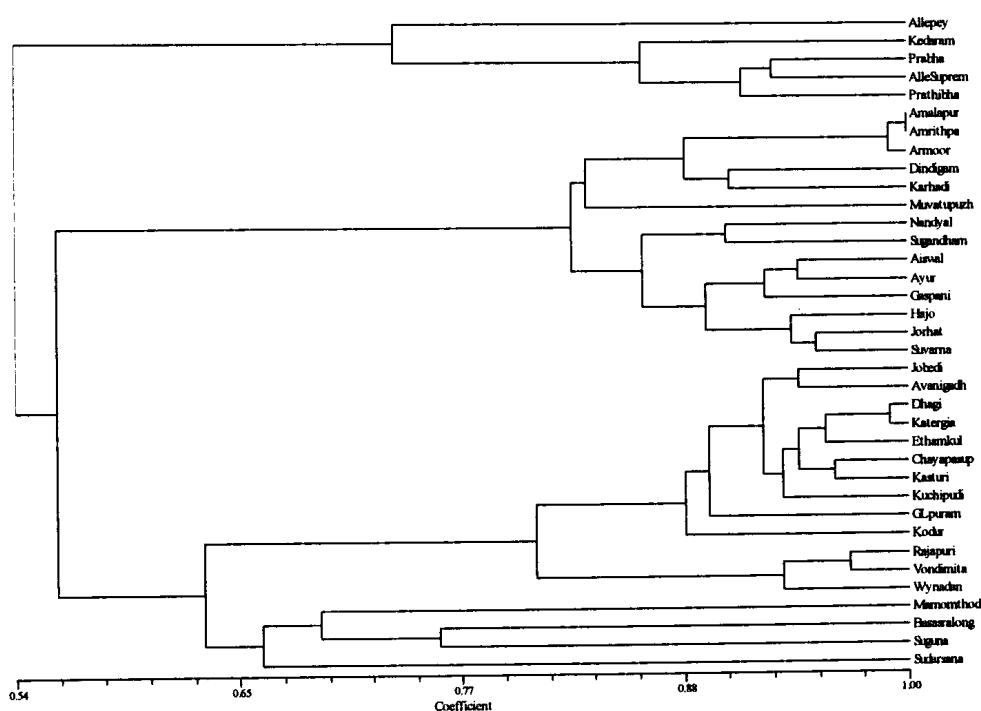


Fig. 59. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on ISSR markers using Jaccard's similarity coefficients.

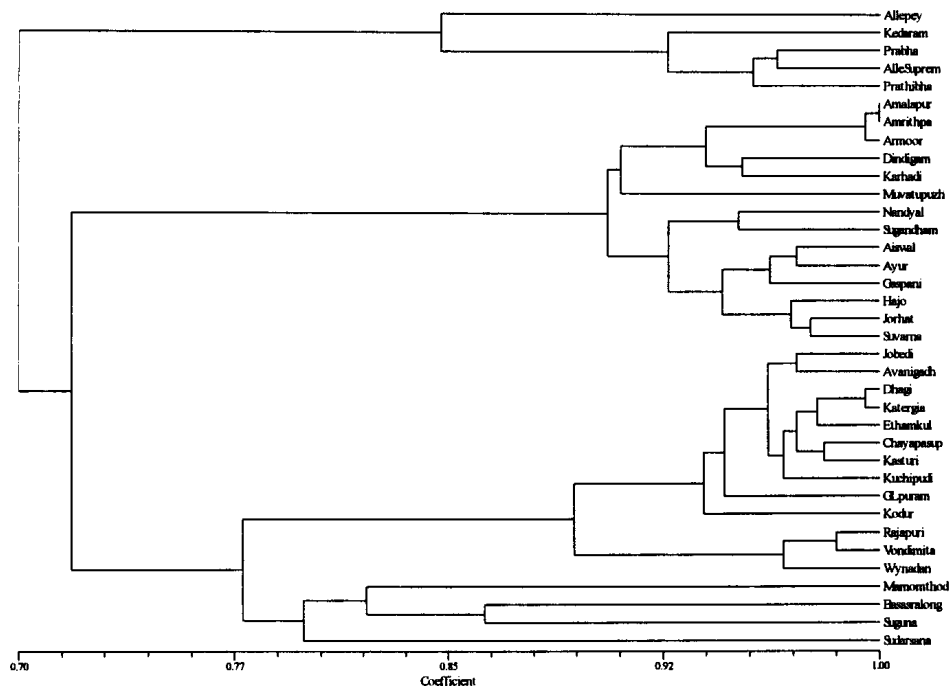


Fig. 60. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on ISSR markers using Sorenson - Dice similarity coefficients.

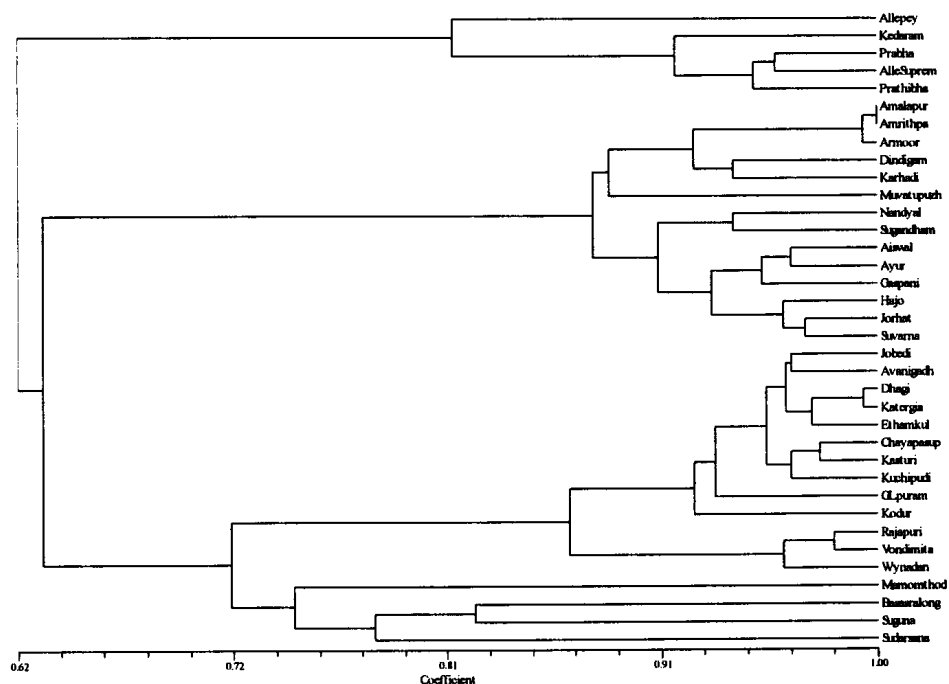


Fig. 61. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on ISSR markers using Simple Matching similarity coefficients.

The different similarity coefficients produced a similar clustering pattern in all the turmeric varieties/cultivars studied, but the degree of similarity of the

accessions in each group varied in three dendrograms. The different groups in the dendrograms produced using Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients from ISSR marker data and the highest and lowest similarities in each group are given in Table 60.

Table 60. Highest and lowest similarities between the varieties and cultivars in the different groups of the dendrogram produced by three similarity coefficients using ISSR marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	'Basar Along' & 'Suguna' (0.76)	'Basar Along' & 'Suguna' (0.86)	'Basar Along' & 'Suguna' (0.82)
	Lowest	'Sudarsana' & 'Mamomthody' (0.61)	'Sudarsana' & 'Mamomthody' (0.76)	'Sudarsana' & 'Mamomthody' (0.69)
II	Highest	'Dhagi' & 'Katergia' (0.99)	'Dhagi' & 'Katergia' (0.99)	'Dhagi' & 'Katergia' (0.99)
	Lowest	'Wynad' & 'GL Puram' (0.78)	'Wynad' & 'GL Puram' (0.88)	'Wynad' & 'GL Puram' (0.84)
III	Highest	'Amalapuram' & 'Amrithapani' (1.00)	'Amalapuram' & 'Amrithapani' (1.00)	'Amalapuram' & 'Amrithapani' (1.00)
	Lowest	'Nandyal' & 'Dindigam' (0.76)	'Nandyal' & 'Dindigam' (0.86)	'Nandyal' & 'Dindigam' (0.82)
IV	Highest	'Prathibha' & 'Alleppey Supreme' (0.93)	'Prathibha' & 'Alleppey Supreme' (0.95)	'Prathibha' & 'Alleppey Supreme' (0.96)
	Lowest	'Alleppey' & 'Prathibha' (0.69)	'Alleppey' & 'Prathibha' (0.82)	'Alleppey' & 'Prathibha' (0.79)

4.2.9. Characterization of turmeric varieties/cultivars using combined RAPD and ISSR markers

Pooled over the two markers, a total of 658 bands were produced and 512 (77.8 %) are polymorphic among the thirty six cultivars/varieties implying profound intraspecific variation among the germplasm studied.

4.2.9. 1. Cluster analysis

The similarity matrices obtained using Jaccard's, Sorensen – Dice and Simple Matching coefficients are given in Tables 61-63. In all the similarity matrices, the cultivar 'Armoor' & 'Amalapuram' showed maximum similarity (0.99) with the cultivar 'Amrithapani'. The least similarity was observed between the variety 'Prathibha' and the cultivar 'Ayur' (0.45, 0.62 and 0.57 in Jaccard's, Sorensen – Dice and Simple Matching coefficients, respectively).

Table 61. Similarity matrix obtained from combined RAPD and ISSR data using Jaccard's similarity coefficients in turmeric varieties/cultivars.

Row/Col	Alleppey	Anamalai	Anantpur	Anoor	Johai	Dhaji	Katanga	Chayras	Aravali	Dudhga	Elakhal	Glennan	Kashik	Karni	Kohr	Kuchipudi	Mammoth	Muruga	Kandyl	Rajapur	Sagatha	Vandini	Wynad	Aimal	Ayer	Banashan	Gopani	Ego	Johai	Serana	Selena	Kadum	Saga	Prabha	Prabha	Alleppey	
Alleppey	1.000000																																				
Anamalai	0.5317919	1.000000																																			
Anantpur	0.5296551	0.9975610	1.000000																																		
Anoor	0.5279383	0.9951220	0.9975530	1.000000																																	
Johai	0.4770115	0.4990594	0.5000000	0.5009452	1.000000																																
Dhaji	0.4886364	0.5138879	0.5140187	0.5121495	0.5242054	1.000000																															
Katanga	0.4924242	0.5111524	0.5092937	0.5102421	0.5290954	0.9900990	1.000000																														
Chayras	0.4838757	0.5074674	0.5055556	0.5064935	0.5126329	0.9730392	0.9779412	1.000000																													
Aravali	0.4869421	0.5111111	0.5092993	0.5102041	0.5223301	0.9539952	0.9588378	0.9518072	1.000000																												
Dudhga	0.5123810	0.4784404	0.4761468	0.4781609	0.4776952	0.4917127	0.4899083	0.4862888	0.4899452	1.000000																											
Elakhal	0.4867424	0.5083799	0.5065177	0.5074627	0.4803828	0.9347826	0.9396135	0.9188544	0.9009434	0.4953789	1.000000																										
Glennan	0.4943182	0.5018430	0.5000000	0.4981530	0.5221411	0.5073629	0.9123223	0.8967136	0.9148936	0.4862888	0.9188544	1.000000																									
Kashik	0.5028571	0.4627002	0.4604119	0.4622853	0.4821764	0.4907407	0.4916821	0.4907749	0.4917127	0.9039913	0.4888889	0.4852941	1.000000																								
Karni	0.4838757	0.5102041	0.5083488	0.5092937	0.4800534	0.9212411	0.9200143	0.9192399	0.8925234	0.4917431	0.9562044	0.9101655	0.4907749	1.000000																							
Kohr	0.4821092	0.5121043	0.5102421	0.5111940	0.4852842	0.9210536	0.9212411	0.9190476	0.8878305	0.5046382	0.9222614	0.8700696	0.5065421	0.9373000	1.000000																						
Kuchipudi	0.4847328	0.5093985	0.5075188	0.5084746	0.4722962	0.9318735	0.9530388	0.9297821	0.9023810	0.4962687	0.9436275	0.8976190	0.4953896	0.9439024	0.9485294	1.000000																					
Mammoth	0.5442043	0.4880734	0.4862285	0.4844037	0.5833010	0.5912698	0.5952381	0.6033976	0.6107784	0.4781022	0.5988824	0.6100000	0.4743590	0.6100000	0.5964215	0.6072874	1.000000																				
Muruga	0.5093037	0.4940494	0.4836222	0.4840301	0.4804469	0.4889706	0.4899083	0.4944853	0.4899452	0.4867887	0.4926199	0.4808743	0.4804119	0.4890110	0.5046382	0.5046904	0.5000000	1.000000																			
Kandyl	0.4981203	0.4813514	0.4832731	0.4810138	0.4888060	0.5000000	0.4981618	0.5027624	0.4981685	0.4980872	0.4981530	0.4809437	0.4961538	0.5000000	0.5102041	0.5046729	0.5000000	0.9048724	1.000000																		
Rajapur	0.4465291	0.5018868	0.5028355	0.5009452	0.48079625	0.4817647	0.4852127	0.48372093	0.48333333	0.4859813	0.48738930	0.48372093	0.4805680	0.4867623	0.4897653	0.4881055	0.5931174	0.4887640	0.5142315	1.000000																	
Sagatha	0.4760077	0.4804545	0.4802272	0.48041002	0.4893617	0.4980916	0.4990476	0.4980980	0.5019011	0.4814643	0.4876660	0.4812030	0.4811493	0.4867925	0.5038881	0.4942308	0.4867424	0.4818873	0.48511628	0.4922481	1.000000																
Vandini	0.4550562	0.5046904	0.5056391	0.5063913	0.48135198	0.48615023	0.4861972	0.48511628	0.4847222	0.4860633	0.4857143	0.4841014	0.4894334	0.48729412	0.48594848	0.4860476	0.4891784	0.4944020	0.5198488	0.4949847	0.4951830	1.000000															
Wynad	0.4644195	0.5053970	0.5037313	0.5046729	0.48251748	0.48732394	0.48779343	0.48594887	0.48587963	0.4925926	0.4841608	0.48456221	0.48711963	0.48671329	0.48711944	0.48764846	0.4891984	0.4981413	0.5093284	0.5191176	0.4990403	0.4981481	1.000000														
Aimal	0.50257471	0.4973904	0.4993151	0.48512386	0.4933336	0.5018727	0.5028037	0.4990689	0.5084112	0.48409091	0.4971963	0.4990689	0.48212670	0.4933065	0.49253445	0.4981132	0.5046904	0.4802247	0.48187919	0.4990476	0.48253814	0.5018939	0.5200000	1.000000													
Ayer	0.4952562	0.4889655	0.4870977	0.4888636	0.4747664	0.4806630	0.4788891	0.4753199	0.4844037	0.4866667	0.4815498	0.4753199	0.4866819	0.4753199	0.4823046	0.4788889	0.4916512	0.4812898	0.4849886	0.4914934	0.4834988	0.4943608	0.4933271	0.5007392	1.000000												
Banashan	0.5187377	0.4722222	0.4730983	0.4739777	0.5929752	0.5846774	0.5887097	0.5843373	0.6077726	0.4568807	0.5922921	0.6036385	0.4450549	0.5939394	0.5803213	0.5788933	0.7823129	0.4622468	0.4786642	0.6029106	0.4592030	0.5987654	0.5939184	0.4802260	0.4784240	1.000000											
Gopani	0.4933078	0.48486239	0.48005747	0.4825346	0.47748930	0.4814126	0.4823748	0.4787431	0.4834399	0.4833130	0.4795339	0.4678889	0.5006306	0.4732965	0.4803335	0.4803002	0.4897196	0.48348387	0.4660308	0.4980843	0.4839626	0.4932362	0.4962264	0.4846467	0.4868224	0.4880451	1.000000										
Ego	0.5056604	0.4747153	0.4767123	0.4787185	0.4851301	0.4908425	0.4917733	0.4881603	0.4972578	0.4853535	0.4862888	0.4827386	0.4842438	0.4827386	0.4845173	0.4788991	0.4900425	0.48513514	0.4873000	0.5018797	0.48491879	0.4990689	0.5000000	0.4755760	0.4888889	0.4805195	0.5216152	1.000000									
Johai	0.510163	0.4735632	0.4712644	0.4889655	0.5009488	0.5065421	0.5046555	0.5009756	0.5102421	0.48333333	0.4907407	0.4981481	0.48261851	0.4880871	0.4916821	0.4888889	0.4923926	0.48333333	0.48481463	0.5030823	0.4817974	0.4981203	0.5018727	0.4800696	0.47803704	0.4849424	0.4814933	0.9383886	1.000000								
Serana	0.5153257	0.4867269	0.4849886	0.4869725	0.5028463	0.5055970	0.5065177	0.5027829	0.5121043	0.4827343	0.4953618	0.4944649	0.48161435	0.4917127	0.4962963	0.4974579	0.4861878	0.48314807	0.48382022	0.4971645	0.4806530	0.5028249	0.5039433	0.48637413	0.4855046	0.4868421	0.48321940	0.9180328	0.9495192	1.000000							
Selena	0.4797571	0.47994521	0.4803922	0.4813380	0.5913043	0.5859873	0.5802235	0.5822785	0.5960030	0.4833006	0.5906183	0.5889831	0.4880952	0.5889831	0.5780591	0.5824411	0.6133609	0.5009940	0.5009901	0.5913043	0.4989733	0.5974026	0.5910064	0.4940000	0.4980000	0.6426966	0.4988889	0.4940945	0.4990220	0.4980000	1.000000						
Kadum	0.8008589	0.4980989	0.4961977	0.4971429	0.4921775	0.4980789	0.5048170	0.4980843	0.5078923	0.4933336	0.4961538	0.5009597	0.4838710	0.4952199	0.4913938	0.4970874	0.5517928	0.4877127	0.4877389	0.4941176	0.4722753	0.4846743	0.4898610	0.4876190	0.5290581	0.4884615	0.5124283	0.5145068	0.5282651	0.4958678	1.000000						
Saga	0.5694165	0.4770642	0.4779412	0.4788214	0.4803861	0.5951904	0.6024096	0.5948104	0.6181818	0.4565217	0.5992976	0.6044177	0.4537374	0.4012024	0.5940000	0.5887097	0.7571744	0.4644809	0.4677227	0.5882731	0.4788015	0.5708383	0.5745527	0.4713494	0.4723247	0.7449664	0.4567219	0.4664247	0.4780148	0.4778398	0.6377181	0.5755510	1.000000				
Prabha	0.8052257	0.4811521	0.4820416	0.4829545	0.4950884	0.4951800	0.5019305	0.4952015	0.5048170	0.4876660	0.4990329	0.5038610	0.4808886	0.4952015	0.4913628	0.4941634	0.5366337	0.4848485	0.4793223	0.4836615	0.4824219	0.4637405	0.4704762	0.4809160	0.4704694	0.5109344	0.4826923	0.4924242	0.4857143	0.4961686	0.4922536	0.4891598	0.5976289	1.000000			
Prabha	0.7500000	0.4615385	0.4624277	0.4633305	0.4749499	0.4783465	0.4852071	0.4842530	0.4825329	0.4795332	0.4851485	0.4942530	0.4784314	0.4901186	0.4832347	0.4860000	0.5110664	0.4880851	0.4882081	0.4574257	0.4768612	0.4549020	0.4561404	0.4639376	0.4536680	0.4759036	0.4722277	0.4729730	0.4803482	0.4594595	0.5200000	0.4804516	0.5488565	0.8979038	1.000000		
Alleppey	0.7464455	0.4652720	0.4634615	0.4643545	0.4819277	0.4852071	0.4920949	0.4825529	0.4891945	0.4834308	0.4920635	0.4911243	0.4785625	0.4940711	0.4871795	0.490000																					

Table 62. Similarity matrix obtained from combined RAPD and ISSR data using Sorensen - Dice similarity coefficients in turmeric varieties/cultivars

Row\Col	Alboppy	Amalpur	Amizyan	Amoor	Johi	Daji	Kataga	Chayga	Aravind	Dudigan	Ethambal	Glycan	Karkhi	Kartzi	Koti	Kuchip	Mananthol	Morapga	Hudyal	Rajapur	Sagadha	Vendant	Wynad	Aimal	Ayer	Basakha	Gopani	Pajo	Jashat	Serana	Sekurus	Kodusan	Sigma	Prakha	Prathib	Alboppy			
Alboppy	1.000000																																						
Amalpur	0.6943396	1.000000																																					
Amizyan	0.6926952	0.9987790	1.000000																																				
Amoor	0.6910467	0.9975530	0.9987790	1.000000																																			
Johi	0.6439144	0.6638291	0.6666667	0.6675063	1.000000																																		
Daji	0.6564885	0.6806412	0.6790123	0.6773795	0.9606099	1.000000																																	
Kataga	0.6598985	0.6765068	0.6748768	0.6757090	0.9632446	0.9930249	1.000000																																
Chayga	0.6539924	0.6732187	0.6715867	0.6724138	0.9544304	0.9863354	0.9888476	1.000000																															
Aravind	0.6548673	0.6764706	0.6748466	0.6756757	0.9595960	0.9764560	0.9789864	0.9753086	1.000000																														
Dudigan	0.6773819	0.9353869	0.9339653	0.9351285	0.6465409	0.6592393	0.6578355	0.6543665	0.6576687	1.000000																													
Ethambal	0.6547771	0.6740741	0.6724351	0.6732673	0.9363868	0.9662921	0.9688667	0.9577114	0.9478808	0.6625464	1.000000																												
Glycan	0.6615970	0.6883047	0.6666667	0.6630246	0.9594937	0.9515528	0.9541512	0.9453446	0.9555536	0.6543665	0.9577114	1.000000																											
Karkhi	0.6692015	0.9262899	0.9260692	0.9261084	0.6506329	0.6583851	0.6592317	0.6584158	0.6592593	0.9495695	0.6567164	0.6534653	1.000000																										
Kartzi	0.6539924	0.6736757	0.6704667	0.6748768	0.9567089	0.9500062	0.9615861	0.9579208	0.9432099	0.6592866	0.9776119	0.9529703	0.6584158	1.000000																									
Koti	0.6505718	0.6733399	0.6757090	0.6765432	0.9263959	0.9580941	0.9590062	0.9578164	0.9405941	0.6707768	0.9600998	0.9505211	0.6724566	0.9677419	1.000000																								
Kuchip	0.6529563	0.6749889	0.6723187	0.6741573	0.9319641	0.9647355	0.9648241	0.9636326	0.9488859	0.6633416	0.9709962	0.9460477	0.6624843	0.9711418	0.9735949	1.000000																							
Mananthol	0.7048346	0.6598003	0.6543210	0.6526576	0.7369759	0.7431421	0.7462687	0.7503106	0.7383643	0.6469136	0.7490637	0.7577640	0.6434783	0.7577640	0.7471980	0.7556675	1.000000																						
Morapga	0.6730630	0.9133089	0.9119804	0.9130967	0.6490566	0.6567901	0.6576355	0.6617466	0.6576687	0.9193154	0.6600742	0.6494465	0.9249692	0.6588266	0.6707768	0.6708229	0.6666667	1.000000																					
Hudyal	0.6649937	0.9197080	0.9208283	0.9195122	0.6566416	0.6666667	0.6653007	0.6691176	0.6670367	0.9133201	0.6630246	0.6495208	0.9166667	0.6666667	0.6756757	0.6708075	0.6666667	0.9500609	1.000000																				
Rajapur	0.6173800	0.6683417	0.6691824	0.6675063	0.8937824	0.9199492	0.9001521	0.9113924	0.9090909	0.6540881	0.9338422	0.9113924	0.6382278	0.9291139	0.9187817	0.9293967	0.7445997	0.6566038	0.6791980	1.000000																			
Sagadha	0.6449935	0.8916877	0.8902800	0.8914141	0.6571429	0.6464082	0.6638196	0.6649746	0.6683544	0.8978562	0.6556122	0.6497462	0.8959391	0.6548223	0.6892112	0.6615187	0.6547771	0.9003783	0.9195980	0.6597403	1.000000																		
Vendant	0.6524826	0.6708229	0.6716604	0.6723000	0.8971722	0.9233990	0.9283019	0.9195980	0.9172952	0.6541823	0.9393939	0.9085477	0.6680840	0.9321608	0.9244332	0.9299563	0.7414880	0.6616729	0.6840796	0.9742951	0.6623711	1.000000																	
Wynad	0.6394211	0.6716233	0.6699752	0.6708075	0.9042146	0.9523308	0.9350000	0.9238452	0.9240249	0.6600996	0.9385194	0.9163546	0.6641698	0.9288390	0.9311640	0.9341772	0.7493734	0.6630124	0.6749073	0.9578544	0.6658131	0.9733840	1.000000																
Aimal	0.6715557	0.9173859	0.9185185	0.9196539	0.6687370	0.6683292	0.6681542	0.6658385	0.6741016	0.9135802	0.6641698	0.6583865	0.9018634	0.6608096	0.6625156	0.6649874	0.6708229	0.9012246	0.9002890	0.6638196	0.9044586	0.6683480	0.6842105	1.000000															
Ayer	0.6624365	0.9288873	0.9310345	0.9297164	0.6438530	0.6499237	0.6476427	0.6443618	0.6526576	0.9257814	0.6500823	0.6443618	0.9169765	0.6443618	0.6509317	0.6457286	0.6592040	0.9137931	0.9278074	0.6590621	0.9097840	0.6616352	0.6625000	0.9477612	1.000000														
Basakha	0.6831169	0.6412094	0.6423174	0.6431274	0.7444877	0.7379135	0.7411168	0.7376426	0.7560051	0.6272040	0.7434940	0.7528517	0.6159696	0.7452071	0.7344346	0.7326478	0.8778626	0.6322418	0.6474279	0.7522698	0.6293888	0.7490347	0.7468031	0.6488530	0.6472081	1.000000													
Gopani	0.6606914	0.9181141	0.9192547	0.9203980	0.6419437	0.6499573	0.6508135	0.6475000	0.6508728	0.9093168	0.6482412	0.6575000	0.9075000	0.6423000	0.6541353	0.6489227	0.6574655	0.9217391	0.9282178	0.6494616	0.9128205	0.6624365	0.6633039	0.9134253	0.9286608	0.6576440	1.000000												
Pajo	0.6716792	0.9317313	0.9340066	0.9354446	0.6533166	0.6584767	0.6593137	0.6560598	0.6642247	0.9224111	0.6543665	0.6511628	0.9155447	0.6511628	0.6527407	0.6476427	0.6584767	0.9197080	0.9333333	0.6683354	0.9184442	0.6638385	0.6666667	0.9336609	0.9411765	0.6491228	0.9592089	1.000000											
Jashat	0.6729494	0.9325153	0.9312039	0.9298893	0.6675095	0.6724566	0.6707921	0.6674907	0.6757090	0.9090909	0.6583851	0.6650185	0.9048828	0.6674000	0.6592317	0.6564416	0.6604096	0.9080909	0.9179927	0.6700379	0.8989753	0.8649937	0.6883292	0.9305211	0.9306951	0.6531646	0.9313338	0.9482152	1.000000										
Serana	0.6801517	0.9289216	0.9278074	0.9287469	0.6691919	0.6716233	0.6724351	0.6691358	0.6773399	0.9055215	0.6625310	0.6617284	0.8987654	0.9592593	0.6633663	0.6608260	0.6542751	0.9079755	0.9119804	0.6641414	0.8911392	0.6691729	0.6699875	0.9268897	0.9221261	0.6540673	0.9201995	0.9572650	0.9741060	1.000000									
Sekurus	0.6484268	0.6481481	0.6490066	0.6498674	0.7431694	0.7389538	0.7422321	0.7360000	0.7473404	0.6516536	0.7426273	0.7413333	0.6560000	0.7413333	0.7326203	0.7361299	0.7603748	0.6675497	0.6675462	0.7431694	0.6657534	0.7479975	0.7429341	0.6613119	0.6648865	0.7624887	0.6657882	0.6613966	0.6657790	0.6648936	1.000000								
Kodusan	0.8930401	0.6647946	0.6632783	0.6641221	0.6569859	0.6649551	0.6780934	0.6649616	0.6734694	0.6607730	0.6633291	0.6675192	0.6521739	0.6624041	0.6589744	0.6640726	0.7111682	0.6555544	0.6556962	0.6413613	0.6614173	0.6415584	0.6529032	0.6700899	0.6555898	0.8920052	0.6563307	0.6776233	0.6794361	0.6913265	0.6629634	1.000000							
Sigma	0.7256410	0.6439627	0.6467662	0.6475716	0.7528809	0.6422312	0.7518797	0.7459324	0.7640449	0.6268657	0.7498855	0.7534418	0.7452949	0.7411168	0.6618090	0.6543284	0.6569269	0.7247119	0.6290116	0.7268107	0.7297980	0.6407035	0.6416040	0.8538462	0.6270544	0.6361386	0.6450000	0.6466916	0.7955223	0.7322122	1.000000								
Prakha	0.8921023	0.6498915	0.6525102	0.6512410	0.6622865	0.6623711	0.6683805	0.6623877	0.6709347	0.6556122	0.6638065	0.6700899	0.6495507	0.6623877	0.6580447	0.6614583	0.6984526	0.6530612	0.6480305	0.6533771	0.6580564	0.6536375	0.6580864	0.6494845	0.6401028	0.6763138	0.6511025	0.6598985	0.6338462	0.6625252	0.6601942	0.9428951	0.7402597	1.000000					
Prathib	0.8571429	0.6515789	0.6520111	0.6520454	0.6440217	0.6471372	0.6530865	0.6525199	0.6507937	0.6482213	0.6533333	0.6525199	0.6472149	0.6578049	0.6513957	0.6541059	0.6764314	0.6576812	0.6577953	0.6277174	0.6457766	0.6253369	0.6265060	0.6538216	0.6241700	0.6448980	0.6461126	0.6422018	0.6504636	0.6296286	0.6666667	0.8928571	0.7087248	0.9462089	1.0000				

Table 63. Similarity matrix obtained from combined RAPD and ISSR data using Simple Matching similarity coefficients in turmeric varieties/cultivars.

Row/Col	Alhappay	Amalapur	Amritpur	Armoor	Johali	Dhaji	Katanga	Chayapa	Aravindal	Dindigul	Ethambal	GLyanam	Kahali	Kotai	Kolar	Kuchipudi	Manmadh	Muratsap	Ramyl	Rajapur	Saguntha	Yendim	Wyal	Aiswal	Ayer	Basankin	Gupani	Haj	Jochai	Servana	Julesan	Kolaram	Sagun	Prabha	Pratik	Alhappay	
Alhappay	1.000000																																				
Amalapur	0.6295732	1.000000																																			
Amritpur	0.6280488	0.9984756	1.000000																																		
Armoor	0.6265244	0.9989512	0.9984756	1.000000																																	
Johali	0.5838415	0.5945122	0.5802366	0.5975610	1.000000																																
Dhaji	0.5884146	0.6051829	0.6036385	0.6021341	0.9327439	1.000000																															
Katanga	0.5914634	0.5930854	0.5975610	0.5930854	0.9557927	0.9939024	1.000000																														
Chayapa	0.5838415	0.5945122	0.5929878	0.5945122	0.9451220	0.9832317	0.9862805	1.000000																													
Aravindal	0.5838415	0.5975610	0.5802366	0.5975610	0.9512195	0.9718366	0.9740854	0.9695122	1.000000																												
Dindigul	0.6097561	0.9192073	0.9178829	0.9192073	0.5716463	0.5792683	0.5762195	0.5716463	0.5746951	1.000000																											
Ethambal	0.5868902	0.5975610	0.5802366	0.5975610	0.9237805	0.9388415	0.94618902	0.9481707	0.9359756	0.5838415	1.000000																										
GLyanam	0.5929878	0.5884146	0.5889902	0.5833639	0.9512195	0.9405488	0.9433976	0.9329268	0.9451220	0.5716463	0.9481707	1.000000																									
Kahali	0.6021341	0.9082366	0.9078122	0.9082366	0.5792683	0.5807927	0.5807927	0.5792683	0.5792683	0.9373000	0.5792683	0.5731707	1.000000																								
Kotai	0.5838415	0.5975610	0.5802366	0.5975610	0.9237805	0.9496951	0.9527439	0.9481707	0.9298780	0.5777439	0.9725610	0.9420732	0.5792683	1.000000																							
Kolar	0.5807927	0.6008098	0.5930854	0.6008098	0.9115854	0.9496951	0.9496951	0.9481707	0.9268293	0.5929878	0.9512195	0.9146341	0.5975610	0.9802366	1.000000																						
Kuchipudi	0.5884146	0.6021341	0.6008098	0.6021341	0.9192073	0.9573171	0.9573171	0.9573171	0.9373000	0.5884146	0.9649390	0.9345112	0.5899290	0.9649390	0.5679878	1.000000																					
Manmadh	0.6463415	0.5746951	0.5731707	0.5716463	0.6844512	0.6839796	0.6890244	0.6933976	0.7027439	0.5640244	0.6933976	0.7027439	0.5623000	0.7027439	0.6905488	0.7046341	1.000000																				
Muratsap	0.6067073	0.8917683	0.8902439	0.8917683	0.5746951	0.5762195	0.5762195	0.5807927	0.5746951	0.8993902	0.5807927	0.5655488	0.9078122	0.5746951	0.5929878	0.5975610	0.5884146	1.000000																			
Ramyl	0.5929878	0.8993902	0.9009146	0.8993902	0.5823171	0.5868902	0.5838415	0.5884146	0.5823171	0.8917683	0.5833639	0.5640244	0.8963415	0.5833639	0.5975610	0.5868902	0.9373000	1.000000																			
Rajapur	0.5503049	0.5975610	0.5990854	0.5975610	0.8750000	0.9039634	0.9039634	0.8952927	0.8902439	0.5807927	0.9207317	0.8952927	0.5884146	0.9146341	0.9024390	0.9161585	0.8933976	0.5838415	0.6097561	1.000000																	
Saguntha	0.5838415	0.8889024	0.8673780	0.8889024	0.5975610	0.5990854	0.5990854	0.5975610	0.6008098	0.7862344	0.5884146	0.5792683	0.8750000	0.5833639	0.6026385	0.5990854	0.5868902	0.8795732	0.9024390	0.6008098	1.000000																
Yendim	0.5564024	0.5975610	0.5990854	0.6008098	0.8780488	0.9100610	0.9131098	0.9024390	0.8993902	0.5777439	0.9268293	0.8902439	0.5884146	0.9178829	0.9082366	0.9161585	0.8875000	0.5868902	0.6128049	0.9695122	0.6008098	1.000000															
Wyal	0.5640244	0.5960266	0.5945122	0.5960266	0.8856707	0.9178829	0.9207317	0.9078122	0.9078122	0.5823171	0.9253049	0.8978639	0.5899390	0.9131098	0.9161585	0.9207317	0.8951220	0.5884146	0.5990854	0.9496951	0.6021341	0.9479878	1.000000														
Aiswal	0.6067073	0.8978639	0.8993902	0.9009146	0.5929878	0.5945122	0.5945122	0.5899390	0.5990854	0.8932927	0.5899390	0.5899390	0.8795732	0.5838415	0.5868902	0.5945122	0.5975610	0.8780488	0.8762344	0.5990854	0.8856707	0.5990854	0.6158237	1.000000													
Ayer	0.5945122	0.9131098	0.9146341	0.9131098	0.5716463	0.5701220	0.5670732	0.5623000	0.5716463	0.9115854	0.5716463	0.5623000	0.8978639	0.5623000	0.5716463	0.5701220	0.5823171	0.8952927	0.9100610	0.5899390	0.8917683	0.5899390	0.5884146	0.9359756	1.000000												
Basankin	0.6280488	0.5655488	0.5670732	0.5683976	0.6996951	0.6839796	0.6890244	0.6844512	0.7057927	0.5487805	0.6933976	0.7027439	0.5381098	0.6933976	0.6814024	0.6829268	0.8356385	0.5548780	0.5716463	0.7088415	0.5655488	0.7027439	0.6981707	0.5792683	0.5762195	1.000000											
Gupani	0.5802366	0.8993902	0.9009146	0.9024390	0.5731707	0.5746951	0.5746951	0.5701220	0.5731707	0.8887195	0.5731707	0.5792683	0.8871951	0.5640244	0.5792683	0.5777439	0.5838415	0.9039634	0.9115854	0.6008098	0.8963415	0.5945122	0.3929878	0.8948171	0.9131098	0.5683976	1.000000										
Haj	0.6008098	0.9161585	0.9178829	0.9192073	0.5777439	0.5762195	0.5762195	0.5716463	0.5807927	0.9024390	0.5716463	0.5655488	0.8948171	0.5655488	0.5683976	0.5670732	0.5762195	0.8993902	0.9161585	0.5802366	0.9009146	0.5899390	0.5884146	0.9178829	0.9268293	0.5731707	0.9496951	1.000000									
Jochai	0.6097561	0.9161585	0.9146341	0.9131098	0.5990854	0.5975610	0.5945122	0.5899390	0.5990854	0.8871951	0.5807927	0.5868902	0.8826220	0.5777439	0.5807927	0.5823171	0.5823171	0.8871951	0.8978639	0.6021341	0.8795732	0.5929878	0.5945122	0.9146341	0.9146341	0.5823171	0.9161585	0.9802366	1.000000								
Servana	0.6143293	0.9115854	0.9100610	0.9115854	0.6008098	0.5802366	0.5802366	0.5914634	0.6008098	0.8826220	0.5833639	0.5823171	0.8750000	0.5792683	0.5833639	0.5868902	0.5746951	0.8856707	0.8902439	0.5945122	0.8890244	0.5975610	0.5802366	0.9100610	0.9029634	0.5838415	0.9024390	0.9466463	0.9679878	1.000000							
Julesan	0.6082317	0.5945122	0.5802366	0.5975610	0.7134146	0.7027439	0.7057927	0.6981707	0.7103639	0.5990854	0.703171	0.7042683	0.6067073	0.7042683	0.6951220	0.7027439	0.7271341	0.6173780	0.6138337	0.7134146	0.6280488	0.7146341	0.7088415	0.6143293	0.6173780	0.7576220	0.6219512	0.6082317	0.6173780	0.6158237	1.000000						
Kolaram	0.7862344	0.5975610	0.5802366	0.5975610	0.6036385	0.6021341	0.6082317	0.6008098	0.6097561	0.5929878	0.6008098	0.6036385	0.5833639	0.5975610	0.5945122	0.6051829	0.6270122	0.5868902	0.5833639	0.5823171	0.6067073	0.5792683	0.5899390	0.6082317	0.5899390	0.6417683	0.5945122	0.6112805	0.6173780	0.6310976	0.6280488	1.000000					
Sagun	0.6737805	0.5655488	0.5670732	0.5683976	0.7057927	0.6970732	0.6981707	0.6905488	0.7118902	0.5426829	0.6966463	0.6966951	0.5440273	0.6966463	0.6905488	0.6890244	0.8232171	0.5518293	0.5533337	0.6722561	0.5394512	0.6722561	0.6737805	0.5640244	0.5640244	0.8262195	0.5302049	0.5518293	0.5670732	0.5683976	0.7667683	0.6844512	1.000000				
Prabha	0.8793000	0.3807927	0.3823171	0.3838415	0.6082317	0.6008098	0.6067073	0.5990854	0.6082317	0.5884146	0.6051829	0.6082317	0.3838415	0.5990854	0.5802366	0.6036385	0.6432927	0.5833639	0.5777439	0.5746951	0.5802366	0.5716463	0.5762195	0.5833639	0.5731707	0.6220000	0.3899390	0.5914634	0.5884146	0.5990854	0.6262344	0.9345112	0.6951220	1.000000			
Pratik	0.8399390	0.5731707	0.5746951	0.5762195	0.6008098	0.5802366	0.6021341	0.6008098	0.5975610	0.5929878	0.6036385	0.6008098	0.6082317	0.6295732	0.5807927	0.5792683	0.5823171	0.6036385	0.5762195	0.5746951	0.5807927	0.5683976	0.6021341	0.5975610	0.5838415	0.5746951	0.5731707	0.6463415	0.8810976	0.6692073	0.9405488	1.000000					
Alhappay	0.8368902	0.5731707	0.5746951	0.5762195	0.6067073	0.6021341	0.6082317	0.5975610	0.6036385	0.5802366	0.6097561	0.6067073	0.5914634	0.6097561	0.6036385	0.6112805	0.6295732	0.5807927	0.5823171	0.5833639	0.6097561	0.5792683	0.5777439	0.5838415	0.5716463	0.6051829	0.6008098										

The UPGMA dendrogram constructed using the genetic similarities obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on the combined RAPD and ISSR markers are given in Figs. 62 – 64. In all the three dendrograms, the clustering pattern was similar and produced four different groups by splitting at coefficients 0.62, 0.76 and 0.71, respectively. The different members in the groups are:

- Group I - 'Alleppey', 'Kedaram', 'Prabha', 'Prathibha', and 'Alleppey Supreme'
- Group II - 'Jabedi', 'GL Puram', 'Dhagi', 'Katergia', 'Chayapasupu', 'Avanigadda', 'Ethamkula', 'Kasturi', 'Kodur', 'Kuchipudi', 'Rajapuri', 'Vondimita' and 'Wynad local'
- Group III - 'Mamomthody', 'Basar Along', 'Suguna' and 'Sudarsana'
- Group IV - 'Amalapuram', 'Amrithapani', 'Armoor', 'Dindigam', 'Karhadi local', 'Aizwal', 'Ayur', Gaspani, Hajo, 'Jorhat', 'Suvarna', 'Muvatupuzha', 'Nandyal' and 'Sugandham'

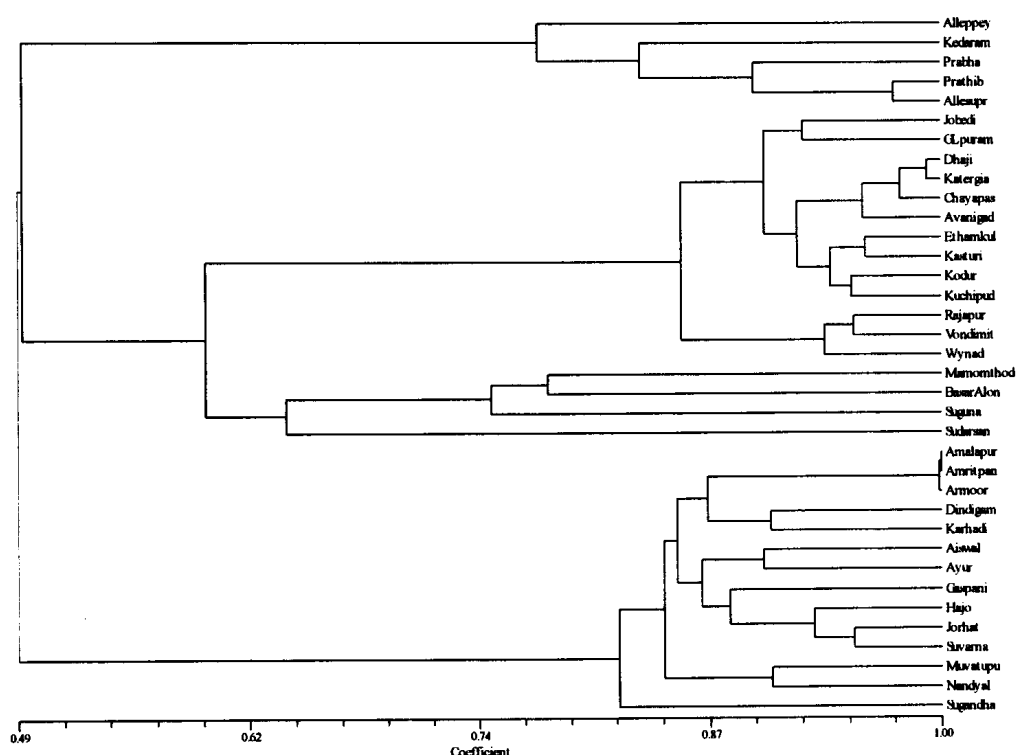


Fig. 62. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD and ISSR markers using Jaccard's similarity coefficients.

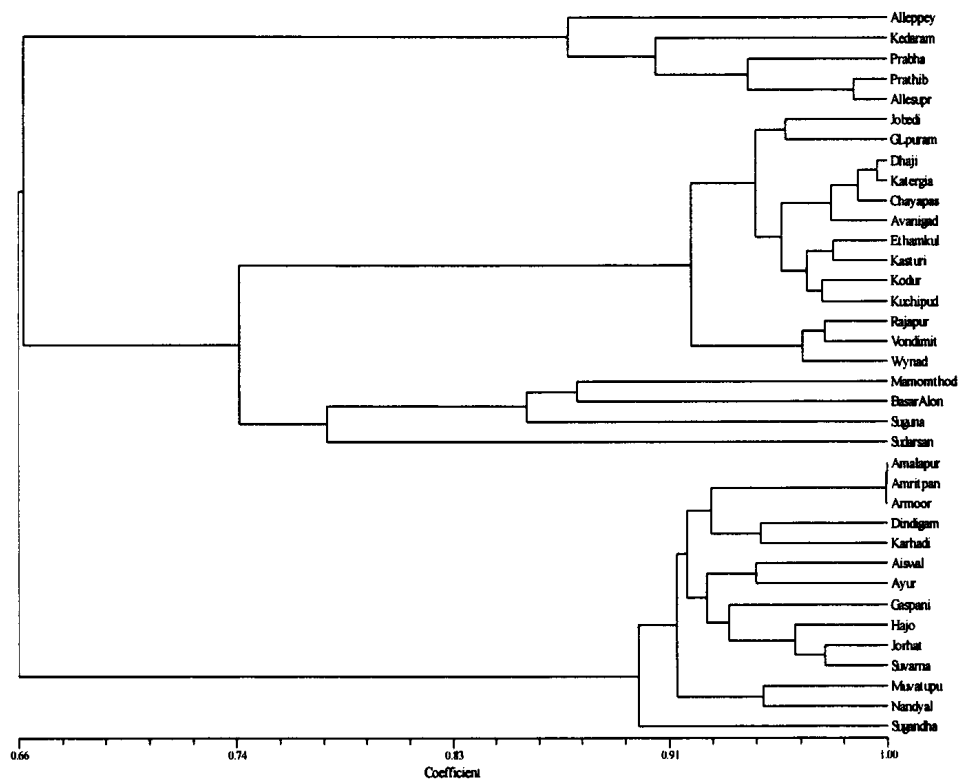


Fig. 63. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD and ISSR markers using Sorensen - Dice similarity coefficients.

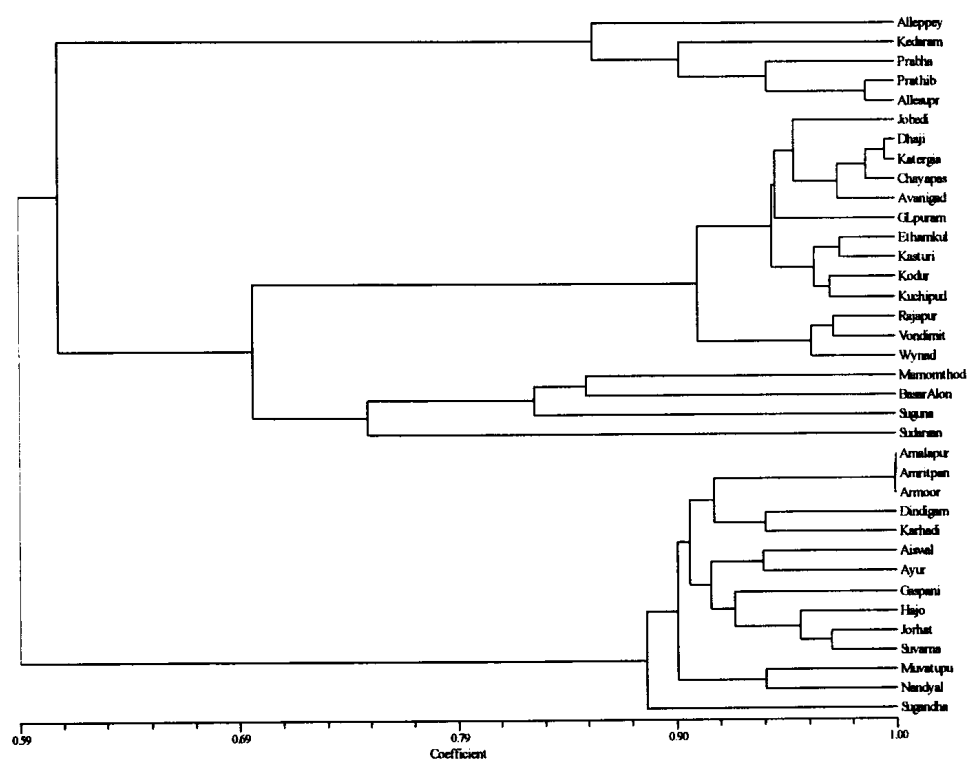


Fig. 64. Dendrogram of genetic similarity between 36 turmeric varieties/cultivars based on RAPD and ISSR markers using Simple Matching similarity coefficients.

The degree of similarity of the accessions in the different groups of the dendrogram constructed using three similarity coefficients from the combined RAPD and ISSR marker data are given in Table. 64.

Table 64. Highest and lowest similarities between the varieties and cultivars in the different groups of the dendrogram produced by three similarity coefficients using RAPD and ISSR marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	'Armoor', 'Amalapuram' & 'Amrithapani' (0.99)	'Armoor', 'Amalapuram' & 'Amrithapani' (0.99)	'Armoor', 'Amalapuram' & 'Amrithapani' (0.99)
	Lowest	'Sugandham' & 'Armoor' (0.80)	'Sugandham' & 'Armoor' (0.89)	'Sugandham' & 'Armoor' (0.87)
II	Highest	'Mamomthody' & 'Basar Along' (0.78)	'Mamomthody' & 'Basar Along' (0.88)	'Mamomthody' & 'Basar Along' (0.85)
	Lowest	'Sudarsana' & 'Mamomthody' (0.61)	'Sudarsana' & 'Mamomthody' (0.76)	'Sudarsana' & 'Mamomthody' (0.73)
III	Highest	'Dhagi' & 'Katergia' (0.99)	'Dhagi' & 'Katergia' (0.99)	'Dhagi' & 'Katergia' (0.99)
	Lowest	'Rajapuri' & 'Jabedi' (0.81)	'Rajapuri' & 'Jabedi' (0.89)	'Rajapuri' & 'Jabedi' (0.86)
IV	Highest	'Prathibha' & 'Alleppey Suprem (0.97)	'Prathibha' & 'Alleppey Suprem (0.99)	'Prathibha' & 'Alleppey Suprem (0.98)
	Lowest	'Alleppey' & 'Alleppey Supreme' (0.75)	'Alleppey' & 'Alleppey Supreme' (0.85)	'Alleppey' & 'Alleppey Supreme' (0.84)

4.2.10. Characterization of turmeric varieties/cultivars using rhizome DNA

A total of sixteen RAPD primers were used for amplifying the DNA isolated from the fresh mature rhizomes of turmeric to characterize the varieties/cultivars. The different primers studied produced a total of one hundred and fourty three scorable bands among the twelve samples and one hundred and ten were polymorphic bands amounting to 76.92 % of the bands produced. Maximum number of bands and polymorphic bands (93.33) were produced by primer OPC 07. The least number of bands were produced by the primers OPC 05 & OPC 06 with five scorable bands each and the least polymorphic primer was OPA 18 with 50% polymorphic bands (Figs. 65 - 66).

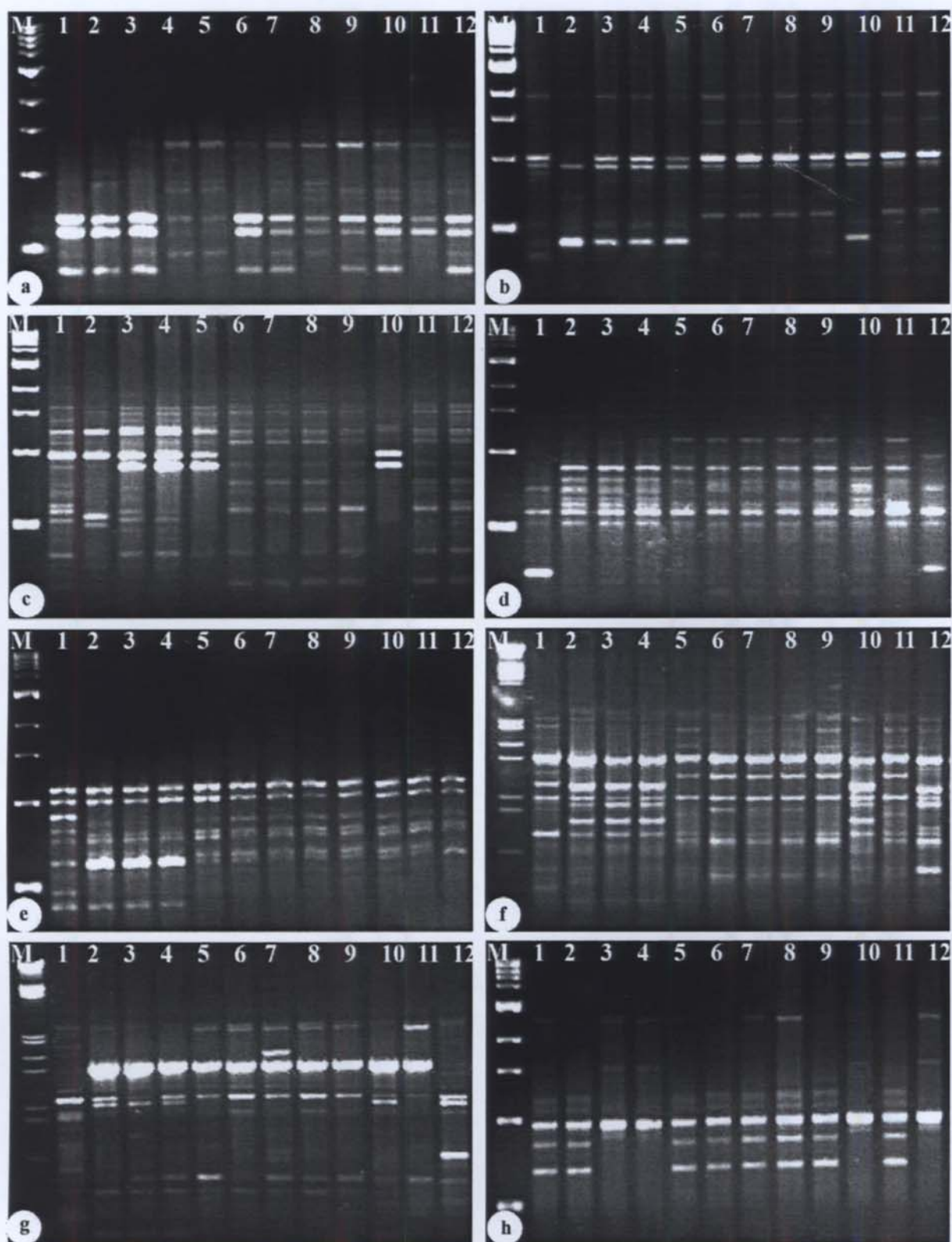


Fig. 65. a-h are the RAPD profiles of turmeric rhizome DNA using primers OPA 03, OPA 08, OPA 12, OPA 17, OPA 18, OPB 11, OPB 12 and OPB 14. Lane 1-12 are 1) 'Alleppey', 2) 'Amalapuram', 3) 'Armoor', 4) 'Amrithapani', 5) 'Dhagi', 6) 'Jabedi', 7) 'Prabha', 8) 'Katergia', 9) 'Prathibha', 10) 'Suvarna', 11) 'Suguna' and 12) 'Sudarsana', respectively. M - Marker 1 Kb ladder (Biogene, USA).

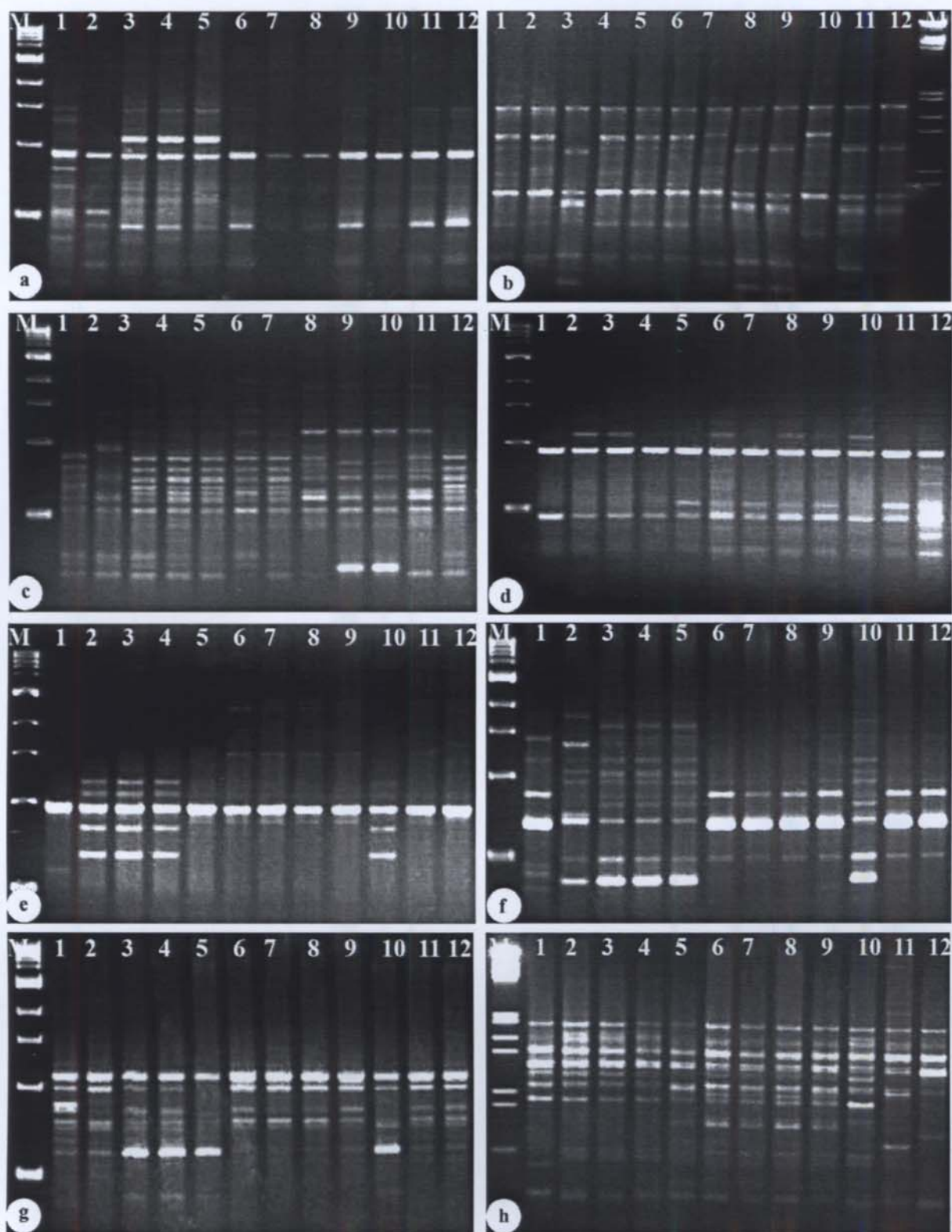


Fig. 66. a-h are the RAPD profiles of turmeric rhizome DNA using primers OPB 18, OPB 19, OPC 03, OPC 05, OPC 06, OPC 07, OPC 09 and OPC 12. Lane 1-12 are 1) 'Alleppey', 2) 'Amalapuram', 3) 'Armoor', 4) 'Amrithapani', 5) 'Dhagi', 6) 'Jabedi', 7) 'Prabha', 8) 'Katergia', 9) 'Prathibha', 10) 'Suvarna', 11) 'Suguna' and 12) 'Sudarsana', respectively. M - Marker 1 Kb ladder (Biogene, USA).

Table 65 gives the details of the RAPD primers used with sequences, total number of bands generated by each primer, number of polymorphic bands and percentage polymorphism.

Table 65. Sequence of RAPD primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in turmeric varieties/ cultivars using rhizome DNA

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	OPA 03	AGTCAGCCAC	8	6	75.0
2	OPA 08	GTGACGTAGG	6	4	66.67
3	OPA 12	TCGGCGATAG	14	13	92.86
4	OPA 17	GACCGCTTGT	8	7	87.5
5	OPA 18	AGGTGACCGT	8	4	50.0
6	OPB 11	GTAGACCCGT	10	7	70.0
7	OPB 12	CCTTGACGCA	9	8	88.89
8	OPB 14	TCCGCTCTGG	6	5	83.33
9	OPB 18	CCACAGCAGT	8	6	75.0
10	OPB 19	ACCCCCGAAG	9	7	77.78
11	OPC 03	GGGGGTCTTT	11	8	72.72
12	OPC 05	GATGACCGCC	5	3	60.0
13	OPC 06	GAACGGACTC	5	4	80.0
14	OPC 07	GTCCCGACGA	15	14	93.33
15	OPC 09	CTCACCGTCC	6	4	66.67
16	OPC 12	TGTCATCCCC	15	10	66.67
Total			143	110	76.92

4.2.10. 1. Cluster analysis

The genetic similarity matrices obtained using Jaccard's, Sorensen – Dice and Simple Matching coefficients are given in Table 66-68. In all the similarity matrices, the cultivar 'Amrithapani' & 'Armoor' showed maximum similarity between them (0.88, 0.93 and 0.92, respectively). The least similarity was observed between the variety 'Prathibha' and the cultivar 'Amrithapani' (0.47, 0.64 and 0.55 in Jaccard's, Sorensen – Dice and Simple Matching coefficients, respectively).

Table 66. Similarity matrix obtained from RAPD data using Jaccard's similarity coefficients in turmeric varieties/ cultivars using rhizome DNA.

Rows\Cols	Suvarna	Suguna	Prabha	Prathubha	Alleppey	Jabedi	Katergia	Amrithapani	Armoor	Sudarsana	Dhagi	Amalapuram
Suvarna	1.0000000											
Suguna	0.5130435	1.0000000										
Prabha	0.5803571	0.6194690	1.0000000									
Prathubha	0.5714286	0.6106195	0.8686869	1.0000000								
Alleppey	0.5636364	0.5614035	0.6160714	0.6981132	1.0000000							
Jabedi	0.6226415	0.5614035	0.5338983	0.5254237	0.6923077	1.0000000						
Katergia	0.5137615	0.5132743	0.4871795	0.4913793	0.6732673	0.7789474	1.0000000					
Amrithapani	0.4910714	0.5043478	0.5042735	0.4705882	0.6442308	0.8191489	0.8222222	1.0000000				
Armoor	0.5454545	0.5304348	0.5565217	0.4957983	0.6111111	0.8315789	0.7765957	0.8777778	1.0000000			
Sudarsana	0.4736842	0.6415094	0.6238532	0.6296296	0.5925926	0.5636364	0.5420561	0.5321101	0.5596330	1.0000000		
Dhagi	0.5277778	0.4741379	0.5000000	0.4913793	0.6095238	0.7422680	0.7608696	0.8222222	0.7956989	0.4864865	1.0000000	
Amalapuram	0.5688073	0.4628099	0.5652174	0.5431034	0.5217391	0.6355140	0.6310680	0.6190476	0.6634615	0.5000000	0.6633663	1.0000000

Table 67. Similarity matrix obtained from RAPD data using Sorensens - Dice similarity coefficients in turmeric varieties/ cultivars using rhizome DNA.

Rows\Cols	Suvarna	Suguna	Prabha	Prathubha	Alleppey	Jabedi	Katergia	Amrithapani	Armoor	Sudarsana	Dhagi	Amalapuram
Suvarna	1.0000000											
Suguna	0.6781609	1.0000000										
Prabha	0.7344633	0.7650273	1.0000000									
Prathubha	0.7272727	0.7582418	0.9297297	1.0000000								
Alleppey	0.7209302	0.7191011	0.7624309	0.8222222	1.0000000							
Jabedi	0.7674419	0.7191011	0.6961326	0.6888889	0.8181818	1.0000000						
Katergia	0.6787879	0.6783626	0.6551724	0.6589595	0.8047337	0.8757396	1.0000000					
Amrithapani	0.6586826	0.6705202	0.6704545	0.6400000	0.7836257	0.9005848	0.9024390	1.0000000				
Armoor	0.7058824	0.6931818	0.7150838	0.6629213	0.7586207	0.9080460	0.8742515	0.9349112	1.0000000			
Sudarsana	0.6428571	0.7816092	0.7683616	0.7727273	0.7441860	0.7209302	0.7030303	0.6946108	0.7176471	1.0000000		
Dhagi	0.6909091	0.6432749	0.6666667	0.6589595	0.7573964	0.8520710	0.8641975	0.9024390	0.8862275	0.6545455	1.0000000	
Amalapuram	0.7251462	0.6327684	0.7222222	0.7039106	0.6857143	0.7771429	0.7738095	0.7647059	0.7976879	0.6666667	0.7976190	1.0000000

Table 68. Similarity matrix obtained from RAPD data using Simple Matching similarity coefficients in turmeric varieties/ cultivars using rhizome DNA.

Rows\Cols	Suvarna	Suguna	Prabha	Prathubha	Alleppey	Jabedi	Katergia	Amrithapani	Armoor	Sudarsana	Dhagi	Amalapuram
Suvarna	1.0000000											
Suguna	0.6083916	1.0000000										
Prabha	0.6643357	0.6923077	1.0000000									
Prathubha	0.6573427	0.6853147	0.9090909	1.0000000								
Alleppey	0.6573427	0.6433566	0.6993007	0.7762238	1.0000000							
Jabedi	0.7202797	0.6503497	0.6083916	0.6013986	0.7692308	1.0000000						
Katergia	0.6293706	0.6153846	0.5734266	0.5804196	0.7622378	0.8531469	1.0000000					
Amrithapani	0.6013986	0.6013986	0.5874126	0.5524476	0.7342657	0.8811189	0.8881119	1.0000000				
Armoor	0.6503497	0.6223776	0.6363636	0.5734266	0.6993007	0.8881119	0.8531469	0.9230769	1.0000000			
Sudarsana	0.5804196	0.7342657	0.7062937	0.7132867	0.6853147	0.6643357	0.6573427	0.6433566	0.6643357	1.0000000		
Dhagi	0.6433566	0.5734266	0.5874126	0.5804196	0.7062937	0.8251748	0.8461538	0.8881119	0.8671329	0.6013986	1.0000000	
Amalapuram	0.6713287	0.5454545	0.6433566	0.6223776	0.6083916	0.7272727	0.7342657	0.7202797	0.7552448	0.6013986	0.7622378	1.0000000

The UPGMA dendrogram constructed using the genetic similarities obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on the RAPD markers are given in Fig. 67 – 69. In all the three dendrograms, the clustering pattern was similar and produced four different groups by splitting at coefficients 0.60, 0.75 and 0.70, respectively. The different members in the groups are

- Group I - 'Suvarna'
 Group II - 'Jabedi', 'Amrithapani', 'Armoor', 'Katergia', 'Dhagi' and 'Amalapuram'.
 Group III - 'Suguna' and 'Sudarsana'
 Group IV - 'Prabha', 'Prathibha' & 'Alleppey'

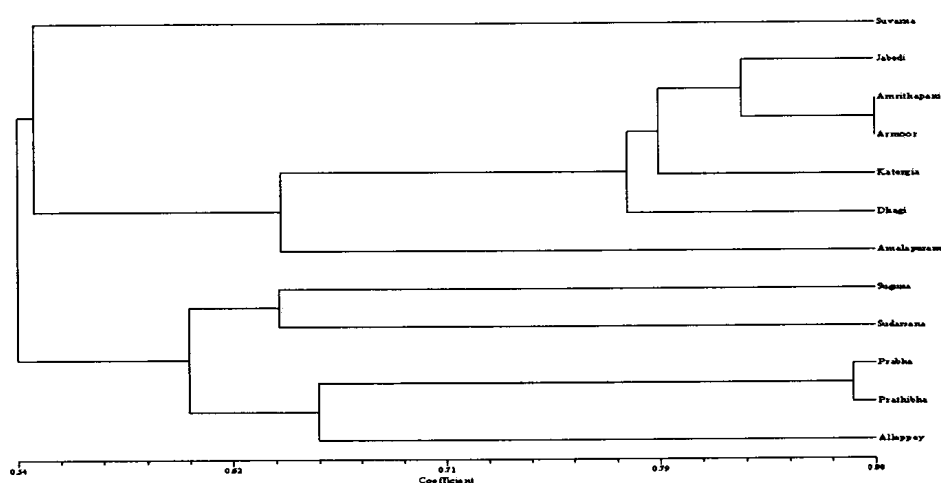


Fig. 67. Dendrogram of genetic similarity between 12 turmeric varieties/cultivars based on RAPD analysis using Jaccard's similarity coefficients.

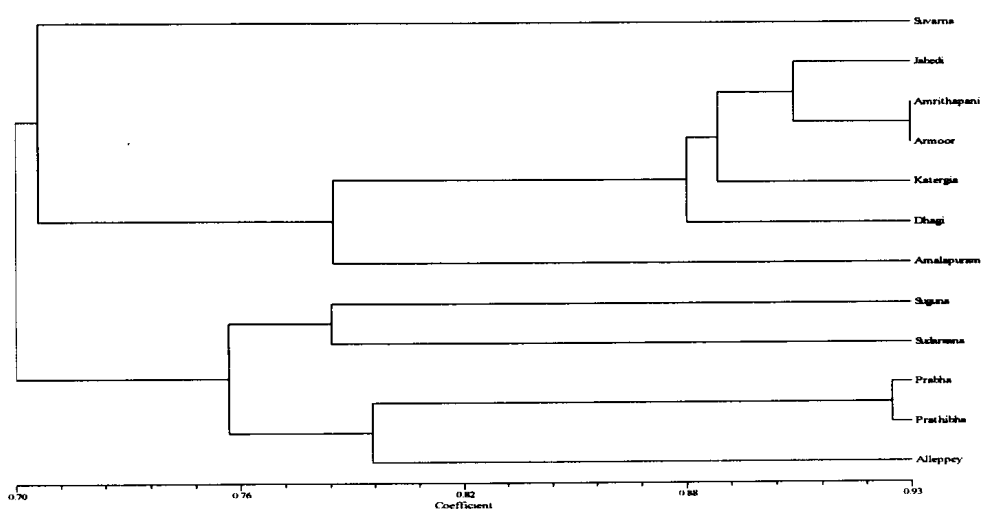


Fig. 68. Dendrogram of genetic similarity between 12 turmeric varieties/cultivars based on RAPD analysis using Sorensens - Dice similarity coefficients.

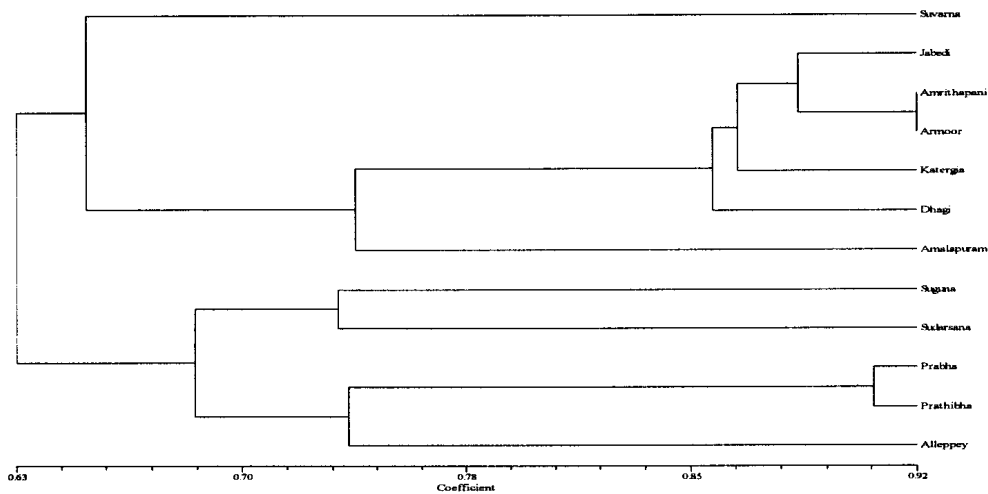


Fig. 69. Dendrogram of genetic similarity between 12 turmeric varieties/cultivars based on RAPD analysis using Simple Matching similarity coefficients.

Eventhough the grouping of varieties/cultivars were same, the degree of similarity of the accessions in the different groups of the dendrogram constructed using three similarity coefficients showed variation (Table. 69).

Table 69. Highest and lowest similarities between the varieties and cultivars in the different groups of the dendrogram produced by three similarity coefficients using RAPD marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	'Suvarna'	'Suvarna'	'Suvarna'
	Lowest	-	-	-
II	Highest	'Amrithapani' & 'Armoor' (0.88)	'Amrithapani' & 'Armoor' (0.93)	'Amrithapani' & 'Armoor' (0.92)
	Lowest	'Jabedi' & 'Amalapuram' (0.64)	'Jabedi' & 'Amalapuram' (0.78)	'Jabedi' & 'Amalapuram' (0.73)
III	Highest	'Suguna' & 'Sudarsana' (0.64)	'Suguna' & 'Sudarsana' (0.78)	'Suguna' & 'Sudarsana' (0.73)
	Lowest	-	-	-
IV	Highest	'Prabha' & 'Prathibha' (0.87)	'Prabha' & 'Prathibha' (0.93)	'Prabha' & 'Prathibha' (0.91)
	Lowest	'Alleppey' & 'Prabha' (0.87)	'Alleppey' & 'Prabha' (0.76)	'Alleppey' & 'Prabha' (0.70)

4.2.11. Characterization of open pollinated progenies of turmeric using RAPD markers

Fifty two random decamer primers produced a total of 553 scorable bands in the twelve open pollinated progenies studied out of which 328 (59.31%) were polymorphic. The percentage of polymorphism ranged from a maximum of 90.9% percent showed by primers OPC 09 & OPC 20 to a minimum of 11.1% showed by primer OPD 07. The sequence of the RAPD primers used for the molecular genetic fingerprinting and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table – 70 and Figs. 70 – 76.

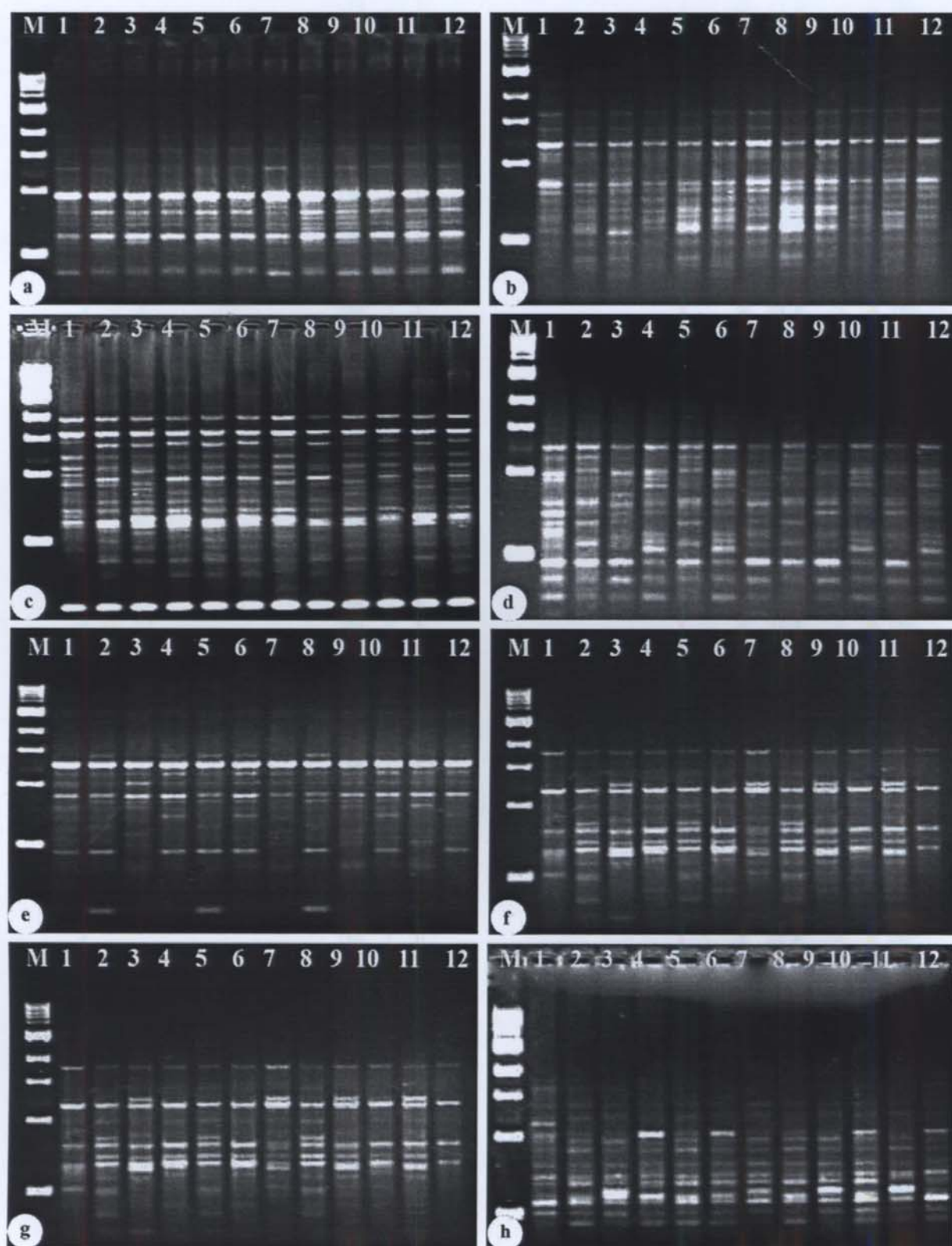


Fig. 70. a-h are the RAPD profile of open pollinated progenis of turmeric using primers OPA 02, OPA 03, OPA 04, OPA 05, OPA 07, OPA 08, OPA 09 and OPA 11. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M- Marker 1 Kb ladder (Biogene, USA)

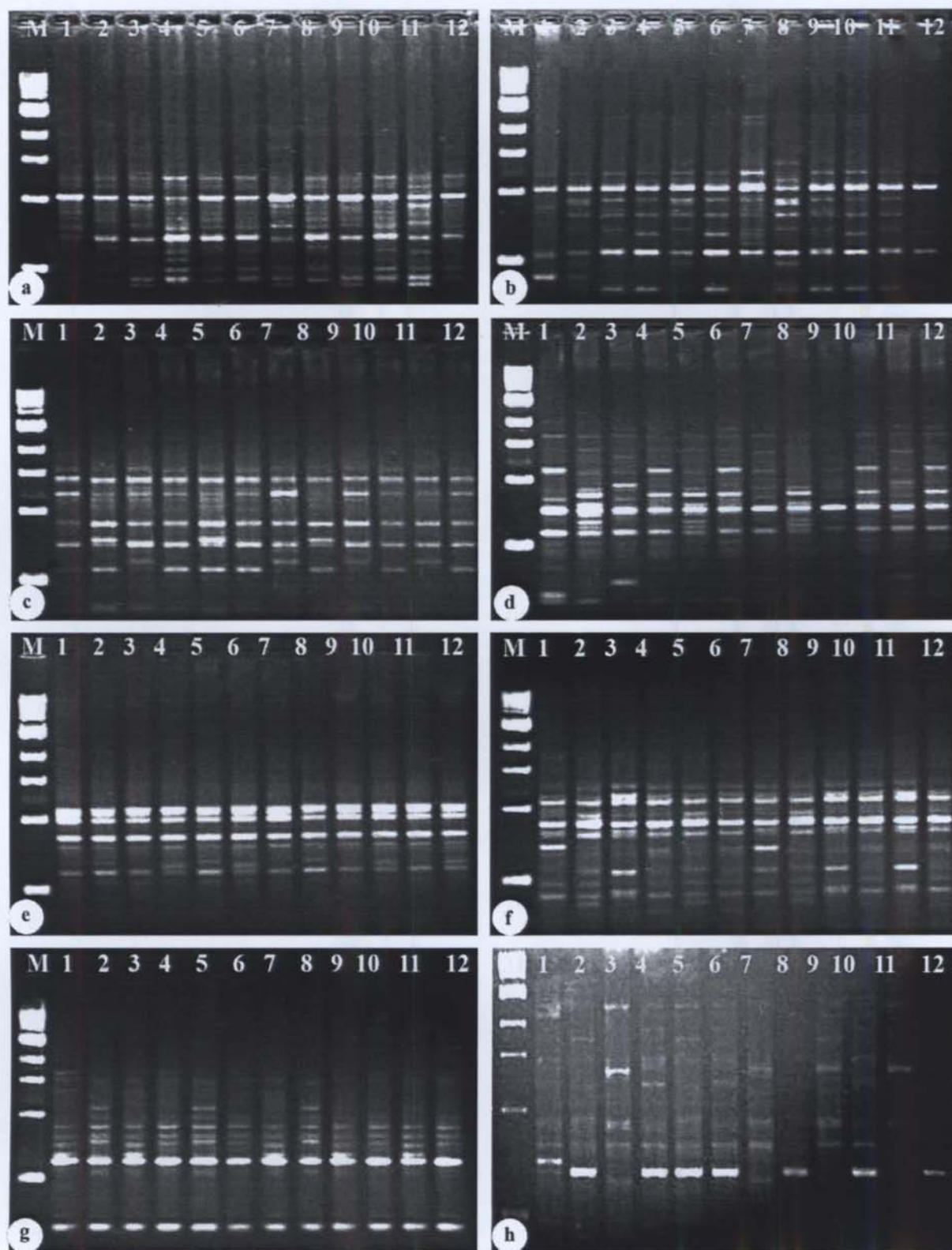


Fig. 71. a-h are the RAPD profile of open pollinated progenies of turmeric using primers OPA 13, OPA 14, OPA 15, OPA 17, OPA 18, OPA 19, OPA 20 and OPb09. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

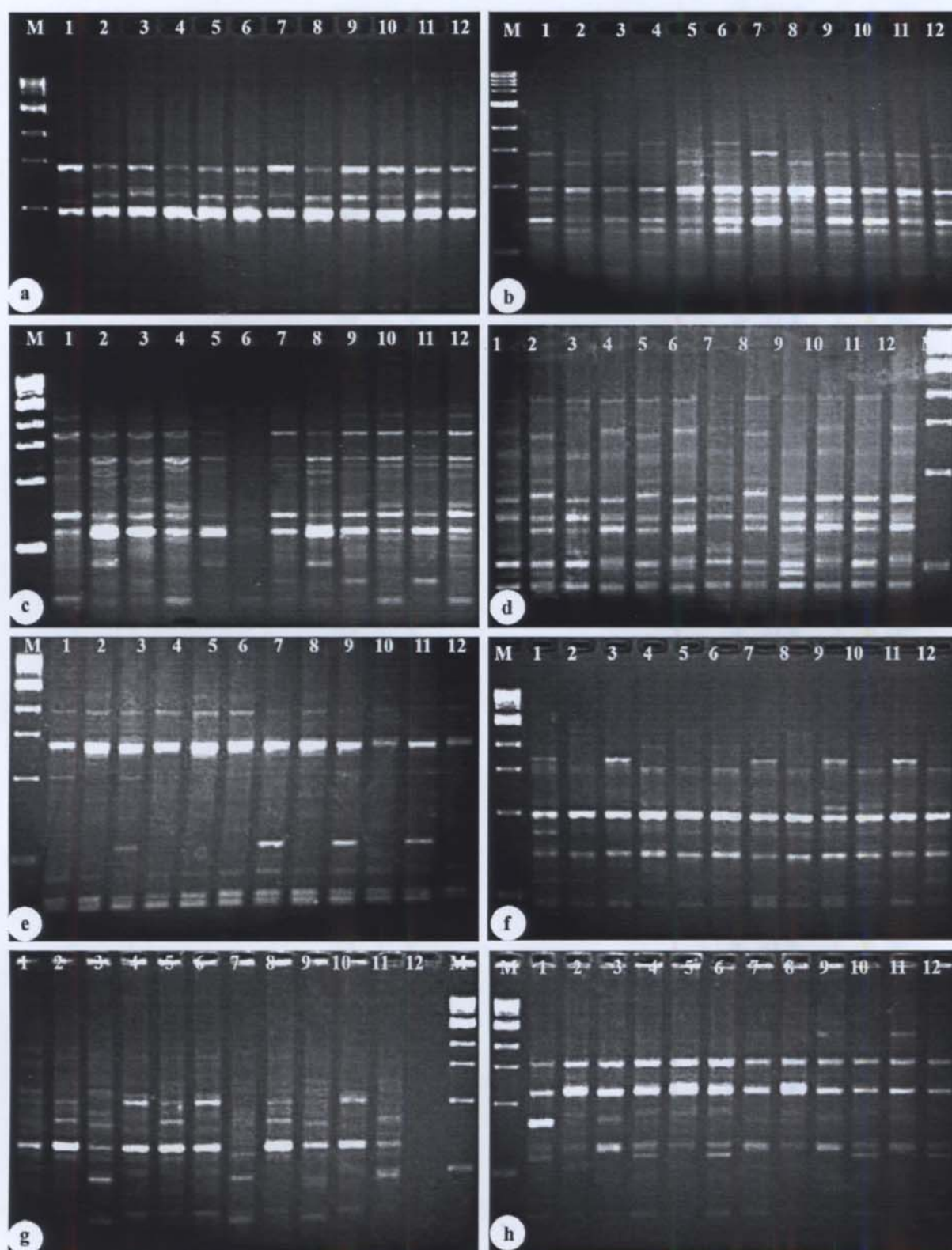


Fig. 72. a-h are the RAPD profile of open pollinated progenis of turmeric using primers OPB 01, OPB 02, OPB 10, OPB 11, OPB 12, OPB 14, OPB 19 and OPA 11. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Am-ruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

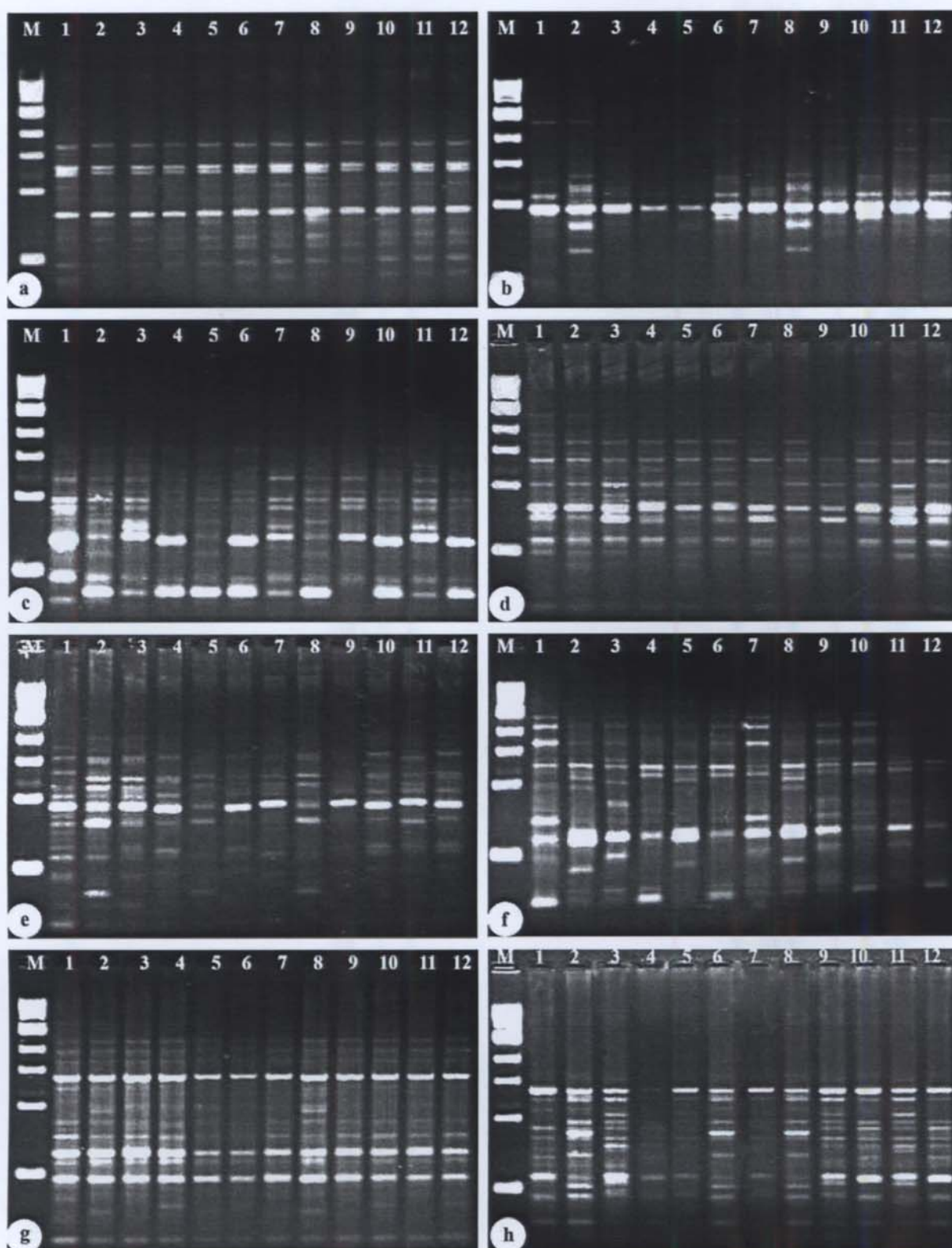


Fig. 73. a-h are the RAPD profile of open pollinated progenis of turmeric using primers OPC 05, OPC 06, OPC 07, OPC 08, OPC 09, OPC 10, OPC 11 and OPC 12. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

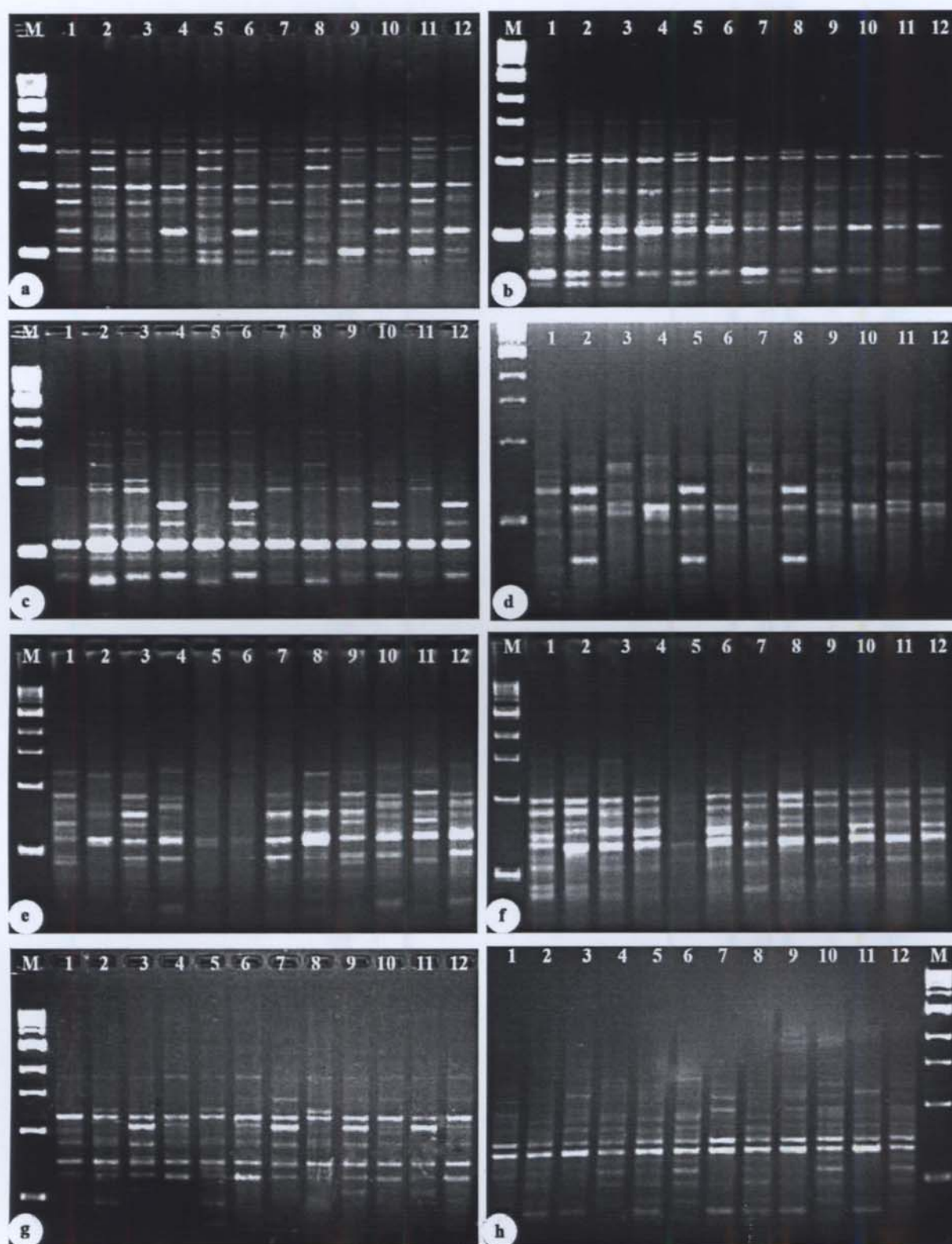


Fig. 74. a-h are the RAPD profile of open pollinated progenies of turmeric using primers OPC 13, OPC 14, OPC 16, OPC 20, OPD 01, OPD 02, OPD 04 and OPD 05. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

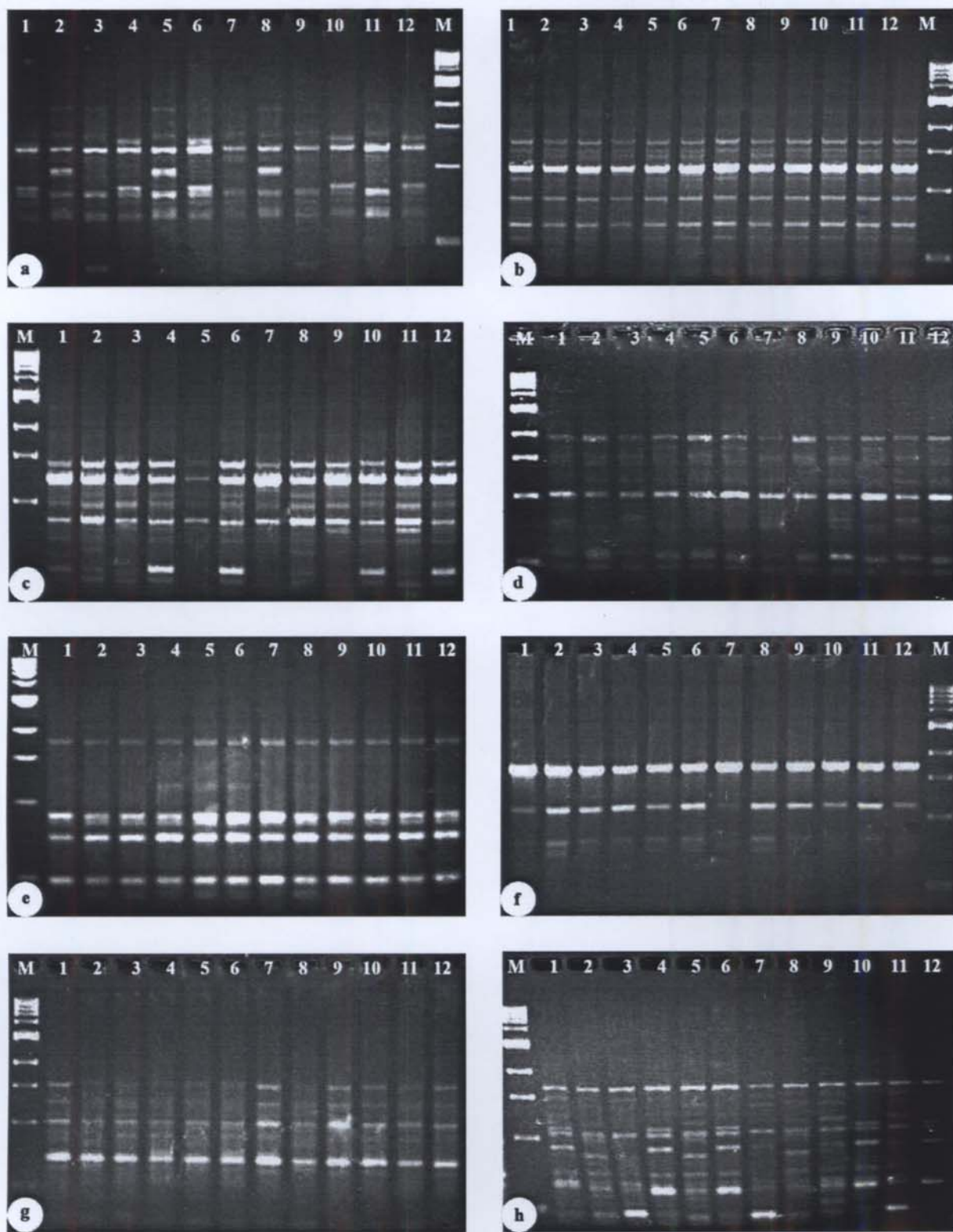


Fig. 75. a-h are the RAPD profile of open pollinated progenis of turmeric using primers OPD 06, OPD 07, OPD 08, OPD 09, OPD 11, OPD 12, OPD 13 and OPD 14. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

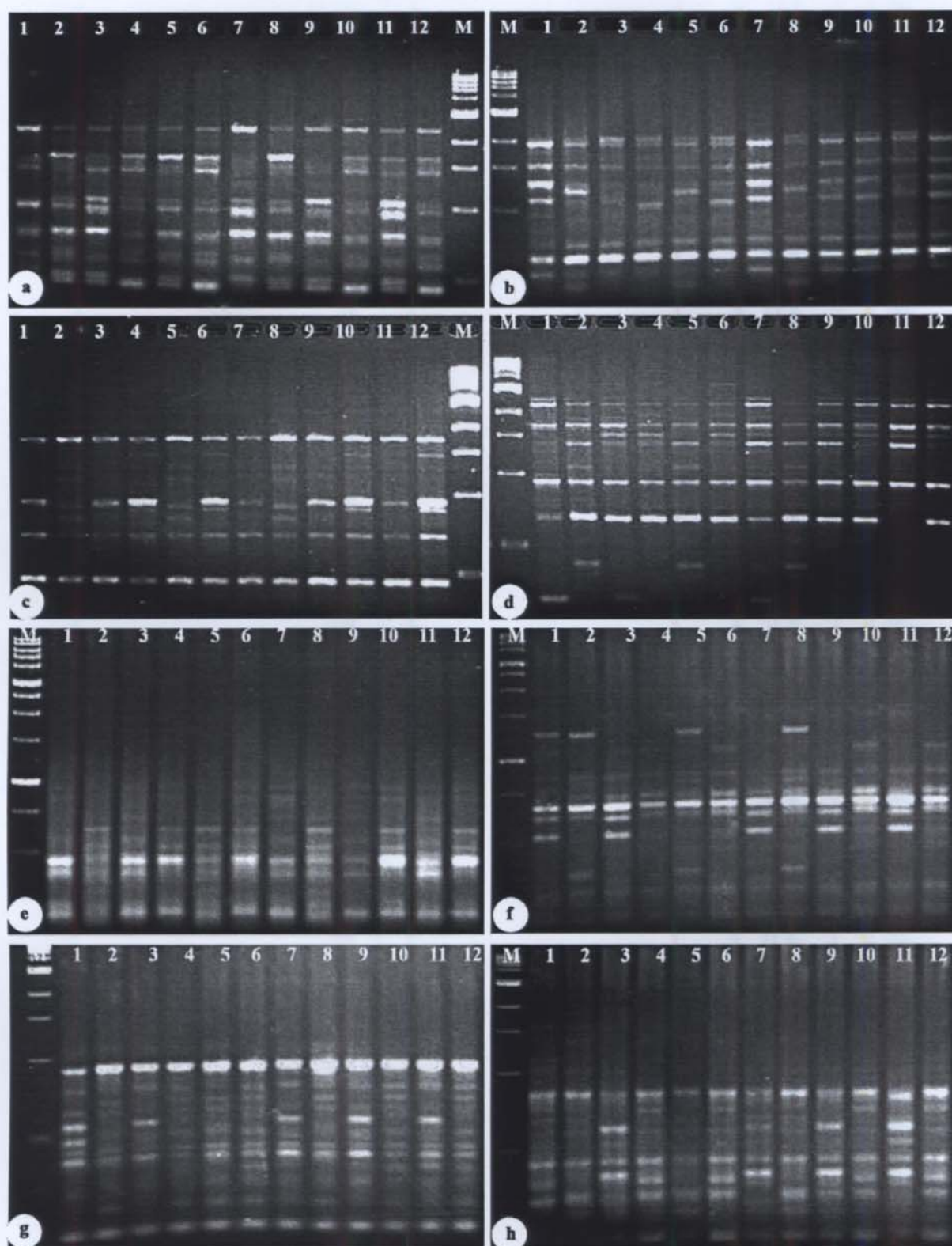


Fig. 76. a-d are the RAPD profile of open pollinated progenis of turmeric using primers OPD 15, OPD 16, OPJ 07 and OPJ 09. e - h are profile using primers ISSR 03, ISSR 04, ISSR 05 and ISSR 07. Lane 1-12 are 1) OP 'Muvatu-puzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

Table 70. Sequence of RAPD primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in OP progenies of turmeric and their mother parents

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	OPA 02	TGCCGAGCTG	10	03	30.00
2	OPA 03	AGTCAGCCAC	16	07	45.75
3	OPA 04	AATCGGGCTG	15	10	66.67
4	OPA 05	AGGGGTCTTG	17	10	58.82
5	OPA 07	GAAACGGGTG	12	10	83.33
6	OPA 08	GTGACGTAGG	19	06	31.58
7	OPA 09	GGGTAACGCC	12	09	75.00
8	OPA 11	CAATCGCCGT	13	10	76.92
9	OPA 13	CAGCACCCAC	10	07	70.00
10	OPA 14	TCTGTGCTGG	13	11	84.62
11	OPA 15	TTCCGAACCC	09	05	55.56
12	OPA 17	GACCGCTTGT	17	14	82.35
13	OPA 18	AGGTGACCGT	07	02	28.58
14	OPA 19	CAAACGTCGG	12	06	50.00
15	OPA 20	GTTGCGATCC	09	03	33.33
16	OPB 01	GTTTCGCTCC	05	02	40.00
17	OPB 02	TGATCCCTGG	10	05	50.00
18	OPB 09	TGGGGGACTC	06	04	66.67
19	OPB 10	CTGCTGGGAC	13	08	61.54
20	OPB 11	GTAGACCCGT	12	07	58.33
21	OPB 12	CCTTGACGCA	07	02	28.57
22	OPB 14	TCCGCTCTGG	07	04	57.14
23	OPB 18	CCACAGCAGT	09	08	88.89
24	OPB 19	ACCCCCGAAG	09	05	55.56
25	OPC 05	GATGACCGCC	09	05	55.56
26	OPC 06	GAACGGACTC	09	07	77.78
27	OPC 07	GTCCCGACGA	13	10	76.92
28	OPC 08	TGGACCGGTG	12	07	58.33
29	OPC 09	CTCACCGTCC	11	10	90.91
30	OPC 10	TGTCTGGGTG	11	06	54.55
31	OPC 11	AAAGCTGCGG	08	01	12.5
32	OPC 12	TGTCATCCCC	16	10	62.5
33	OPC 13	AAGCCTCGTC	11	06	54.55
34	OPC 14	TGCGTGCTTG	08	04	50.00
35	OPC 16	CACACTCCAG	13	10	76.92
36	OPC 20	ACTTCGCCAC	11	10	90.91
37	OPD 01	ACCGCGAAGG	13	10	76.92
38	OPD 02	GGACCCAACC	08	04	50.00
39	OPD 04	TCTGGTGAGG	14	10	71.43
40	OPD 05	TGAGCGGACA	14	11	78.57
41	OPD 06	ACCTGAAACG	10	08	80.00
42	OPD 07	TTGGCACGGG	09	01	11.11
43	OPD 08	GTGTGCCCCA	10	05	50.00
44	OPD 09	CTCTGGAGAC	09	03	33.33
45	OPD 11	AGCGCCATTG	05	01	20.00
46	OPD 12	CACCGTATCC	05	01	20.00
47	OPD 13	GGGGTGACGA	05	01	20.00
48	OPD 14	CTTCCCCAAG	12	06	50.00
49	OPD 15	CATCCGTGCT	10	07	70.00
50	OPD 16	AGGGCGTAAG	09	05	55.56
51	OPJ 07	CCTCTCGACA	09	06	66.67
52	OPJ 09	TGAGCCTCAC	10	05	50.00
			553	328	59.31

Table 73. Similarity matrix obtained from RAPD data using Simple Matching similarity coefficients generated in OP progenies of turmeric and their mother parents.

Row/Col	OPMuvapuzha	OPAlleppey	OPAmrith	OPKatargia	OPDindigam	OPKasturi	OPAyur	OPSugandha	OPAizwal	OPAmalpur	OPJabedi	OPAvanigadda	OPAlleppey	Amalapuram	Amrithapani	Jabedi	Katargia	Avanigadda	Dindigam	Kasturi	Muvapuzha	Sugandham	Aizwal	Ayur
OPMuvapuzha	1.000000																							
OPAlleppey	0.6807818	1.000000																						
OPAmrith	0.6125212	0.6453648	1.000000																					
OPKatargia	0.6463621	0.6613162	0.6366667	1.000000																				
OPDindigam	0.6156406	0.7014218	0.7442623	0.6360656	1.000000																			
OPKasturi	0.6790541	0.6730769	0.6322795	0.7720466	0.6252046	1.000000																		
OPAyur	0.6269165	0.6365105	0.7852349	0.6114497	0.7524752	0.6197655	1.000000																	
OPSugandha	0.6203390	0.6943338	0.7445743	0.6544240	0.8013136	0.6800000	0.7663866	1.000000																
OPAizwal	0.5965517	0.6437988	0.6994907	0.6451613	0.7212020	0.6101695	0.7316239	0.7414966	1.000000															
OPAmalpur	0.6144010	0.6475430	0.9752441	0.6387960	0.7532895	0.6343907	0.7979798	0.7537688	0.7120954	1.000000														
OPJabedi	0.6260575	0.6613162	0.6466667	0.7900000	0.6327869	0.6955075	0.6140940	0.6143573	0.6757216	0.6387960	1.000000													
OPAvanigadda	0.6953642	0.6635220	0.6286900	0.8026101	0.6292135	0.8208449	0.8975534	0.6699546	0.6378738	0.6353045	0.7014482	1.000000												
Alleppey	0.6722026	0.8913043	0.6602254	0.6666667	0.6529319	0.6784566	0.6450567	0.6612803	0.6327869	0.6623586	0.6666667	0.6715243	1.000000											
Amalapuram	0.6043406	0.6497623	0.9276316	0.6546053	0.7666990	0.6371100	0.8410596	0.7874794	0.7470687	0.9471947	0.6348684	0.6376812	0.6645469	1.000000										
Amrithapani	0.5949580	0.6411483	0.9105960	0.6589404	0.7783016	0.6413225	0.8500000	0.7992367	0.7521079	0.9269103	0.6291391	0.6385727	0.6624000	0.9803922	1.000000									
Jabedi	0.6031196	0.6402841	0.6245734	0.8771331	0.6174497	0.7700170	0.6185567	0.6461538	0.6330435	0.6301570	0.8225526	0.7646077	0.6490939	0.6531987	0.6610169	1.000000								
Katargia	0.6166951	0.6625386	0.6275168	0.9285302	0.6303630	0.7906198	0.6114865	0.6420168	0.6391162	0.6286296	0.8087248	0.7914614	0.6677472	0.6499066	0.6333333	0.9430172	1.000000							
Avanigadda	0.6182432	0.6570513	0.6322795	0.9051381	0.6362979	0.7864032	0.6231156	0.6533333	0.6644068	0.6343907	0.8053343	0.8110749	0.6653849	0.6568144	0.6611570	0.9268976	0.9715243	1.000000						
Dindigam	0.5780731	0.6402785	0.8183306	0.6415712	0.8470309	0.6078431	0.8270181	0.7901639	0.7333333	0.8407225	0.6088380	0.6230000	0.6582278	0.8788388	0.8878049	0.6130653	0.6280297	0.6339889	1.000000					
Kasturi	0.6216216	0.6602564	0.6356075	0.8783358	0.6252046	0.8308870	0.6231156	0.6400000	0.6271186	0.6377255	0.8119800	0.7654723	0.6688103	0.6568144	0.6611570	0.9255605	0.9413735	0.9206638	0.6241830	1.000000				
Muvapuzha	0.5876461	0.6529319	0.8380158	0.6381579	0.7799332	0.6208887	0.8478821	0.7507743	0.7303183	0.8547835	0.6184211	0.6280193	0.6677266	0.9089809	0.9117647	0.6397308	0.6357616	0.6483941	0.8722748	0.6436782	1.000000			
Sugandham	0.5783972	0.6171617	0.7958834	0.6482785	0.7588333	0.6061644	0.8383782	0.8281787	0.7307692	0.8089501	0.6174957	0.6241611	0.6192853	0.8395601	0.8756388	0.6467487	0.6424870	0.6541084	0.8385829	0.6438256	0.8954010	1.000000		
Aizwal	0.5806452	0.6247987	0.8227425	0.6454989	0.7631579	0.6210351	0.8518519	0.8140704	0.7870528	0.8389262	0.6187291	0.6219313	0.6397415	0.8877888	0.9036545	0.6541096	0.6464646	0.6577829	0.8804289	0.6410884	0.8910891	0.8915663	1.000000	
Ayur	0.5012162	0.6346154	0.8319468	0.6256240	0.7575774	0.6046512	0.9112228	0.8100000	0.7525424	0.8514190	0.5956739	0.8028039	0.6527331	0.8946317	0.9123967	0.6305237	0.6164154	0.6279070	0.8856209	0.9064039	0.8938356	0.9282137	1.000000	

The UPGMA dendrogram constructed using the genetic similarities obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on the RAPD markers are given in Fig. 77 – 79. In all the three dendrograms, the clustering pattern was similar and produced four different groups by splitting at coefficients 0.49, 0.64 and 0.60, respectively. The different members in the groups are

- Group I - OP 'Muvapuzha', OP 'Alleppey' and 'Alleppey'
- Group II - OP 'Katargia', 'Jabedi', 'Katargia', 'Avanigadda', 'Kasturi', OP 'Jabedi', OP 'Kasturi' and OP 'Avanigadda'
- Group III - OP 'Amrithapani', OP 'Amalapuram', 'Amalapuram', 'Amrithapani', 'Dindigam', 'Muvapuzha', 'Aizwal', 'Ayur', 'Sugandham', OP 'Dindigam', OP 'Sugandham', OP 'Ayur' and OP 'Aizwal'

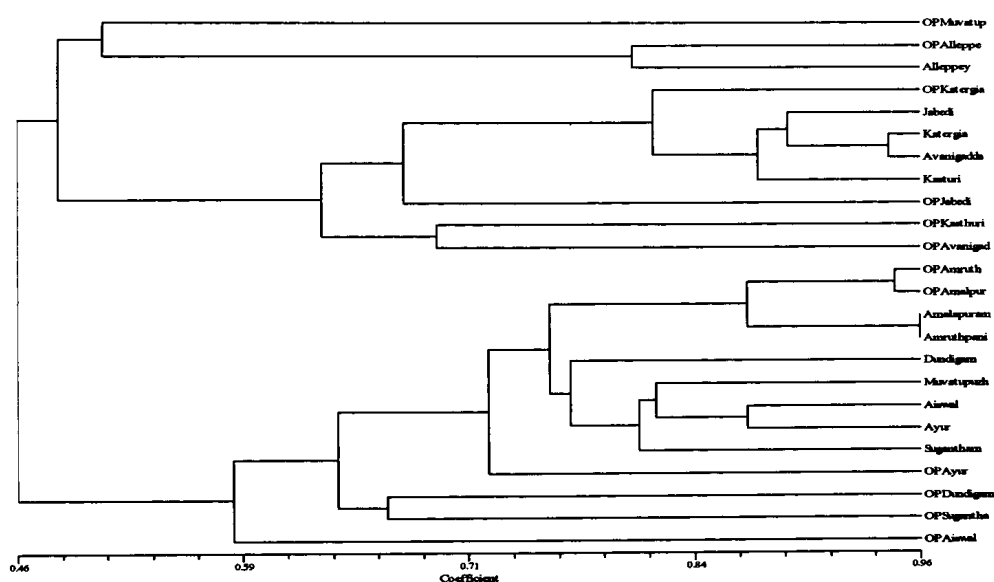


Fig. 77. Dendrogram of genetic similarity between open pollinated progenies of turmeric varieties/cultivars along with their parents based on RAPD analysis using Jaccard's similarity coefficients.

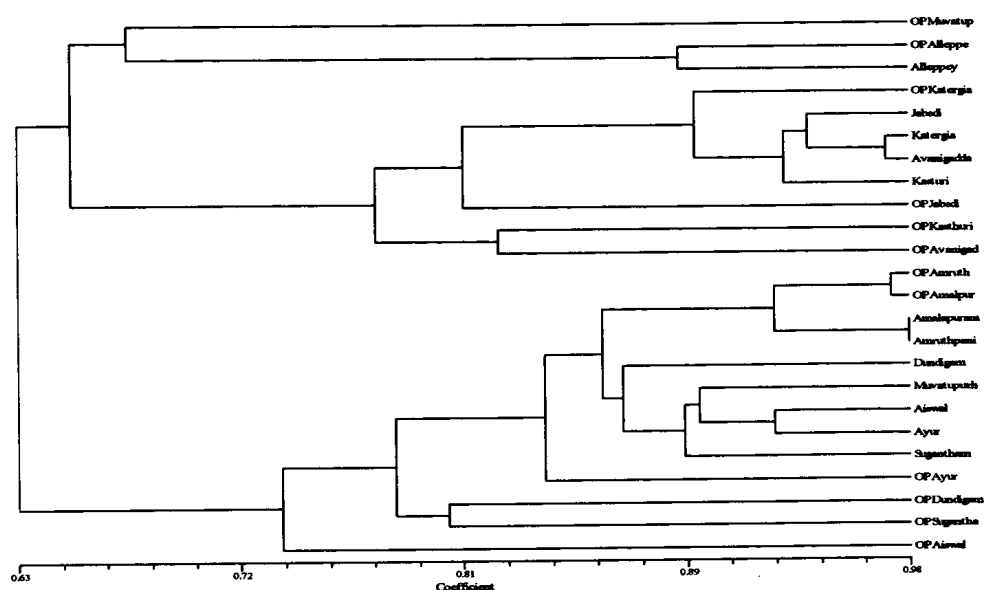


Fig. 78. Dendrogram of genetic similarity between open pollinated progenies of turmeric varieties/cultivars along with their parents based on RAPD analysis using Sorensen - Dice similarity coefficients.

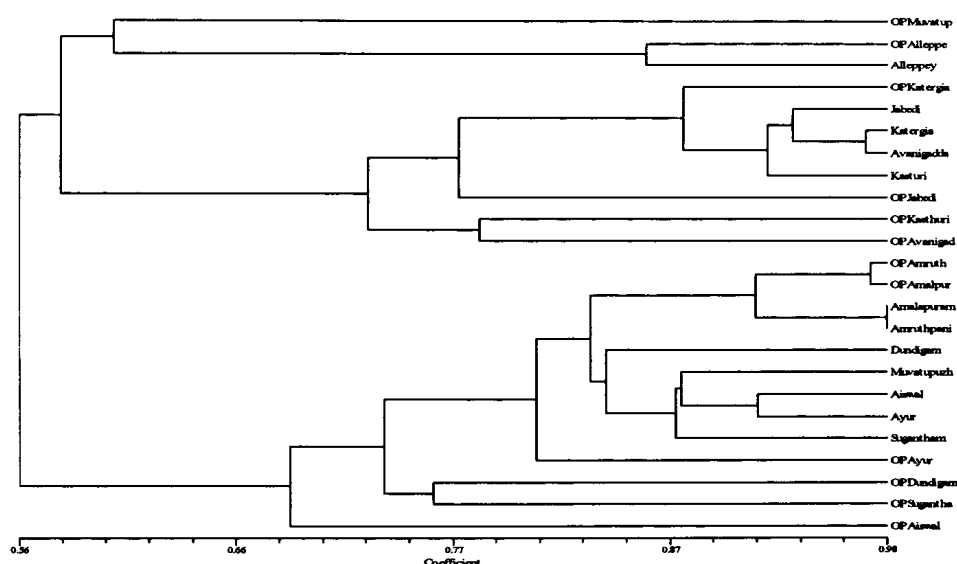


Fig. 79. Dendrogram of genetic similarity between open pollinated progenies of turmeric varieties/cultivars alongwith their parents based on RAPD analysis using Simple Matching similarity coefficients.

The grouping of OP progenies and their mother parents were same in the different groups of the dendrogram constructed using three similarity coefficients, the degree of similarity of the accessions in each showed variation (Table. 74).

Table 74. The highest and lowest similarities between open pollinated progenies of turmeric varieties/cultivars and their mother parents different groups of the dendrogram produced by three similarity coefficients using RAPD marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	OP 'Alleppey' & 'Alleppey' (0.80)	OP 'Alleppey' & 'Alleppey' (0.86)	OP 'Alleppey' & 'Alleppey' (0.89)
	Lowest	OP 'Muvattupuzha' & 'Alleppey' (0.51)	OP 'Muvattupuzha' & 'Alleppey' (0.60)	OP 'Muvattupuzha' & 'Alleppey' (0.67)
II	Highest	'Katergia' & 'Avanigadda' (0.94)	'Katergia' & 'Avanigadda' (0.90)	'Katergia' & 'Avanigadda' (0.97)
	Lowest	OP 'Jabedi' & OP 'Kasturi' (0.53)	OP 'Jabedi' & OP 'Kasturi' (0.63)	OP 'Jabedi' & OP 'Katergia' (0.79)
III	Highest	'Amalapuram' & 'Amrithapani' (0.96)	'Amalapuram' & 'Amrithapani' (0.98)	'Amalapuram' & 'Amrithapani' (0.98)
	Lowest	OP 'Aizwal' & OP 'Amrithapani' (0.54)	OP 'Aizwal' & OP 'Amrithapani' (0.64)	OP & OP 'Amrithapani' (0.70)

4.2.12. Characterization of open pollinated progenies of turmeric using ISSR markers

Twelve ISSR primers produced a total of 109 scorable bands in the 12 open pollinated progenies studied out of which 63 (58.76%) were polymorphic. The percentage of polymorphism ranged from a maximum of 80.0 percent showed by primer ISSR 11 to a minimum of 40.0 percent revealed by primer ISSR 04. The sequence of the ISSR primers used, total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism are presented in Table 75 and Fig. 80.

Table 75. Sequence of ISSR primers, number of bands produced by each primer, number of polymorphic bands and percentage polymorphism generated in OP progenies of turmeric and their mother parents.

Sl. No.	Primer	Sequence (5' – 3')	Total no. of bands	No. of polymorphic bands	% polymorphism
1	ISSR 03	(TCC) ₅ AG	09	05	55.56
2	ISSR 04	(AGC) ₄ GT	10	04	40.00
3	ISSR 05	(CAC) ₃ GC	12	08	66.67
4	ISSR 07	(GACA) ₃	08	04	50.00
5	ISSR 08	(GACA) ₃ GC	07	04	57.14
6	ISSR 11	(AGTG) ₃ TT	05	04	80.00
7	ISSR13	(AGTG) ₃	11	06	54.55
8	ISSR 14	(AG) ₈ T	08	05	62.50
9	ISSR 15	(TC) ₈ C	11	07	63.64
10	ISSR 16	(AC) ₇ T	10	05	50.0
11	ISSR 17	(GA) ₇ A	10	05	50.0
12	ISSR 18	(GA) ₈ T	08	06	75.00
		Total	109	63	58.76

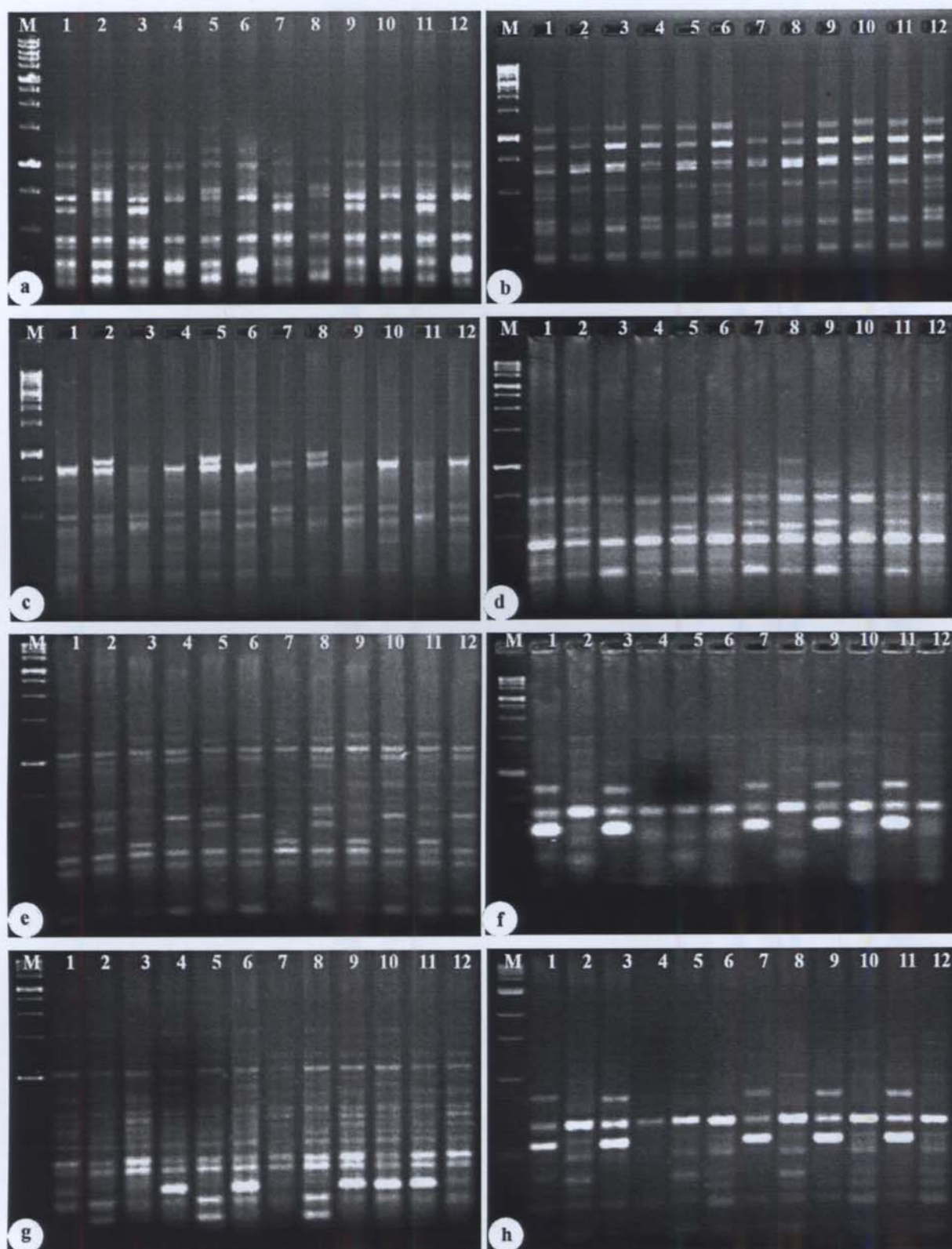


Fig. 80. a-h are the ISSR profile of the open pollinated progenis of turmeric using primers ISS R08, ISSR 11, ISSR 13, ISSR 14, ISSR 15, ISSR 16, ISSR 17 and ISSR 18. Lane 1-12 are 1) OP 'Muvatupuzha', 2) OP 'Alleppey', 3) OP 'Amruthapani', 4) OP 'Katergia', 5) OP 'Dindigam', 6) OP 'Kasturi', 7) OP 'Ayur', 8) OP 'Sugandham', 9) OP 'Aizwal', 10) OP 'Amalapuram', 11) OP 'Jabedi' and 12) OP 'Avanigadda'. M-Marker 1Kb ladder (Biogene, USA).

4.2.12.1. Cluster analysis

Tables 76 – 78 gives the similarity coefficients of the twelve open pollinated progenies of turmeric along with their mother parents analysed by ISSR markers. In all the three similarity matrices the open pollinated progeny of ‘Katergia’ showed maximum similarity with the parent (0.99 in Jaccard’s, Sorensen – Dice and Simple Matching similarity coefficients) and the least similarity between the progeny of ‘Amalapuram’ with the parent (0.51, 0.68 and 0.55, respectively).

Table 76. Similarity matrix obtained from ISSR data using Jaccard’s similarity coefficients in OP progenies of turmeric and their mother parents

Chole	Alleppey	Amalapur	Amritkila	Jahuli	Katergia	Aravindgathi	Dindigun	Kuruti	Moravagathi	Sugantham	Aimal	Ayer	OPMunnap	OPAlleppey	OPAmuth	OPKatergia	OPDindigun	OPKuruti	OPAyer	OPSegunda	OPAimal	OPAmalapur	OPJahuli	OPAravindgathi
Alleppey	1.000000																							
Amalapur	0.5977011	1.000000																						
Amritkila	0.5977011	1.000000	1.000000																					
Jahuli	0.4851304	0.5952381	0.5952381	1.000000																				
Katergia	0.5053763	0.6117647	0.6117647	0.9285714	1.000000																			
Aravindgathi	0.5368817	0.6162791	0.6162791	0.9285775	0.9444444	1.000000																		
Dindigun	0.5395258	0.8356164	0.8356164	0.5803614	0.6071429	0.6117647	1.000000																	
Kuruti	0.4946237	0.6000000	0.6000000	0.9430220	0.9853072	0.9905556	0.6144578	1.000000																
Moravagathi	0.5384615	0.8266667	0.8266667	0.5882253	0.6046512	0.6091954	0.8750000	0.5930223	1.000000															
Sugantham	0.5617978	0.7894737	0.7894737	0.5764706	0.5930223	0.5977011	0.7402597	0.5813953	0.8026316	1.000000														
Aimal	0.5305618	0.8495151	0.8495151	0.5833333	0.6000000	0.6046512	0.7972975	0.5882253	0.8378378	0.8495151	1.000000													
Ayer	0.5747126	0.8611111	0.8611111	0.5529412	0.5897674	0.5747126	0.8082192	0.5381395	0.8263243	0.8356164	0.9272362	1.000000												
OPMunnap	0.5000000	0.5161290	0.5161290	0.5795455	0.5777778	0.5824176	0.5108696	0.5666667	0.5777778	0.5000000	0.5217291	0.5108696	1.000000											
OPAlleppey	0.7261905	0.5804396	0.5804396	0.4893617	0.5052652	0.5267138	0.5217391	0.4947368	0.5376344	0.4791667	0.5494305	0.5384615	0.6153846	1.000000										
OPAmuth	0.5464305	0.8400000	0.8400000	0.5632184	0.5795455	0.5842697	0.7000000	0.5681818	0.7160494	0.6829268	0.7341772	0.7215190	0.5888889	0.5483871	1.000000									
OPKatergia	0.4946237	0.6000000	0.6000000	0.9142857	0.9853072	0.9905556	0.5932281	0.9710145	0.5930223	0.5813953	0.5882253	0.5581395	0.5842697	0.5106363	0.5681818	1.000000								
OPDindigun	0.5326087	0.8157895	0.8157895	0.3813653	0.6162791	0.6206897	0.8888889	0.6046512	0.8051948	0.7230000	0.7792208	0.7894737	0.5376344	0.5652174	0.6867470	0.6046512	1.000000							
OPKuruti	0.5053763	0.6117647	0.6117647	0.9014085	0.9436260	0.8918919	0.6265060	0.9571429	0.6046512	0.3930223	0.6000000	0.5697674	0.5777778	0.5025263	0.5977011	0.9285775	0.6162791	1.000000						
OPAyer	0.5053763	0.8913380	0.8913380	0.5168529	0.5333333	0.5384615	0.6666667	0.5222222	0.7230000	0.8513514	0.7662338	0.7533468	0.4947368	0.5052652	0.6162791	0.5222222	0.6547619	0.5333333	0.7307892	1.000000				
OPSegunda	0.5842697	0.8157895	0.8157895	0.6000000	0.6162791	0.6206897	0.8195335	0.6235294	0.8051948	0.8157895	0.9285775	0.8650137	0.5212766	0.5652174	0.7283951	0.6046512	0.7721519	0.6547619	0.8630137	0.7375000	1.000000			
OPAimal	0.5245255	0.5100000	0.5100000	0.4900000	0.5000000	0.5247525	0.4900000	0.5100000	0.5200000	0.4930495	0.5151515	0.5030505	0.6956522	0.6702128	0.5148515	0.5252525	0.5300000	0.5353535	0.5304082	0.5353535	0.5300000	1.000000		
OPAmalapur	0.4800000	0.4948454	0.4948454	0.5212766	0.5208333	0.5578947	0.4893830	0.5263158	0.5051546	0.5104167	0.4845261	0.4591827	0.6128032	0.5567010	0.5978261	0.5263158	0.5154639	0.5308333	0.4895833	0.5051546	0.5154639	0.6326531	1.000000	
OPJahuli	0.4639175	0.4631579	0.4631579	0.5393258	0.5384615	0.5434783	0.4421053	0.5444444	0.4583333	0.4479167	0.4680851	0.4421053	0.6363636	0.6111111	0.4536082	0.5444444	0.4842105	0.5790057	0.4793183	0.4432990	0.5000000	0.8333333	0.5913978	1.000000

Table 77. Similarity matrix obtained from ISSR data using Sorensen – Dice similarity coefficients in OP progenies of turmeric and their mother parents

Alleppey	Amalapur	Amritkila	Jahuli	Katergia	Aravindgathi	Dindigun	Kuruti	Moravagathi	Sugantham	Aimal	Ayer	OPMunnap	OPAlleppey	OPAmuth	OPKatergia	OPDindigun	OPKuruti	OPAyer	OPSegunda	OPAimal	OPAmalapur	OPJahuli	OPAravindgathi
1.0000000																							
0.7482014	1.0000000																						
0.7482014	1.0000000	1.0000000																					
0.6569343	0.7462487	0.7462487	1.0000000																				
0.6714286	0.7591241	0.7591241	0.9629630	1.0000000																			
0.6901408	0.7623899	0.7623899	0.9633036	0.9714286	1.0000000																		
0.7007299	0.9104478	0.9104478	0.7434342	0.7555556	0.7391241	1.0000000																	
0.6618705	0.7500000	0.7500000	0.9701493	0.9927007	0.9640288	0.7611940	1.0000000																
0.7000000	0.9051095	0.9051095	0.7407407	0.7536232	0.7571429	0.9333333	0.7445255	1.0000000															
0.7194345	0.8623529	0.8623529	0.7313433	0.7445255	0.7482014	0.8507463	0.7352841	0.8805109	1.0000000														
0.7101449	0.9185185	0.9185185	0.7568421	0.7500000	0.7536232	0.8872180	0.7407407	0.9117647	0.9185185	1.0000000													
0.7299270	0.9253731	0.9253731	0.7121212	0.7259259	0.7299270	0.8939394	0.7164179	0.9037037	0.9104478	0.9634060	1.0000000												
0.6666667	0.6808511	0.6808511	0.7338129	0.7323944	0.7361111	0.6765290	0.7234043	0.7323944	0.6666667	0.6857143	0.6762290	1.0000000											
0.8413793	0.7183099	0.7183099	0.6571429	0.6713287	0.6884552	0.6637143	0.6693007	0.6478873	0.7092199	0.7000000	0.7619048	1.0000000											
0.7092199	0.9130435	0.9130435	0.7285882	0.7238129	0.7373887	0.8235294	0.7246377	0.8345324	0.8115942	0.8467153	0.8782333	0.7412387	0.7083333	1.0000000									
0.6618705	0.7500000	0.7500000	0.9552239	0.9927007	0.9640288	0.7463687	0.9853841	0.7445255	0.7352841	0.7407407	0.7164179	0.7373887	0.6760562	0.7246377	1.0000000								
0.6930555	0.8985307	0.8985307	0.7552941	0.7623899	0.7638574	0.9411165	0.7536232	0.8870863	0.8405797	0.8739124	0.8823529	0.6993007	0.7222222	0.8142857	0.7536232	1.0000000							
0.6714286	0.7591241	0.7591241	0.9481481	0.9701145	0.9428571	0.7703704	0.9781022	0.7536232	0.7445255	0.7500000	0.7239259	0.7323944	0.6713287	0.7482014	0.9633036	0.7423899	1.0000000						
0.7152385	0.8805970	0.8805970	0.8694987	0.7239259	0.7299270	0.8939394	0.7164179	0.9037037	0.8955224	0.9323308	0.9545455	0.7050260	0.7000000	0.8088235	0.7164179	0.8823529	0.7407407	1.0000000					
0.6714286	0.8175182	0.8175182	0.6814815	0.6956522	0.7000000	0.8000000	0.6861314	0.8405797	0.9197080	0.8676471	0.8592593	0.6619718	0.6713287	0.7623899	0.6861314	0.6956522	0.8444444	1.0000000					
0.7373887	0.8985307	0.8985307	0.7300000	0.7623899	0.7638574	0.8970388	0.7681139	0.8630863	0.8685507	0.9633036	0.9264708	0.6853147	0.7222222	0.8428571	0.7536232	0.8714286	0.7913669	0.9264708	0.8489309	1.0000000			
0.6887117	0.6754967	0.6754967	0.6577181	0.6754967	0.6883117	0.6577181	0.6754967	0.6842105	0.6822517	0.6800000	0.6771409	0.8205128	0.8025478	0.6797386	0.6887417	0.6928105	0.6973684	0.6843638	0.6973684	0.6928105	1.0000000		
0.6486486	0.6630490	0.6630490	0.6853147	0.6849315	0.7162162	0.6572427	0.6884552	0.6712329	0.6738621	0.6522778	0.6295708	0.7600000	0.7152318	0.7482993	0.6886552	0.6802721	0.6849315	0.6572427	0.6712329	0.6802721	0.7750000	1.0000000	
0.6338028	0.6330935	0.6330935	0.7007299	0.7000000	0.7042254	0.6131587	0.7030360	0.6285714	0.6187030	0.6376812	0.6131587	0.7777778	0.7384207	0.6241135	0.7030360	0.6534823	0.7285714	0.6423538	0.6142857	0.6666667	0.9090909	0.7432432	1.0000000

Table 78. Similarity matrix obtained from ISSR data using Simple Matching similarity coefficients in OP progenies of turmeric and their mother parents

	Alleppey	Amalapuram	Amrithapani	Jabedi	Katargia	Avanigadda	Dindigam	Kasturi	Muvatupuzha	Sugandham	Aizwal	Ayur	OPAmalapuram	OPAlleppey	OPAmrithapani	OPKatargia	OPDindigam	OPKasturi	OPAyur	OPJabedi	OPAvanigadda	OPDindigam	OPKasturi	OPAyur	OPJabedi	OPAvanigadda
Alleppey	1.000000																									
Amalapuram	0.6739239	1.000000																								
Amrithapani	0.5648148	0.6851852	1.000000																							
Jabedi	0.5740741	0.6944444	0.6944444	1.000000																						
Katargia	0.5923926	0.6944444	0.6944444	0.9537037	1.000000																					
Avanigadda	0.6202704	0.8088889	0.8088889	0.6851852	0.6944444	1.000000																				
Dindigam	0.5648148	0.6851852	0.6851852	0.9629630	0.9907407	0.9537037	1.000000																			
Kasturi	0.6111111	0.8796296	0.8796296	0.6739239	0.6851852	0.9166667	0.6739239	1.000000																		
Muvatupuzha	0.6388889	0.8518519	0.8518519	0.6666667	0.6739239	0.6739239	0.8148148	0.6666667	0.8611111	1.000000																
Sugandham	0.6296296	0.8981481	0.8981481	0.6739239	0.6851852	0.8611111	0.6739239	0.8988889	0.8981481	1.000000																
Aizwal	0.6374074	0.9074074	0.9074074	0.6481481	0.6374074	0.8703704	0.6481481	0.8796296	0.8888889	0.9537037	1.000000															
Ayur	0.5555556	0.8233333	0.8233333	0.6374074	0.6481481	0.8611111	0.6388889	0.6481481	0.5648148	0.5923926	0.5833333	1.000000														
OPAmalapuram	0.7870370	0.6296296	0.6296296	0.5555556	0.5648148	0.3833333	0.3923926	0.5555556	0.6018519	0.3370370	0.6202704	0.6111111	0.6739239	1.000000												
OPAlleppey	0.6202704	0.8088889	0.8088889	0.6481481	0.6374074	0.8703704	0.7777778	0.6481481	0.7870370	0.7592393	0.8055556	0.7962963	0.6574074	0.6111111	1.000000											
OPAmrithapani	0.5648148	0.6851852	0.6851852	0.9644444	0.9907407	0.9537037	0.6851852	0.9814815	0.6739239	0.6666667	0.6739239	0.6481481	0.6574074	0.5740741	0.6481481	1.000000										
OPKatargia	0.6018519	0.8703704	0.8703704	0.6666667	0.6944444	0.9239239	0.6851852	0.8611111	0.7962963	0.8423926	0.8233333	0.8518519	0.6296296	0.7592393	0.6851852	1.000000										
OPDindigam	0.5740741	0.6944444	0.6944444	0.9531852	0.9629630	0.9239239	0.7129630	0.9722222	0.6851852	0.6739239	0.6851852	0.6574074	0.6481481	0.5648148	0.6739239	0.9537037	1.000000									
OPKasturi	0.6388889	0.8518519	0.8518519	0.6296296	0.6574074	0.8703704	0.6481481	0.8796296	0.8703704	0.9166667	0.9444444	0.6202704	0.6111111	0.7592393	0.6481481	0.8518519	0.6739239	1.000000								
OPAyur	0.5740741	0.7885185	0.7885185	0.6018519	0.6111111	0.7300000	0.6018519	0.7962963	0.8981481	0.8333333	0.8240741	0.5555556	0.5648148	0.6944444	0.6018519	0.7314815	0.6111111	0.8055556	1.000000							
OPJabedi	0.6374074	0.8703704	0.8703704	0.6851852	0.6944444	0.8944444	0.8703704	0.7037037	0.8611111	0.8703704	0.9537037	0.9074074	0.5833333	0.6296296	0.7962963	0.6851852	0.8333333	0.7314815	0.9074074	0.8055556	1.000000					
OPAvanigadda	0.5555556	0.5462963	0.5462963	0.5277778	0.5555556	0.5555556	0.5277778	0.5462963	0.5555556	0.5277778	0.5462963	0.5092393	0.6666667	0.6018519	0.6374074	0.5833333	0.5648148	0.5740741	0.5462963	0.5555556	0.5648148	0.6666667	1.000000			
OPDindigam	0.5185185	0.5277778	0.5277778	0.6202704	0.6111111	0.6111111	0.3892393	0.4203704	0.5185185	0.3892393	0.5370370	0.5092393	0.7037037	0.6739239	0.5092393	0.6202704	0.5462963	0.6481481	0.5462963	0.3000000	0.5648148	0.8703704	0.6481481	1.000000		

The UPGMA dendrogram constructed using the genetic similarity obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on ISSR markers are given in Fig. 81-83. In all the three dendrograms the clustering pattern was similar and produced five different groups. The denrograms were splits at coefficients 0.64, 0.78 and 0.70, respectively in the three similarity coefficient stueied and the different members in the groups are

- Group I - 'Alleppey' and OP 'Alleppey'.
- Group II - OP 'Muvatupuzha', OP 'Amalapuram' and OP 'Avanigadda'
- Group III - OP 'Jabedi'
- Group IV - 'Amalapuram', 'Amrithapani', OP 'Amrithapani', 'Dindigam', OP 'Dindigam', 'Muvatupuzha', 'Aizwal', OP 'Aizwal', 'Ayur', OP 'Ayur', 'Sugandham' and OP 'Sugandham'
- Group V - 'Jabedi', 'Avanigadda', 'Katargia', 'Kasturi', OP 'Katargia' and OP 'Kasturi'.

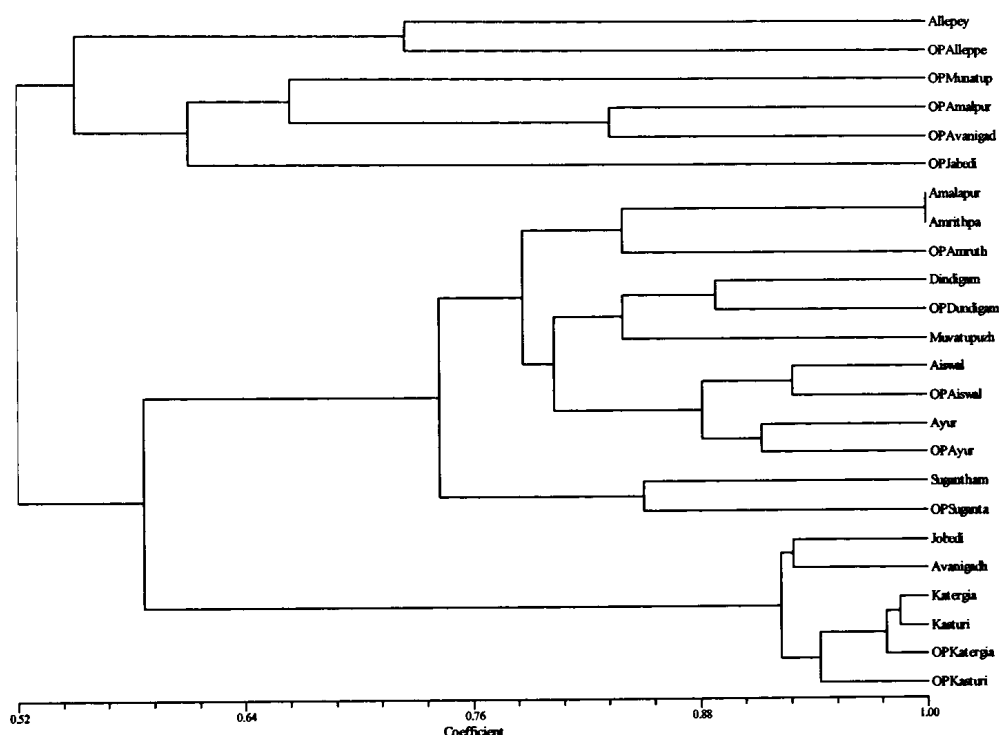


Fig. 81. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars along with their parents based on ISSR analysis using Jaccard's similarity coefficients.

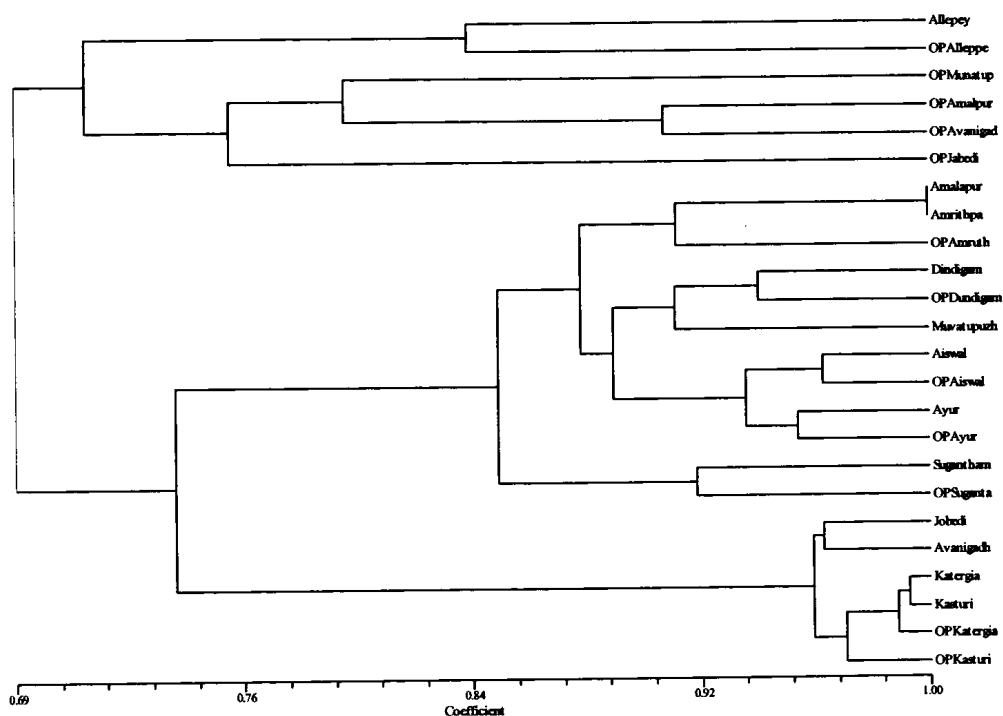


Fig. 82. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars along with their parents based on ISSR analysis using Sorensen - Dice similarity coefficients.

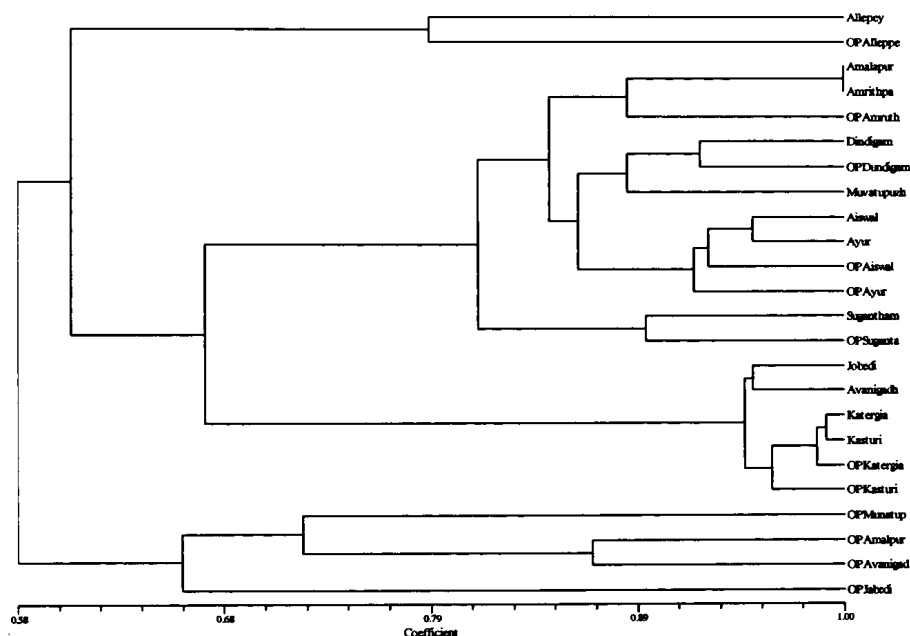


Fig. 83. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars along with their parents based on ISSR analysis using Simple Matching similarity coefficients.

The different similarity coefficients produced a similar clustering pattern in all the OP progenies of turmeric along with their mother parent studied, but the degree of similarity of the accessions in each group was varied in three dendrograms. The different groups in the dendrograms produced using Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients from ISSR marker data and the highest and lowest similarities in each group are given in Table 79.

Table 79. Highest and lowest similarities between open pollinated progenies of turmeric varieties/cultivars and their mother parents different groups of the dendrogram produced by three similarity coefficients using ISSR marker data

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	OP 'Alleppey' & 'Alleppey' (0.73)	OP 'Alleppey' & 'Alleppey' (0.84)	OP 'Alleppey' & 'Alleppey' (0.79)
	Lowest	'Alleppey' & OP 'Avanigadda' (0.46)	'Alleppey' & OP 'Avanigadda' (0.58)	'Alleppey' & OP 'Jabedi' (0.52)
II	Highest	OP 'Jabedi'	OP 'Jabedi'	'Amalapuram' & 'Amrithapani' (1.0)
	Lowest	-	-	OP 'Sugandham' & OP 'Amrithapani' (0.69)
III	Highest	OP 'Amalapuram' & OP 'Avanigadda' (0.83)	OP 'Amalapuram' & OP 'Avanigadda' (0.89)	'Katergia' & 'Kasturi' (0.99)
	Lowest	OP 'Avanigadda' & OP 'Jabedi' (0.59)	OP 'Avanigadda' & OP 'Jabedi' (0.61)	OP 'Kasturi' & 'Avanigadda' (0.93)
IV	Highest	'Amalapuram' & 'Amrithapani' (1.0)	'Amalapuram' & 'Amrithapani' (1.0)	OP 'Amalapuram' & OP 'Avanigadda' (0.87)
	Lowest	OP 'Sugandham' & 'Dindigam' (0.67)	OP 'Sugandham' & 'Dindigam' (0.80)	OP 'Muvatupuzha' & OP 'Avanigadda' (0.70)
V	Highest	'Katergia' & 'Kasturi' (0.99)	'Katergia' & 'Kasturi' (0.99)	OP 'Jabedi'
	Lowest	OP 'Kasturi' & 'Avanigadda' (0.89)	OP 'Kasturi' & 'Avanigadda' (0.94)	-

4.2.13. Characterization of open pollinated progenies of turmeric using combined RAPD and ISSR markers

A total of 662 bands were produced by the combined RAPD and ISSR markers together and 391 (59.06 %) are polymorphic among the twelve open pollinated progenies and their parents studied.

4.2.13.1. Cluster analysis

The similarity matrices obtained using Jaccard's, Sorensen – Dice and Simple Matching coefficients are given in Tables 80-82. In all the similarity matrices the open pollinated progeny 'Katergia' showed maximum similarity (0.85, 0.92 & 0.90 in Jaccard's, Sorensen – Dice and Simple Matching coefficients, respectively) with the parent. The least similarity was observed between the open pollinated progeny 'Muvatupuzha' and its mother parent (0.44, 0.61 and 0.52 in Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients respectively).

Table 80. Similarity matrix obtained from combined RAPD and ISSR data using Jaccard's similarity coefficients in open pollinated progenies of turmeric varieties/cultivars along with their parents

[illegible]

Table 81. Similarity matrix obtained from combined RAPD and ISSR data using Sorensen – Dice similarity coefficients in open pollinated progenies of turmeric varieties/cultivars along with their parents

Row/Col	OP4Shrop	OP4Bpps	OP4Amsth	OP4Ksaga	OP4Dmigan	OP4Krtini	OP4Yer	OP4Gmtha	OP4Alrai	OP4Almep	OP4Isab	OP4Arviga	Al4Beyr	Am4alagum	Am4etipen	Isab	Ksaga	Arv4saga	Dm4igan	Krtini	Shrop4gth	Ag4sthan	Alrai	Yer	
OP4Shrop	1.0000000																								
OP4Bpps	0.8664520	1.0000000																							
OP4Amsth	0.6378022	0.6571036	1.0000000																						
OP4Ksaga	0.6639344	0.6640532	0.6331165	1.0000000																					
OP4Dmigan	0.6317204	0.7852767	0.7573333	0.6577540	1.0000000																				
OP4Krtini	0.6864354	0.6718730	0.6331714	0.8064625	0.6498803	1.0000000																			
OP4Yer	0.6418733	0.6482213	0.7896173	0.6336164	0.7762803	0.6412005	1.0000000																		
OP4Gmtha	0.6284153	0.6901961	0.7479675	0.6802361	0.7996432	0.6837645	0.7808219	1.0000000																	
OP4Alrai	0.6132387	0.6378800	0.7260274	0.6682552	0.7468646	0.6435458	0.7673120	0.7608980	1.0000000																
OP4Almep	0.6577181	0.6786632	0.9134487	0.6488652	0.7611301	0.6462766	0.7132333	0.7423231	0.7071325	1.0000000															
OP4Isab	0.6531714	0.6718730	0.6666667	0.7704698	0.6430079	0.6925134	0.6224628	0.6253804	0.6757120	0.6675462	1.0000000														
OP4Arviga	0.7112299	0.6811780	0.6286672	0.7845745	0.6205079	0.8026490	0.6129410	0.6395743	0.6434731	0.6901194	0.7093596	1.0000000													
Al4Beyr	0.6772487	0.7879394	0.6781040	0.6686211	0.6406318	0.6788991	0.6531724	0.6651379	0.6462766	0.6701164	0.6821990	0.6675239	1.0000000												
Am4alagum	0.6162162	0.6571798	0.9086472	0.6530140	0.7724888	0.6425703	0.8373894	0.7803226	0.7608696	0.8636487	0.6427623	0.6368421	0.6796875	1.0000000											
Am4etipen	0.6086957	0.6201951	0.8848787	0.6394595	0.7739262	0.6468296	0.8448864	0.8000000	0.7602575	0.8791581	0.8301942	0.6375661	0.6780105	0.9639572	1.0000000										
Isab	0.6220250	0.6461948	0.6343490	0.6666667	0.6258831	0.7773167	0.6302351	0.6377778	0.6468474	0.6364720	0.7928669	0.7472826	0.6305376	0.6702397	0.6767956	1.0000000									
Ksaga	0.6364883	0.6682913	0.6394538	0.9167804	0.6416107	0.7989130	0.6299862	0.6521146	0.6537931	0.6434316	0.7043666	0.6692254	0.6692657	0.6729986	0.9483961	1.0000000									
Arv4saga	0.6330068	0.6469351	0.6430578	0.8908603	0.6307304	0.7876264	0.6394538	0.6612686	0.6729427	0.6472149	0.7864667	0.7864764	0.6692810	0.6735474	0.6791946	0.9406897	0.9701897	1.0000000							
Dm4igan	0.3932886	0.6452462	0.8093872	0.6313264	0.8462349	0.6303191	0.8287013	0.7830467	0.7537353	0.8083990	0.6306489	0.6300654	0.6649418	0.6797986	0.8871182	0.6230130	0.6641126	0.6325199	1.0000000						
Krtini	0.6384720	0.6457963	0.6441137	0.8780128	0.6353140	0.8351130	0.6374829	0.6465738	0.6441188	0.6480000	0.7988206	0.7490000	0.6675247	0.6738255	0.6774629	0.9400086	0.9390237	0.9722327	0.6433333	1.0000000					
Shrop4gth	0.6091644	0.6286422	0.7961165	0.6481204	0.7633979	0.6149394	0.6387087	0.6400556	0.7454280	0.7841530	0.6318481	0.6204082	0.6379542	0.6367229	0.6789018	0.6607354	0.6620112	0.6712777	0.634380	0.6611111	0.2816234	1.0000000			
Ag4sthan	0.6000000	0.6038934	0.8206332	0.6512362	0.7894370	0.6329237	0.8343936	0.8117497	0.8016529	0.8065678	0.6294789	0.6213333	0.6317130	0.8921833	0.9303191	0.6685237	0.6468427	0.6728036	0.8394378	0.6382034	0.8904791	0.8933975	1.0000000		
Alrai	0.6019152	0.6439791	0.6826664	0.6804661	0.6257174	0.7791165	0.6124661	0.8996628	0.8081633	0.7675378	0.8152080	0.8021355	0.5992011	0.6561667	0.9017497	0.9147997	0.6453408	0.6366120	0.6459439	0.8825239	0.6446217	0.9046890	0.8969339	0.9331514	1.0000000

Table 82. Similarity matrix obtained from combined RAPD and ISSR data using Simple Matching similarity coefficients in open pollinated progenies of turmeric varieties/cultivars along with their parents

Row	Col	OP4Sharing	OP4Atype	OP4Amount	OP4Kutanga	OP4Dumghe	OP4Kutoni	OP4Yar	OP4Sungwa	OP4Aired	OP4Amaluz	OP4Jehi	OP4vungu	Alt4beyy	Am4mugwa	Am4mugwa	Jehi	Kutanga	Am4mugwa	Dumghe	Kutoni	Mw4mugwa	Sungwa	Aired	Ay
OP4Sharing	1.0000000																								
OP4Atype	0.6175497	1.0000000																							
OP4Amount	0.5594026	0.5645695	1.0000000																						
OP4Kutanga	0.3927152	0.5743033	0.5761389	1.0000000																					
OP4Dumghe	0.5463578	0.6300809	0.6986755	0.5761389	1.0000000																				
OP4Kutoni	0.6300809	0.5827815	0.5743033	0.7635450	0.5645695	1.0000000																			
OP4Yar	0.5695364	0.5579470	0.7430031	0.5394026	0.7251658	0.5645695	1.0000000																		
OP4Sungwa	0.5494689	0.6076139	0.6920530	0.5809277	0.7516536	0.5910396	0.7350993	1.0000000																	
OP4Aired	0.5364238	0.57711921	0.6688742	0.5980265	0.6920530	0.5678808	0.7218543	0.7119305	1.0000000																
OP4Amaluz	0.5778146	0.5808927	0.8923841	0.5645695	0.6738411	0.5380265	0.7235099	0.6804656	0.6407285	1.0000000															
OP4Jehi	0.5743033	0.5794702	0.5877483	0.7168874	0.5315245	0.6192033	0.5380795	0.5380795	0.6043046	0.5827815	1.0000000														
OP4vungu	0.6422841	0.3877483	0.5346238	0.7317881	0.5364238	0.7233113	0.5201788	0.5761389	0.5394026	0.6076139	0.6341080	1.0000000													
Alt4beyy	0.5980265	0.6427152	0.5809277	0.5827815	0.5662252	0.5943708	0.5695364	0.5761389	0.5394026	0.5778146	0.57711921	0.5738477	1.0000000												
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
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Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
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Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
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Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.7417219	0.7086093	0.8482053	0.5546358	0.5430464	0.5927152	1.0000000											
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Am4mugwa	0.5298013	0.5612583	0.8974172	0.5761389	0.7152318	0.5579470	0.8013245	0.74																	

In all the UPGMA dendrograms (Fig. 84 – 86) constructed using the genetic similarities obtained from Jaccard's, Sorensen- Dice and Simple Matching coefficients based on RAPD and ISSR markers together, the clustering pattern of OP progenies and parents were similar. Unlike in the RAPD and ISSR dendrigrams alone the combined dendrogram produced three groups by splitting at coefficients 0.55, 0.69 and 0.61, respectively. The different members in the groups are

- Group I - OP 'Muvatupuzha', OP 'Alleppey' and 'Alleppey'.
- Group II - OP 'Katergia', 'Jabedi', 'Katergia', 'Avanigadda', 'Kasturi', OP 'Jabedi', OP 'Kasturi' and OP 'Avanigadda'
- Group III - OP 'Amrithapani', OP 'Amalapuram', OP 'Ayur', 'Amalapuram', 'Amrithapani', 'Muvatupuzha', 'Aizwal', 'Ayur', 'Sugandham', 'Dindigam', OP 'Dindigam', OP 'Sugandham' and OP 'Aizwal'.

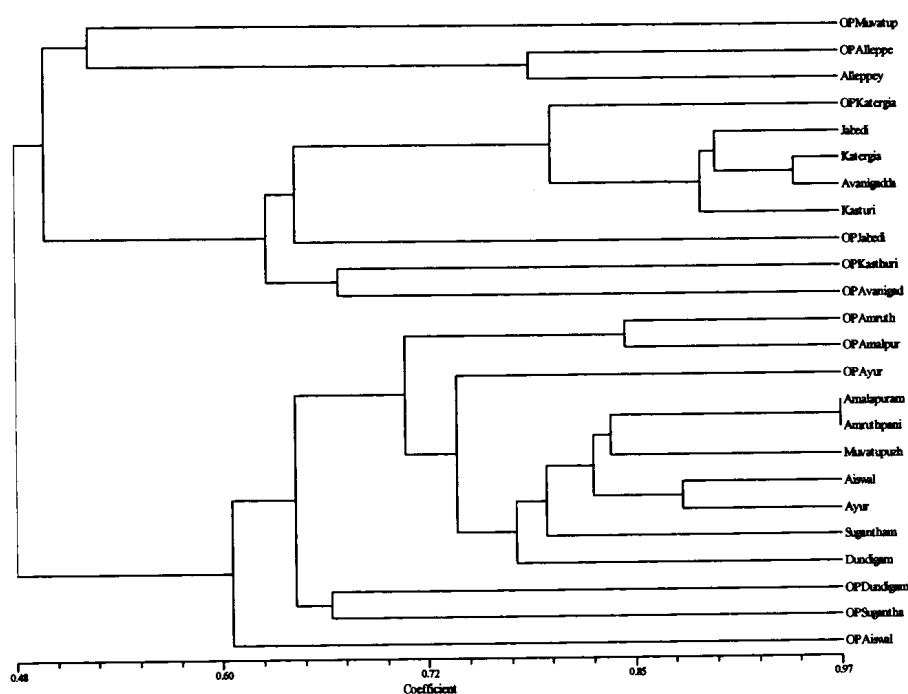


Fig. 84. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars alongwith their parents based on combined RAPD and ISSR data using Jaccard's similarity coefficients.

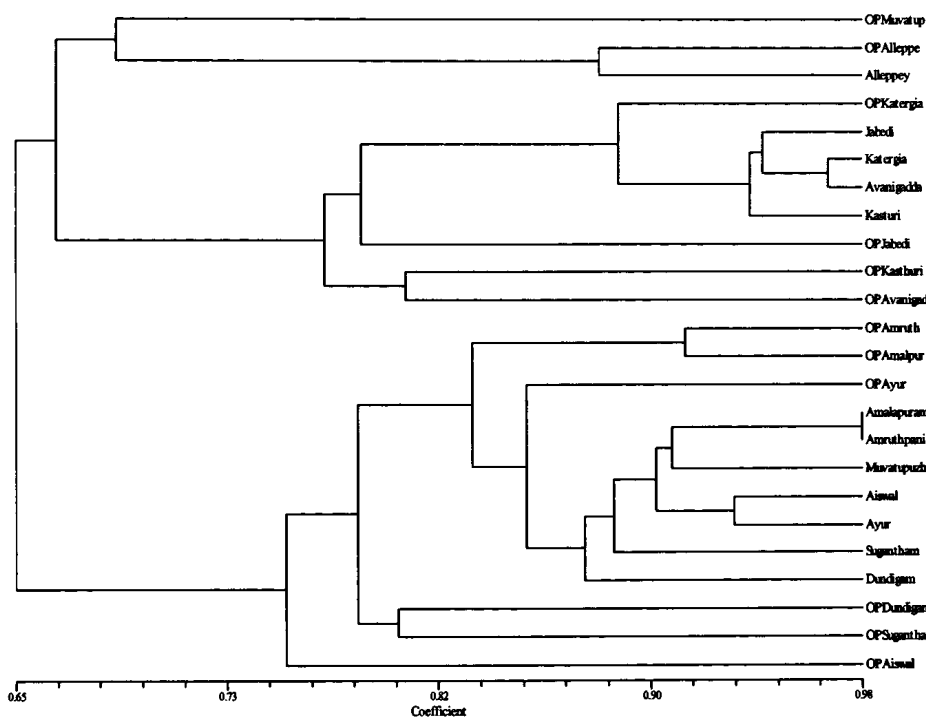


Fig. 85. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars alongwith their parents based on combined RAPD and ISSR data using Sorensen - Dice similarity coefficients.

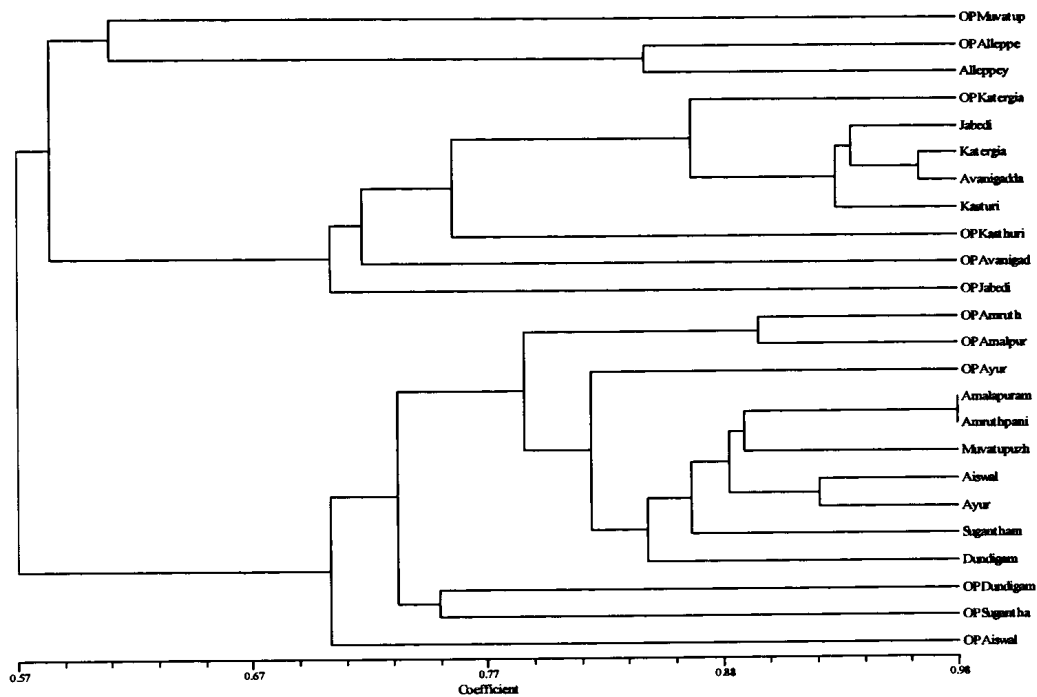


Fig. 86. Dendrogram of genetic similarity between 12 open pollinated progenies of turmeric varieties/cultivars alongwith their based on combined RAPD and ISSR data Simple Matching similarity coefficients.

The different similarity coefficients produced a similar clustering pattern in all the OP progenies of turmeric along with their mother parent studied using the combined RAPD and ISSR data, but the degree of similarity of the accessions in each group was varied in three dendrograms. The different groups in the dendrograms produced using Jaccard's, Sorensen – Dice and Simple Matching similarity coefficients and the highest and lowest similarities in each group are given in Table 83.

Table 83. The Highest and lowest similarities between open pollinated progenies of turmeric varieties/cultivars and their mother parents in the different groups of the dendrogram produced by three similarity coefficients using combined RAPD and ISSR marker data.

Group No.	Similarity	Jaccard's	Sorensen - Dice	Simple Matching
I	Highest	OP 'Alleppey' & 'Alleppey' (0.71)	OP 'Alleppey' & 'Alleppey' (0.88)	OP 'Alleppey' & 'Alleppey' (0.84)
	Lowest	OP 'Muvatupuzha' & 'Alleppey' (0.51)	OP 'Muvatupuzha' & 'Alleppey' (0.68)	OP 'Muvatupuzha' & 'Alleppey' (0.60)
II	Highest	'Katergia' & 'Avanigadda' (0.94)	'Ketergia' & 'Avanigadda' (0.97)	Ketergia & 'Avanigadda' (0.96)
	Lowest	OP 'Avanigadda' & 'Jabedi' (0.55)	OP 'Avanigadda' & 'Jabedi' (0.71)	OP 'Avanigadda' & 'Jabedi' (0.63)
III	Highest	'Amalapuram' & 'Amrithapani' (0.98)	'Amalapuram' & 'Amrithapani' (0.98)	'Amalapuram' & 'Amrithapani' (0.98)
	Lowest	OP 'Aizwal' & 'Sugandham' (0.60)	OP 'Aizwal' & 'Sugandham' (0.74)	OP 'Aizwal' & 'Sugandham' (0.70)

4.3. BIOCHEMICAL CHARACTERIZATION

4.3.1. Essential oil

4.3.1.1. *Curcuma* species

4.3.1.1.1. Estimation of fresh rhizome and leaf oil

Among the twelve *Curcuma* species used for the extraction of fresh rhizome oil, highest oil percentage was observed in *C. aromatica* (4.8 %) followed by *C. caesia* and *C. sylvatica* (3.6 %) and the lowest percentage of oil were recorded in *C. haritha* (2.4 %). The essential oil yield from fresh matured leaves ranged from 0.9 (*C. zedoaria*) to 1.6 % (*C. aromatica*). The details of the *Curcuma* species studied and their essential oil content are given in Table 84 & Fig. 87.

Table 84. Essential oil yield from fresh rhizomes and leaves of *Curcuma* species

Sl. No	Species	Rhizome oil (%)	Leaf oil (%)
1	<i>C. aromatica</i>	4.8	1.6
2	<i>C. amada</i>	2.8	1.2
3	<i>C. caesia</i>	3.6	1.4
4	<i>C. comosa</i>	3.2	1.3
5	<i>C. haritha</i>	2.4	1.4
6	<i>C. malabarica</i>	3.2	1.0
7	<i>C. montana</i>	2.8	1.2
8	<i>C. pseudomontana</i>	2.8	1.3
9	<i>C. raktakanta</i>	2.8	1.1
10	<i>C. sylvatica</i>	3.6	1.2
11	<i>C. zedoaria</i>	3.2	0.9
12	<i>C. longa</i>	3.8	1.0

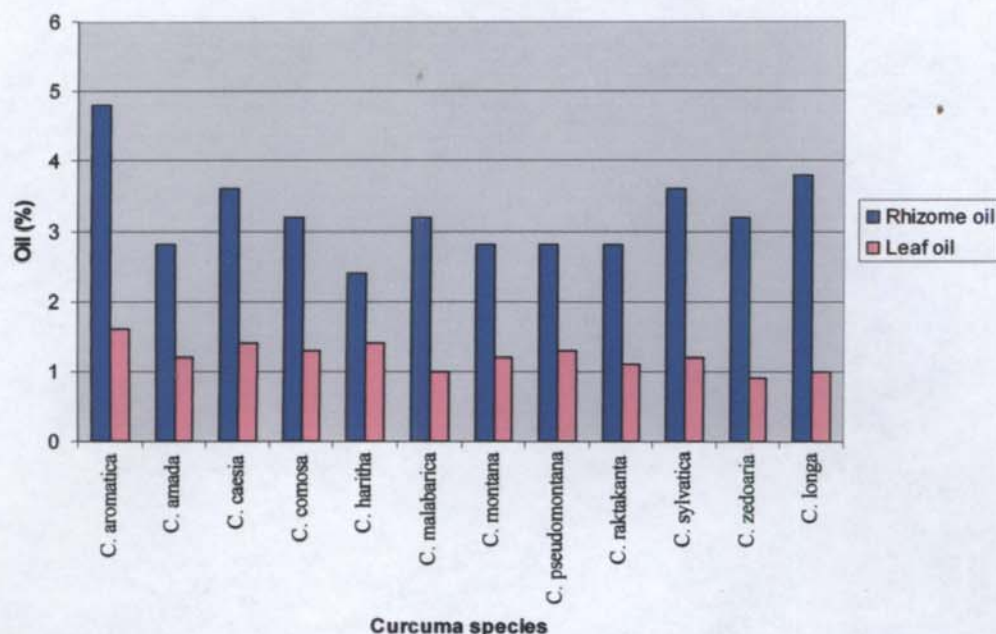


Fig. 87. Histogram showing the percentage of essential oil in fresh rhizomes and leaves of different *Curcuma* spp.

4.3.1.1.2. GC MS analysis of rhizome oil

The GC MS analysis of essential oil from fresh rhizomes of eleven *Curcuma* species placed them in six different groups based on the major compound identified in the GC MS profile. The details of different chemotypes, the highest observed compound and relative level of the highest observed compound are given in Table 85.

Table 85. Highest observed compound and relative level of compounds identified in different *Curcuma* species.

Sl. No	Species	Highest compound	observed	Relative level of highest observed compound (%)
1	<i>C. amada</i>	Myrcene		74.97
2	<i>C. aromatica</i>	L - camphor		22.24
3	<i>C. caesia</i>	1,8 - cineole		25.60
4	<i>C. comosa</i>	Epicurzerenone		29.17
5	<i>C. haritha</i>	ar -turmerone		59.68
6	<i>C. malabarica</i>	Xanthorrhizol		41.87
7	<i>C. montana</i>	Epicurzerenone		29.03
8	<i>C. pseudomontana</i>	ar - turmerone		63.46
9	<i>C. raktakanta</i>	ar - turmerone		36.30
10	<i>C. sylvatica</i>	Epicurzerenone		51.98
11	<i>C. zedoaria</i>	Xanthorrhizol		38.92

Composition of fresh rhizome oils of 11 *Curcuma* species identified by GC MS analysis along with their calculated retention indices are given in Table. 86.

Table. 86. Composition of the fresh rhizome oils of 11 *Curcuma* species and their retention indices (calculated.)

No.	RI	Compound	1	2	3	4	5	6	7	8	9	10	11
1	953	Camphene	-	4.94	2.10	4.94	-	0.28	0.75	-	0.98	1.60	0.78
2	975	β - pinene	7.49	0.55	1.33	0.55	0.72	-	2.87	-	3.98	0.74	0.11
3	991	Myrcene	74.97	0.58	0.30	-	-	-	-	-	-	-	0.10
4	1021	Limonene	0.32	1.23	0.59	0.58	-	-	0.13	-	-	0.24	0.14
5	1033	1,8 - cineole	0.45	9.53	25.6	-	2.38	0.11	-	-	-	-	0.23
6	1050	Z β- ocimene	4.60	-	-	-	-	-	-	-	-	-	-
7	1076	2 nonanone	-	-	0.22	1.23	-	-	0.25	-	-	-	-
8	1100	2 - nonanol	-	-	-	-	-	-	1.02	-	0.94	1.27	-
9	1105	Linalool	-	2.47	-	-	0.60	-	-	0.33	-	-	-
10	1145	Camphor	-	22.24	9.80	9.53	7.93	3.60	6.19	2.24	6.84	11.70	10.26
11	1149	Camphene hydrate	-	0.82	-	-	-	0.16	-	-	-	0.25	0.46
12	1165	Borneol	-	10.89	4.10	2.47	1.83	-	1.87	0.97	2.89	2.84	-
13	1191	α - terpineol	-	1.47	3.01	-	-	0.18	-	-	-	-	0.92
14	1294	Isobornyl acetate	-	-	2.34	-	-	-	-	-	-	-	-
15	1395	β - elemene	-	0.42	0.68	22.24	0.48	-	0.61	0.46	0.77	0.52	-
16	1405	α - longipinene	-	-	-	-	-	0.44	-	-	-	-	-
17	1419	β - caryophyllene	1.20	-	0.24	0.82	0.21	0.30	-	0.51	-	0.63	0.42
18	1443	Aromadendrene	-	-	-	-	-	-	1.21	-	-	-	-
19	1454	γ - curcumene	-	-	-	-	-	0.52	-	-	-	-	0.28
20	1456	γ - selinene	-	-	0.27	-	-	-	-	0.14	-	-	-
21	1460	Alloaromadendrene	-	-	-	0.42	-	-	-	-	-	-	-
22	1460	Calarene	-	-	-	0.36	-	-	0.62	0.38	-	-	-
23	1464	Trans β-farnesene	4.92	-	-	-	-	0.75	-	-	-	-	0.38
24	1469	α - zingiberene	-	-	-	-	-	0.49	-	-	-	-	0.32
25	1477	γ - gurjunene	-	-	0.25	0.74	-	0.31	-	2.30	-	1.87	-
26	1479	β - himachalene	-	-	-	-	-	0.47	-	-	-	-	0.38
27	1481	Germacrene D	-	0.36	0.29	-	-	0.22	-	0.25	-	-	0.89
28	1483	ar - curcumene	-	-	-	-	-	10.08	-	-	-	-	0.48
29	1485	β - selinene	-	0.38	0.25	1.89	0.25	-	0.38	0.46	0.69	0.54	-
30	1494	α - selinene	-	-	0.29	1.47	0.31	-	0.32	0.62	-	0.64	-

31	1497	Curzerene	-	-	0.77	-	-	1.56	-	0.68	-	8.84	
32	1495	Zingiberene	-	-	0.77	-	-	0.28	1.52	-	-	0.28	
33	1507	Germacrene A	-	-	-	-	-	-	-	0.41	-	-	
34	1510	β - bisabolene	-	-	-	-	-	0.87	-	-	-	0.84	
35	1522	δ - cadinene	-	0.51	-	-	0.47	-	-	1.36	-	-	
36	1524	δ - selinene	-	-	-	-	-	-	-	-	1.80	-	
37	1525	β - sesquiphellandrene	-	-	-	-	-	13.24	0.76	-	-	12.88	
38	1529	δ - cadinene	-	0.36	-	-	-	-	-	0.82	-	0.38	
39	1558	Germacrene B	-	0.20	0.36	0.36	0.26	2.21	0.28	0.38	-	1.68	
40	1563	Curcumenol	-	0.63	-	-	-	-	-	-	-	-	
41	1574	Spathulenol	-	0.54	-	-	-	-	-	-	-	-	
42	1583	Caryophyllene oxide	-	-	-	0.38	-	-	0.48	0.26	-	0.68	
43	1588	Cis β - elemenone	-	-	3.4	-	-	-	-	-	-	-	
44	1591	Curzerenone	-	1.22	3.34	1.23	0.80	-	-	-	-	2.83	
45	1596	Epicurzerenone	-	-	16.73	29.17	-	5.67	29.03	-	51.98	4.68	
46	1615	Isocurcumenol	-	-	2.68	0.51	-	-	3.97	4.87	-	-	
47	1641	ar - turnerone	-	-	-	-	59.68	-	-	63.46	-	-	
48	1654	β - eudesmol	-	-	-	-	0.75	0.17	-	0.98	1.49	0.18	
49	1673	β - bisabolol	-	-	-	1.22	-	-	-	-	-	-	
50	1676	α - patchoulene	-	0.13	-	-	1.32	-	-	0.56	-	-	
51	1690	Germacrone	-	2.05	5.68	0.13	8.68	13.67	12.62	15.99	14.30	8.68	
52	1697	Isogermacrone Epoxide	-	-	-	-	-	-	-	6.25	-	-	
53	1701	Curdione	-	16.70	6.28	-	-	-	10.36	-	-	-	
54	1721	E E α - farnesene	-	-	-	-	-	1.06	-	-	-	0.75	
55	1724	α - bisabolone	-	19.24	-	-	-	-	-	-	-	-	
56	1751	Xanthorrhizol	-	-	-	-	-	41.87	-	-	-	38.92	
Total			93.95	97.46	91.67	80.24	86.67	98.51	75.24	85.42	82.53	93.09	97.09

RI – retention index, 1. *C. amada*, 2. *C. aromatica*, 3. *C. caesia*, 4. *C. comosa*, 5. *C. haritha*, 6. *C. malabarica*, 7. *C. montana*, 8. *C. pseudomontana*, 9. *C. raktakanta*, 10. *C. sylvatica* & 11. *C. zedoaria*

The minimum number of identified compounds were present in *C. amada* among the eleven *Curcuma* species profiled for essential oil components using GC MS. *C. amada* showed highest content of myrcene (74.79%) in the fresh rhizome oil followed by β - pinene (7.49%), trans β - farnesene (4.92 %), Z β - ocimene (4.60%) and β - caryophyllene (1.2%). A total of seven compounds amounting 93.95% of the total oil studied were identified using GC MS analysis in this species.

In *C. aromatica*, 97.46% of the total volatile oil constituents identified by GC MS analysis, camphor (22.24%) was the major essential oil component. Other major compounds identified out of the 23 total constituents were α - bisabolone (19.24%), curdione (16.70%), borneol (10.89%), 1, 8 - cineole (9.53%), camphene (4.94%), linalool (2.47%) and germacrone (2.05%).

A total of twenty six compounds amounting 91.67 % of the fresh rhizome oil of *C. caesia* were identified in the GC MS analysis, 1,8 cineole (25.60%) being the major component. Epicurzerenone (16.73%), camphor (9.80%), curdione (6.8%), germacrone (5.68%), borneol (4.10%), curzerenone (3.34%), cis β - elemenone (3.4%), α - terpineol (3.01%), isocurcumenol (2.68%), isobornyl acetate (2.30%) and camphene (2.10%) were the other major volatile oil components identified in *C. caesia*.

Epicurzerenone (29.17%) was found to be the highest observed compound in *C. comosa* followed by β - elemene (22.24%) out of the 20 compounds identified constituting 80.24% of the total oil. Camphor (9.53%), camphene (4.94%), borneol (2.47%), β - selinene (1.89%), α - selinene (1.47%), 2 - nonanone (1.23%), curzerenone (1.23%) and β - bisabolol (1.22%) were the other major compounds.

About 59.68% of the total steam volatile oil from fresh rhizomes of *C. haritha* was constituted by ar - turmerone. A total of 16 compounds were identified in *C. haritha* by GC MS analysis amounting 86.67% of the total oil. Germacrone (8.68%), camphor (7.93%), 1,8 - cineole (2.38%), borneol (1.83%) and α - patchoulene (1.32%) were the other important volatile oil components found in *C. haritha*.

In *C. malabarica* twenty four volatile oil components constituted 98.51 percentage of the total oil was the highest among all the species studied. Xanthorrhizol (41.87%) was the single largest component followed by germacrone (13.67%), β - sesquiphellandrene (13.24%), α - curcumene (10.08%), epicurzerenone (5.67%), camphor (3.60%), germacrone B (2.21%) and curzerene (1.56%).

Curcuma montana showed highest percentage of epicurzerenone (29.03) as the major constituent of fresh rhizome oil. Twenty volatile oil components identified constituted 80.24 % of the total oil and this species showed the least percentage of total oil constituents identified among the 11 species. Other major volatile oil components in *C. montana* identified by GC MS analysis were germacrone (12.62 %), curdione (10.36%), isocurcumenol (3.97%), camphor (6.19%), β - pinene (2.87%), borneol (1.87%), aromadendrene (1.21%) and 2 - nonanol (1.02%).

α - turmerone was the major essential oil component identified in *C. pseudomontana* rhizome oil constituting 63.46% of the total oil as identified by GC MS analysis. A total of 18 compounds were identified in *C. pseudomontana* oil constituting 85.53% of the total oil. Germacrone (9.40%), camphor (2.24%), γ - gurjunene and δ - cadinene (1.36%) were the other major compounds in this species.

Curcuma raktakanta also showed α - turmerone (36.30%) as the major constituent of essential oil among the different components which constituted 85.53% of the total volatile oil .The other major constituents identified were, germacrone (15.99%), camphor (6.84%), isocurcumenol (4.87%), β - pinene (3.98%) and borneol (2.89%).

Highest percentage of epicurzerenone content in the fresh rhizome volatile oils of different *Curcuma* species studied was recorded in *C. sylvatica* (51.98%). A total of 17 compounds comprising 93.09 % of the total volume of the *C. sylvatica* oil were identified. Other major compounds identified were germacrone (14.30 %), camphor (11.70%), borneol (2.84 %), γ - gurjunene (1.87%), camphene (1.60%), 2 - nonanol (1.27%), δ - selinene (1.80%) and β - eudesmol (1.49%).

C. zedoaria recorded maximum number of essential oil components in the fresh rhizome oil of the different *Curcuma* species studied by GC MS analysis. A total of 27 compounds amounting to 97.09% of the total oil were detected. Xanthorrhizol (38.92%) was the highest recorded compound in *C. zedoaria* followed by β - sesquiphellandrene (12.88%), camphor (10.26%), curzerene (8.84%), germacrone (8.68%), epicurzerenone (4.68%), curzerenone (2.83%) and germacrone B (1.68%).

Some essential oil constituents were unique to the particular *Curcuma* species studied. Among the 11 *Curcuma* species fresh rhizome oil profiled using GC MS, $Z \beta$ - ocimene was unique to *C. amada* fresh rhizome oil while spathulenol and curcumol were specific to *C. aromatica*. In *C. caesia*, isobornyl acetate and cis β - elemenone were exceptional and β - bisabolol was specific to *C. comosa*. However no distinct compounds were identified in *C. haritha*, *C. momtana*, *C. pseudomontana* and *C. zedoaria*. *C. raktakanta* had germacrene A and isogermacrone epoxide as the unique compounds while α - longipinene and δ selinene were specific to *C. malabarica* and *C. sylvatica*, respectively.

4.3.1.2. Turmeric varieties/cultivars

4.3.1.2.1. Extraction of rhizome and leaf oil

Essential oil from dried rhizomes and fresh matures leaves of the turmeric cultivars/varieties are given in Table 87. The yield of the rhizome oil ranged from 4.67 % to 7.67 %. Cultivars namely 'Gaspani', 'Vondimita' and 'Jabedi' recorded lowest oil yield while the 'Rajapuri' and 'Ayur' recorded maximum oil yield. Most of the cultivars/varieties recorded one percent fresh leaf essential oil. The highest percentage of fresh leaf oil was recorded in cultivar 'Jabedi' (1.3 %) and the minimum was recorded in the variety 'Sudarsana' (0.6 %).

Table. 87. Essential oil yield from dry rhizomes and fresh leaves of turmeric cultivars/varieties.

Sl. No	Cultivar name	Rhizome oil (l %)	Leaf oil (%)	Sl.No	Cultivar name	Rhizome oil (%)	Leaf oil (%)
1	'Alleppey'	5.0	1.0	19	'Nandyal type'	5.33	1.0
2	'Amalapuram'	6.0	1.0	20	'Rajapuri'	7.67	1.2
3	'Amrithapani'	6.33	1.0	21	'Sugandham'	6.33	1.0
4	'Armoor'	5.33	1.0	22	'Vondimita'	4.67	1.1
5	'Jabedi'	4.67	1.3	23	'Wynadlocal'	6.33	1.0
6	'Dhagi'	5.67	1.0	24	'Aizwal '	5.0	1.0
7	'Katergia'	6.0	1.0	25	'Ayur'	7.67	1.0
8	'Chayapasupu'	6.67	1.0	26	'Basar Along'	5.67	1.0
9	'Avanigadda'	5.0	1.0	27	'Gaspani '	4.67	1.0
10	'Dindigam'	6.33	1.0	28	'Hajo '	5.0	1.0
11	'Ethamkula'	5.0	0.9	29	'Jorhat'	6.33	0.8
12	'GL Puram'	7.0	1.0	30	'Suvarna'	5.67	0.8
13	'Karhadi local'	7.0	0.8	31	'Sudarsana'	5.33	0.6
14	'Kasturi'	5.67	1.1	32	'Kedaram'	7.0	1.0
15	'Kodur'	7.0	1.1	33	'Suguna'	5.33	0.9
16	'Kuchipudi'	6.0	0.8	34	'Prathibha'	7.33	1.0
17	'Mamomthody'	5.33	1.0	35	'Prabha'	6.67	1.0
18	'Muvatupuzha'	8.33	1.1	36	'Alleppey Supreme'	6.33	1.0

4.3.1.2.2. GC MS analysis of leaf oil

The GC MS analysis of essential oil from fresh matured leaves of ten turmeric cultivars/varieties identified a total of 48 compounds. 92.27 to 97.97 % of the total volatile oil constituents were identified and based on the highest observed compound in the GC MS profile, the varieties was placed in five different groups (chemotypes). In six of the samples studied, monoterpene hydrocarbons (α - phellandrene, α -terpinolene and *para* cymene) constituted the major compounds while a sesquiterpene hydrocarbon (zingiberene & β - sesquiphellandrene) was the

major volatile oil compounds in the rest. The details of different chemotypes, the highest observed compound, relative level of the highest observed compound are given in Table 88.

Table 88. Highest observed compound and relative level of compounds identified in different turmeric cultivars/varieties

Sl. No	Variety/Cultivar	Highest observed compound	Relative level of highest observed compound (%)
1	'Alleppey'	α - terpinolene	12.56
2	'Alleppey Supreme'	α - phellandrene	13.92
3	'Kedaram'	α - terpinolene	10.63
4	'Prabha'	α - terpinolene	18.79
5	'Prathibha'	<i>p</i> - cymene	18.96
6	'Rajapuri'	Zingiberene	14.06
7	'Sugandham'	α - terpinolene	15.08
8	'Suguna'	β - sesquiphellandrene	11.17
9	'Sudarsana'	β - sesquiphellandrene	12.90
10	'Wynad'	Zingiberene	19.62

Composition of fresh leaf oils of ten turmeric varieties/cultivars identified by GC MS analysis along with their calculated retention indices are given in Table. 89.

Table. 89. Composition of the fresh leaf oils of selected varieties/cultivars of turmeric and their retention indices (calculated)

No.	RI	Compound	1	2	3	4	5	6	7	8	9	10
1	938	α - pinene	0	0	0	0.68	0	0	1.02	2.03	0	0
2	953	Camphene	0	0	0	0	0	0	2.08	0	0	0
3	975	β - pinene	2.64	3.26	1.82	0	2.45	2.02	0.40	0	3.12	1.82
4	991	Myrcene	1.29	2.89	1.68	1.09	0.98	1.23	2.34	0.96	1.89	0
5	998	α - phellandrene	4.34	13.92	9.86	4.38	6.81	7.68	6.78	5.04	8.12	3.30
6	1019	α - terpinene	9.09	12.08	10.37	2.78	0	3.72	3.41	3.12	6.18	6.98
7	1028	P - cymene	0	0	0	0	18.96	8.96	0	0	0	0
8	1030	Limonene	0	0	0	0	0	0	1.48	0.48	0	0
9	1033	1,8 - Cineole	11.10	7.18	7.45	9.83	0	5.02	8.92	7.08	11.08	8.72
10	1046	β - Ocimene	0.23	0.27	0.28	0.14	0	0	0.42	0.68	0.18	0
11	1063	γ - terpinene	0.21	1.10	0.48	0.20	0	0	0.18	0	0	0
12	1089	α - terpinolene	12.56	5.60	10.63	18.79	9.81	2.42	15.08	10.20	9.18	6.18
13	1098	Linalool	0.67	0.86	1.92	1.69	1.78	0	0.89	0	0.38	0
14	1103	α - thujone	0	0.15	0	0	0	0	0	0	0	0
15	1112	1,3,8 para mentha triene	0.23	0	1.61	0.54	1.38	0	0.60	1.42	0	0
16	1146	δ - terpineol	0.24	0	0	0	0	0	0	0	0.96	0
17	1145	Camphor	1.03	0	1.2	1.02	4.67	5.48	7.92	2.42	1.09	0
18	1150	Camphene hydrate	0	0	0	0.58	0	0	1.02	0	0	0
19	1165	Borneol	0	0	0	0.82	1.58	3.28	1.72	1.24	0.64	0
20	1186	p - cymen -8- ol	0	1.93	4.52	2.86	2.36	0	1.24	5.20	2.68	4.64
21	1191	α - terpineol	0.96	0.26	0	0.34	0	0	0.89	0	0	3.78
22	1245	Piperitone	0	0	0	0.38	0	0	0	0	0	0
23	1269	Geranial	0.59	0	0	0	0	0	0	0	0	0
24	1394	β - elemene	0.27	0	0.98	0.40	1.20	0.80	0.48	1.09	0	2.34
25	1293	2 - undecanone	0	0	0	0.21	0.80	0	0	0.38	0	0
26	1301	Carvacrol	0	0	2.48	0.31	0	0	0	0.86	0	0

27	1341	δ - elemene	0	0	1.11	0.29	0	0	0	0	0	0	0
28	1412	α - cedrene	0	0	0	0.26	0	0	0	0	0	0	0
29	1419	β - caryophyllene	2.32	6.54	3.40	4.20	3.36	6.02	2.20	3.20	1.98	6.48	0
30	1433	γ - elemene	0	0	0.80	0.46	0	0	0	0	0	0	0
31	1449	α - humulene	0	0	0	0.18	0	0	0	0.51	0	0	0
32	1464	Trans β - farnesene	2.56	0	0.48	0.35	1.28	0.82	0	0.92	0	0	0
33	1481	Germacrene D	0.26	0	0	0.29	0	0	0	0	0	0	0
34	1483	ar - curcumene	8.84	3.82	4.21	2.48	4.36	4.08	2.48	1.82	3.18	0	0
34	1495	Zingiberene	7.98	12.03	9.72	12.18	12.87	14.06	8.42	9.82	9.13	19.62	0
36	1511	β - bisabolene	3.62	2.40	0	3.42	0	2.78	0.42	1.86	1.62	1.82	0
37	1524	β - sesquiphellandrene	5.89	6.28	5.28	6.53	7.34	10.32	8.06	11.17	12.90	11.72	0
38	1557	Germacrene B	1.20	2.86	1.21	2.83	0.82	0.19	2.04	2.30	3.18	2.06	0
39	1542	Nerolidol	1.76	0	0	1.03	1.04	0	0	0.96	0	0	0
40	1532	Ethanone	0	0	0	0	1.18	0	0	0	0	0	0
41	1582	Caryophyllene oxide	0	0	0	0.63	0	0	0	0	0	0	0
42	1591	Curzerenone	0	0	3.14	0.35	0	1.89	0	3.20	4.28	0	0
43	1638	ar - turmerone	12.08	2.18	2.28	4.92	5.84	4.02	4.89	5.28	2.48	4.36	0
44	1643	α - turmerone	0	1.40	0.84	0	2.89	2.12	0	1.02	1.89	2.98	0
45	1673	β - turmerone	0	0	0	0	0.93	0.76	0	0.86	0.89	0	0
46	1679	Santalool	1.45	0	0	0	0	0	0	0	0	0	0
47	1694	Germacrone	3.62	8.46	3.60	8.86	2.83	4.08	6.89	5.83	7.06	5.39	0
48	1698	Curdione	0	0	1.28	1.67	0	1.03	0	2.41	0	2.40	0
			97.03	95.47	92.63	97.97	97.52	92.78	92.27	93.36	94.09	94.59	0

RI - Retention index, 1. 'Alleppey', 2. 'Alleppey Supreme', 3. 'Kedaram', 4. 'Prabha', 5. 'Prathibha', 6. 'Rajapuri', 7. 'Sugandham', 8. 'Suguna', 9. 'Sudarsana' & 10. Wynad

α - terpinolene (12.56%) was the highest observed compound in the fresh leaf oil of cultivar 'Alleppey' followed by ar - turmerone (12.08%), 1,8 - Cineole (11.10%), α - terpinene (9.09%), ar - curcumene (8.84%), zingiberene (7.98%), β - sesquiphellandrene (5.89%), α - phellandrene (4.34%), germacrone (3.62%), β - bisabolene (3.62%), α - pinene (2.64%), trans β - farnesene (2.56%), β - caryophyllene (2.32%), nerolidol (1.76%), germacrene B (1.20%), β - turmerone (1.45%), β - myrcene (1.29%) and camphor (1.03%). A total of twenty seven compounds amounting to 97.03% of the total volatile oil studied were identified using GC MS analysis in this cultivar.

A total of 22 compounds comprising 95.47 % of the total oil were identified by GC MS analysis in 'Alleppey' Supreme'. In this improved variety α - phellandrene (13.92%) was the major essential oil component. Other major compounds identified out of the 22 total constituents were α - terpinene (12.08%), zingiberene (12.0%), germacrone (8.46%), 1,8 - cineole (7.18%), β - caryophyllene (6.54%), β - sesquiphellandrene (6.28%), α - terpinolene (5.60%), ar - curcumene (3.82%), β - pinene (3.26%), myrcene (2.89%), germacrene B (2.86%), β - bisabolene (2.40%), ar - turmerone (2.18%), p - cymen -8- ol (1.93%), α - turmerone (1.40%) and γ - terpinene (1.10%).

Twenty seven compounds amounting to 92.63% of the fresh leaf oil were identified in the variety 'Kedaram', α - terpinolene (10.63%) being the single largest compound followed by α - phellandrene (9.86%), zingiberene (9.72%), 1,8 - cineole (7.45%), β - sesquiphellandrene (5.28%), p - cymen -8- ol (4.52%), ar - curcumene (4.21%), germacrone (3.60%), β - caryophyllene (3.40%), curzerenone (3.14%), carvacrol (2.48%), ar - turmerone (2.28%), linalool (1.92%), β - pinene (1.82%), myrcene (1.68%), 1,3,8 para mentha triene (1.61%), curdione (1.28%), germacrene B (1.21%), camphor (1.20%) and δ - elemene (1.11%),.

The variety 'Prabha' showed highest percentage of α - terpinolene (18.79%), as the major constituent of fresh leaf oil. Thirty seven volatile oil components constituted 97.97 % of the total oil and this variety had the highest number of essential oil components identified from the fresh leaf oil among the different

varieties studied. Other major volatile oil components in 'Prabha' identified by GC MS analysis were zingiberene (12.18%), 1,8 - cineole (9.83%), germacrone (8.86%), β - sesquiphellandrene (6.53%), ar - turmerone (4.92%), α - phellandrene (4.38%), β - caryophyllene (4.20%), β - bisabolene (3.42%), p - cymen -8- ol (2.86%), germacrene B (2.83%), α - terpinene (2.78%), ar - curcumene (2.48%), linalool (1.69%), curdione (1.67%), myrcene (1.09%), nerolidol (1.03%) and camphor (1.02%).

About 18.96% of the total steam volatile oil from fresh leaves variety 'Prathibha' was constituted by p - cymene. A total 24 compounds were identified by the GC MS analysis amounting 97.52% of the total oil. Zingiberene (12.87%), α -terpinolene (9.81%), β - sesquiphellandrene (7.34%), α - phellandrene (6.81%), ar - turmerone (5.84%), camphor (4.67%), ar - curcumene (4.36%), β - caryophyllene (3.36%), α - turmerone (2.89%), germacrone (2.83%), β - pinene (2.45%), p - cymen - 8- ol (2.36%), linalool (1.78%), borneol (1.58%), 1,3,8 para mentha triene (1.38%), trans β - farnesene (1.28%), β - elemene (1.20%), ethanone (1.18%) and nerolidol (1.04%) were the other important volatile oil components observed in the variety 'Prathibha'

The sesquiterpene hydrocarbon, zingiberene (14.06%), was found to be the highest observed compound in the famous cultivar 'Rajapuri'. Out of the 24 compounds identified, constituting 97.52% of the total oil, β - sesquiphellandrene (10.32%), p - cymene (8.96%), α - phellandrene (7.68%), β - caryophyllene (6.02%), camphor (5.48%), 1,8 - cineole (5.02%), ar - curcumene (4.08%), germacrone (4.08%), ar - turmerone (4.02%), borneol (3.28%), α - terpinene (3.72%), β -bisabolene (2.78%), α - terpinolene (2.42%), β - pinene (2.02%), curzerenone (1.89%), α - turmerone (2.12%) and curdione (1.03%) were the other major compounds.

The highest percentage of α - terpinolene (15.08%) content in the fresh leaf volatile oil of different cultivars/varieties studied was recorded in the variety 'Sugandham'. A total of 27 compounds comprising 92.27 % of the total volume of the "Sugandham" oil were identified. Other major compounds identified were 1,8 - cineole (8.92%), zingiberene (8.42%), β - sesquiphellandrene (8.06%), camphor

(7.92%), germacrone (6.89%), α - phellandrene (6.78%), ar - turmerone (4.89%), α - terpinene (3.41%), ar - curcumene (2.48%), myrcene (2.34%), β - caryophyllene (2.20%), camphene (2.08%), germacrene B (2.04%), borneol (1.72%), limonene (1.48%), p - cymen -8- ol (1.24%), camphene hydrate (1.02%) and α - pinene (1.02%).

β - sesquiphellandrene (11.17%) was the highest observed compound in the fresh leaf oil of the variety 'Suguna' followed by α - terpinolene (10.20%), zingiberene (9.82%), 1,8 - cineole (7.08%), germacrone (5.83%), ar - turmerone (5.28%), p - cymen -8- ol (5.20%), α - phellandrene (5.04%), β - caryophyllene (3.20%), curzerenone (3.20%), α - terpinene (3.12%), camphor (2.42%), curdione (2.41%), germacrene B (2.30%), α - pinene (2.03%), β - bisabolene (1.86%), ar - curcumene (1.82%), 1,3,8 para mentha triene (1.42%), borneol (1.24%), β - elemene (1.09%) and α - turmerone (1.02%). A total of thirty compounds amounting to 93.39% of the total oil studied were identified in the GC MS analysis.

The improved variety 'Sudarsana' showed highest content of β -sesquiphellandrene (12.90%), in the fresh leaf oil studied followed by 1,8 - cineole (11.08%), α - terpinolene (9.18%), zingiberene (9.13%), α - phellandrene (8.12%), germacrone (7.06%), α - terpinene (6.18%), curzerenone (4.28%), ar - curcumene (3.18%), germacrene B (3.18%), β - pinene (3.12%), p cymen -8- ol (2.68%), ar - turmerone (2.48%), β - caryophyllene (1.98%), α - turmerone (1.89%), myrcene (1.89%), β - bisabolene (1.62%) and camphor (1.09%). A total of 23 compounds amounting to 94.09% of the total oil studied were identified in the GC MS analysis.

The least number of essential oil components in the fresh leaf oil was identified in the cultivar 'Wynad'. A total of 17 compounds amounting to 94.59% of the total oil were identified in his cultivar. Zingiberene (19.62%) was the highest recorded compound followed by β - sesquiphellandrene (11.72%), 1,8 - cineole (8.72%), α - terpinene (6.98%), β - caryophyllene (6.48%), α - terpinolene (6.18%), germacrone (5.39%), p - cymen -8- ol (4.64%), ar - turmerone (4.36%), α -terpineol (3.78%), α - phellandrene (3.30%), α - turmerone (2.98%), curdione (2.40%), β - elemene (2.34%), germacrene B (2.06%), β - pinene (1.82%) and β - bisabolene (1.82%).

Out of the ten varieties/cultivars studied, five showed cultivar/variety specific compounds. Geranial and santalool were unique to the cultivar 'Alleppey' fresh leaf oil while α - cedrene and caryophyllene oxide were specific to the variety 'Prabha'. In the variety 'Alleppey Supreme', α - thujone was the specific compound while ethanone and camphene were specific to variety 'Prathibha' and 'Sugandham', respectively.

4.3.2. Oleoresin

4.3.2.1. Turmeric varieties/cultivars

The oleoresin content of the varieties/cultivars ranged from 10.4 % ('Hajo') to 15.2 % ('Prathibha'). The released varieties showed a higher value for oleoresin content. The percentage oleoresin content in different turmeric varieties/cultivars are given in Table 90. Fig. 88 shows the percentage of oleoresin, rhizome and leaf essential oil from 36 turmeric varieties/cultivars studied.

Table 90. Mean oleoresin yield from dry rhizomes of turmeric cultivars/varieties.

Sl. No	Cultivar name	Oleoresin (%)	Sl.No.	Cultivar name	Oleoresin (%)
1	'Alleppey'	14.6	19	'Nandyal type'	12.8
2	'Amalapuram'	13.0	20	'Rajapuri'	13.4
3	'Amrithapani'	13.2	21	'Sugandham'	13.6
4	'Armoor'	11.5	22	'Vondimita'	11.9
5	'Jabedi'	11.9	23	'Wynad' local'	13.2
6	'Dhagi'	12.0	24	'Aizwal '	12.9
7	'Katergia'	12.4	25	'Ayur'	14.0
8	'Chayapasupu'	13.0	26	'Basar Along'	11.2
9	'Avanigadda'	11.8	27	'Gaspani '	10.8
10	'Dindigam'	11.6	28	'Hajo '	10.4
11	'Ethamkula'	12.2	29	'Jorhat'	13.0
12	'GL Puram'	12.8	30	'Suvarna'	12.8
13	'Karhadi local'	12.6	31	'Sudarsana'	13.8
14	'Kasturi'	13.8	32	'Kedaram'	14.9
15	'Kodur'	14.2	33	'Suguna'	12.6
16	'Kuchipudi'	12.3	34	'Prathibha'	15.2
17	'Mamomthody'	13.4	35	'Prabha'	14.8
18	'Muvatupuzha'	14.2	36	'Alleppey Supreme'	15.0

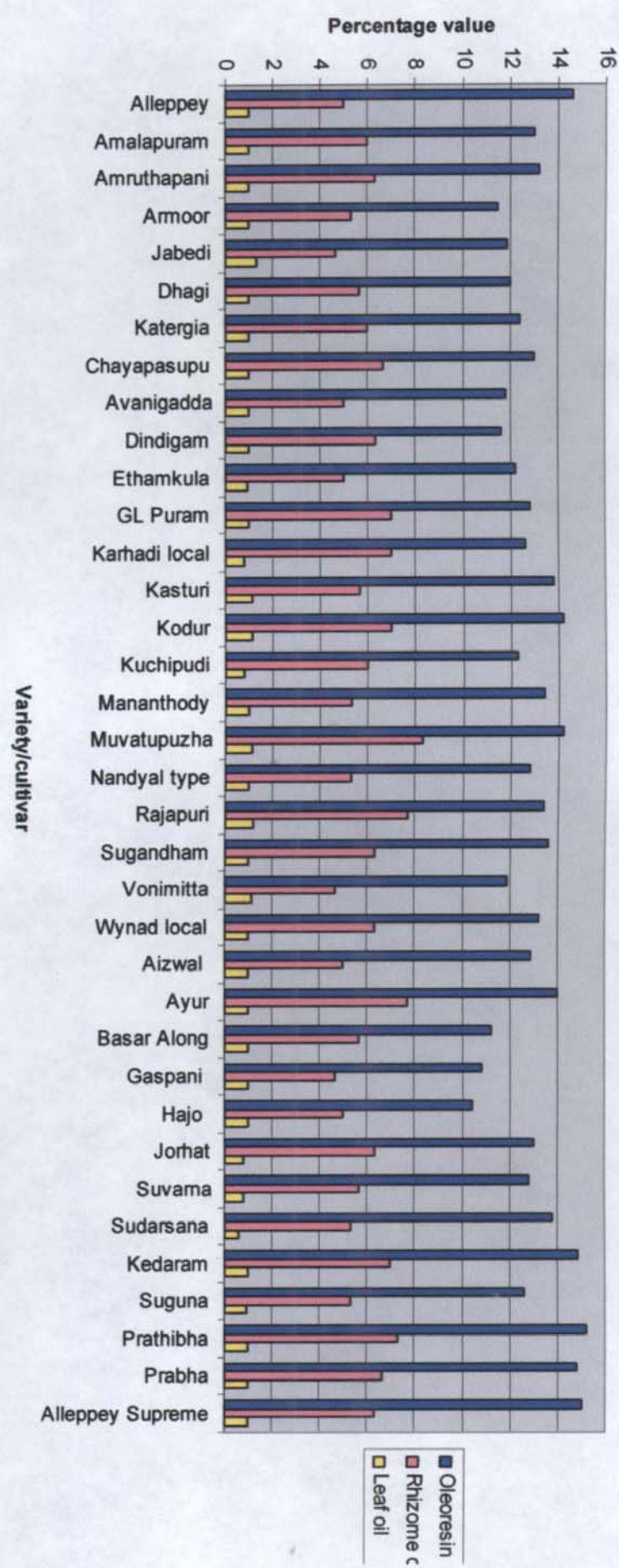


Fig. 88. Histogram showing the percentage of oleoresin, rhizome and leaf essential oil from 36 turmeric varieties/cultivars

4.3.3. Curcumin

4.3.3.1. *Curcuma* species

4.3.3.1.1. Estimation of curcumin

Six *Curcuma* species with light yellow to yellow orange colour to the rhizome were used for the extraction of curcumin. The spectrophotometric analysis showed maximum curcumin content in *C. longa* (6.41 %) and a minimum was recorded in *C. raktakanta* (0.069 %). Table 91 shows the total curcumin content in six *Curcuma* species estimated spectrophotometrically.

Table.91. Total curcumin content in different *Curcuma* species

Sl. No.	Species	Total curcumin (%)
1	<i>C. comosa</i>	0.083
2	<i>C. longa</i>	6.41
3	<i>C. malabarica</i>	1.16
4	<i>C. montana</i>	0.10
5	<i>C. raktakanta</i>	0.069
6	<i>C. zedoaria</i>	1.24

4.3.3.1.2. Curcuminoid profiling by HPLC

The level of curcuminoids in different *Curcuma* species was determined using HPLC. The percentage of three curcuminoids in six *Curcuma* species was given in Table. 92. The highest level of curcumin I, curcumin II and curcumin III was recorded in *C. longa* (1.66, 1.43 and 0.91, respectively) and the lowest level of curcumin I and curcumin II was recorded in *C. raktakanta* (0.026 and 0.011). Four species namely *C. comosa*, *C. malabarica*, *C. raktakanta* and *C. zedoaria* recorded the lowest level of curcumin III (0.001) content. The curcuminoids pattern of different *Curcuma* species alongwith standard curcumin are given in Fig. 89.

Table.92. Percentage of total curcumin, curcumin I, curcumin II, curcumin III and total curcuminoids in *Curcuma* species.

Sl. No.	Species	Total curcumin %	Curcumin I %	Curcumin II %	Curcumin III %	Curcuminoids total %
1	<i>C. comosa</i>	0.083	0.030	0.015	0.001	0.046
2	<i>C. longa</i>	6.41	1.66	1.43	0.907	4.00
3	<i>C. malabarica</i>	1.16	0.059	0.021	0.001	0.081
4	<i>C. montana</i>	0.102	0.039	0.018	0.004	0.057
5	<i>C. raktakanta</i>	0.069	0.026	0.011	0.001	0.041
6	<i>C. zedoaria</i>	1.24	0.061	0.028	0.001	0.090

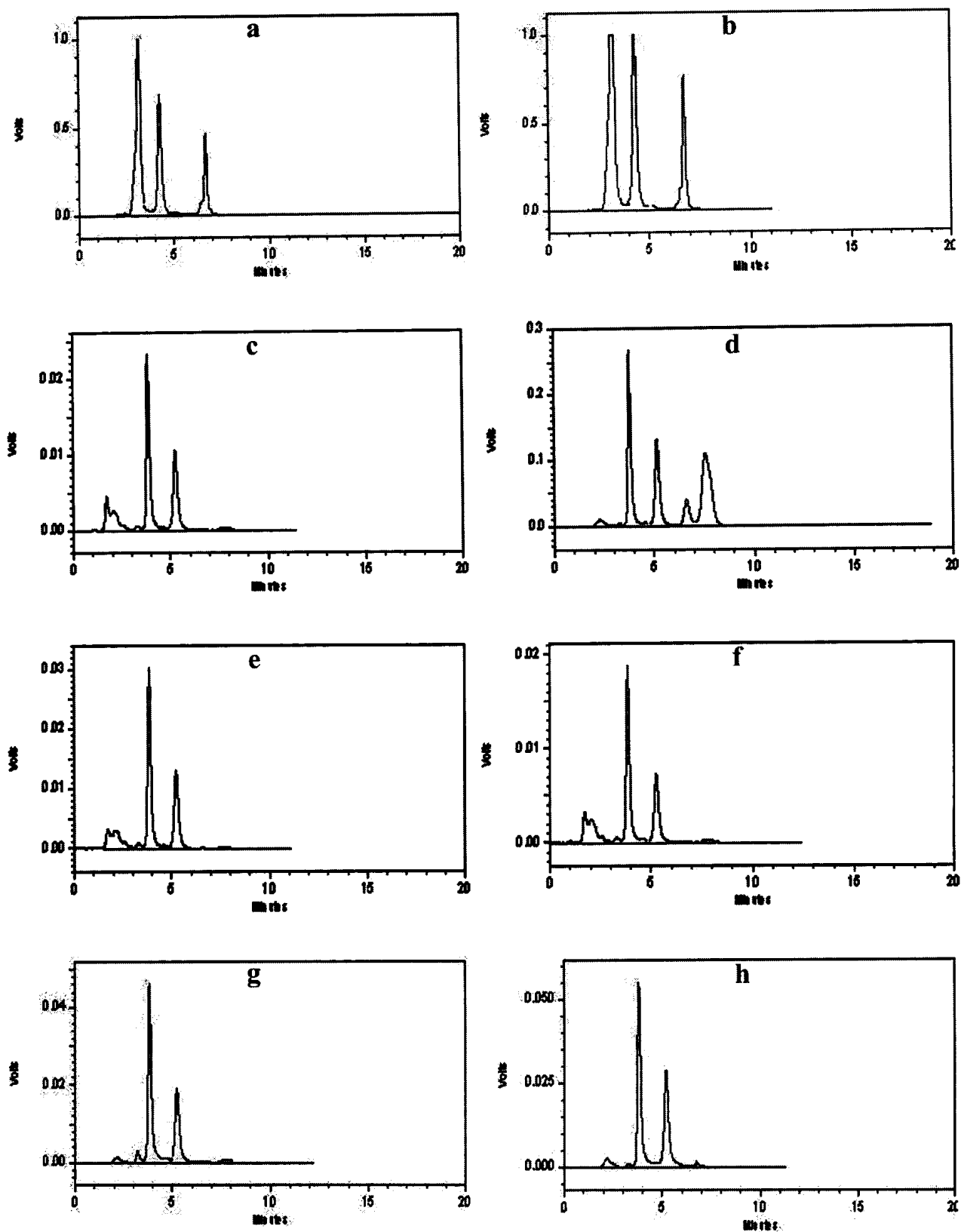


Fig. 89. HPLC analysis of curcuminoids. a & b are curcuminoid profile of standard curcumin in concentrations 20 and 40 ng, respectively. c - h are the curcuminoid profiles of *Curcuma* species viz, *C. comosa*, *C. longa*, *C. montana*, *C. raktakanta*, *C. malabarica* and *C. zedoaria*, respectively.

4.3.3.2. Turmeric varieties/cultivars

4.3.3.2.1. Estimation of curcumin

The total curcumin content of the turmeric varieties/cultivars was estimated spectrophotometrically for three years. The percentage curcumin content ranged from 1.9 % ('Vondimitta') to 6.5 % ('Prathibha'). The mean values for the percentage of curcumin content in turmeric varieties/cultivars over three years were given in Table. 93.

Table 93. Total curcumin content in turmeric varieties/cultivars

Sl. No	Cultivar name	Mean total curcumin (%)	Sl.No.	Cultivar name	Mean total curcumin (%)
1	'Alleppey'	5.80	19	'Nandyal type'	2.45
2	'Amalapuram'	3.20	20	'Rajapuri'	2.30
3	'Amrithapani'	3.20	21	'Sugandham'	2.50
4	'Armoor'	2.95	22	'Vondimita'	1.90
5	'Jabedi'	2.20	23	'Wynad local'	2.30
6	'Dhagi'	2.10	24	'Aizwal '	3.05
7	'Katergia'	2.25	25	'Ayur'	3.75
8	'Chayapasupu'	2.65	26	'Basar Along'	4.25
9	'Avanigadda'	2.65	27	'Gaspani '	2.50
10	'Dindigam'	3.05	28	'Hajo '	2.65
11	'Ethamkula'	2.40	29	'Jorhat'	3.40
12	'GL Puram'	2.35	30	'Suvarna'	3.35
13	'Karhadi local'	3.25	31	'Sudarsana'	4.50
14	'Kasturi'	2.25	32	'Kedaram'	5.95
15	'Kodur'	2.00	33	'Suguna'	3.30
16	'Kuchipudi'	2.45	34	'Prathibha'	6.50
17	'Mamomthody'	4.10	35	'Prabha'	5.90
18	'Muvatupuzha'	2.70	36	'Alleppey Supreme'	5.35

4.3.3.2.2. Curcuminoid profiling by HPLC

Total curcumin from thirty six cultivars/varieties were analyzed using HPLC to determine the levels of different curcuminoids (Figs. 90 - 91).

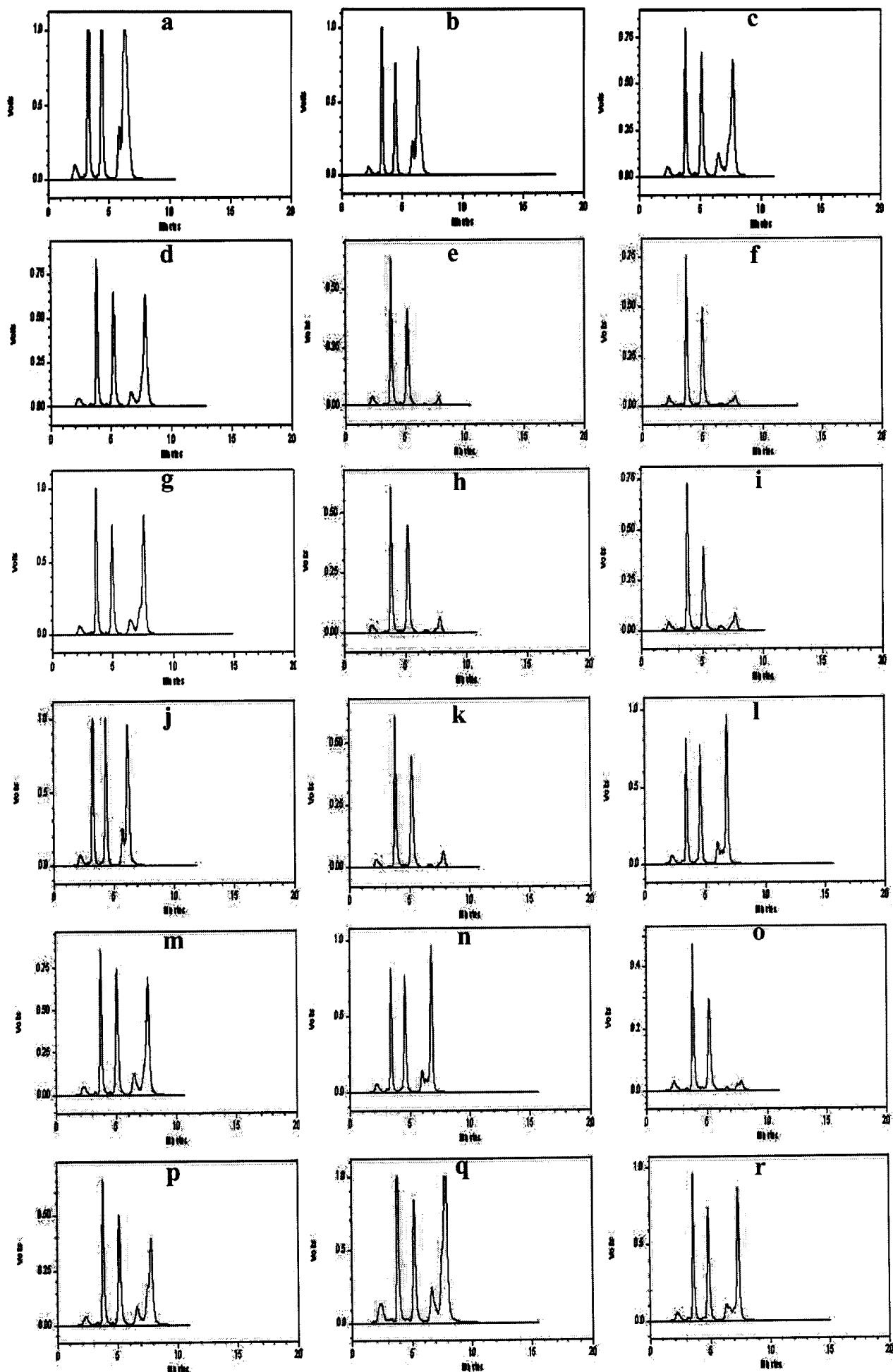


Fig. 90. Curcuminod profiles of turmeric varieties/cultivars. a- r are 'Alleppey', 'Amalapuram', 'Amrithapani', 'Armoor', 'Jabedi', 'Dhagi', 'Katergia', 'Chayapasupu', 'Avanigadda', 'Dindigam', 'Ethamkula', 'GL Puram', 'Karhadi', 'Kasturi', 'Kodur', 'Kuchipudi', 'Mamomthody' and 'Muvatupuzha', respectively.

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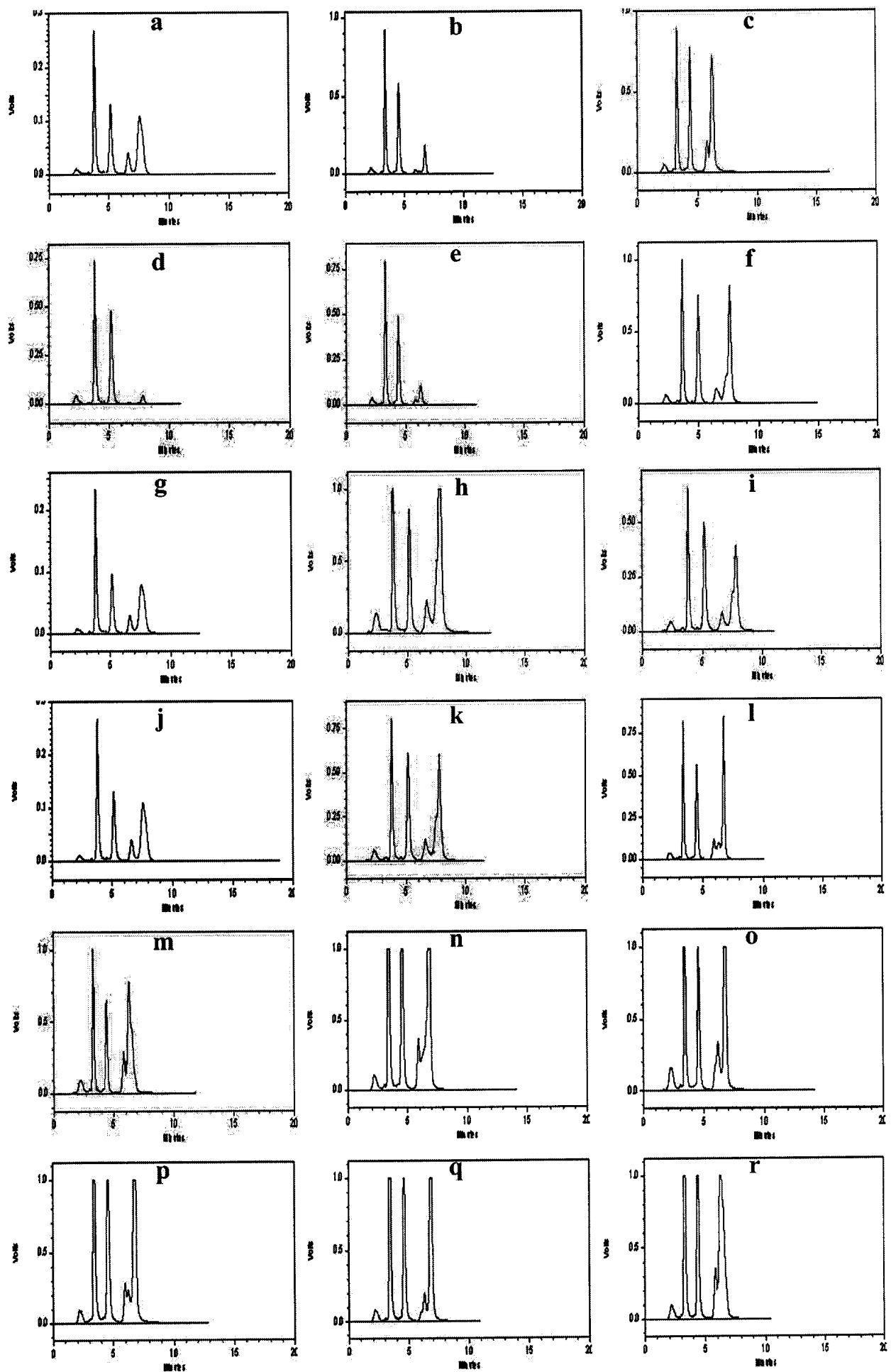


Fig. 91. Curcuminoid profiles of turmeric varieties/cultivars. a - r are 'Nandyal', 'Rajapuri', 'Sugandham', 'Vondimita', 'Wynad', 'Aizwal', 'Ayur', 'Basar Along', 'Gaspani', 'Hajo', 'Jorhat', 'Suvarna', 'Sudarsana', 'Kedaram', 'Suguna', 'Prathibha', 'Prabha' and 'Alleppey Supreme', respectively.

The percentage of total curcuminoids ranged from 0.866 % ('Kuchipudi') to 4.32 % ('Kedaram'). The level of curcumin I was maximum in the improved variety 'Kedaram' (1.78 %) and minimum in cultivar 'Kuchipudi' (0.51 %). Variety 'Prathibha' showed highest content of curcumin II (1.48 %) and 'Kuchipudi' (0.32 %) had the lowest content of curcumin II. The cultivar 'Basar Along' had the highest content of curcumin III (1.18 %) while the least curcumin III content was recorded in 'Jabedi' (0.026 %). The variety 'Sudarsana' followed by the cultivars 'Mamomthody' and 'Basar Along' showed higher curcumin III content when compared to the curcumin II content in these accessions. In all other varieties/cultivars studied curcumin I was the major curcuminoids followed by curcumin II and curcumin III. The percentage of total curcumin, curcumin I, curcumin II, curcumin III and total curcuminoids in turmeric cultivars/varieties are given in Table. 94 & Fig. 92. The percentage of total curcumin in turmeric varieties/cultivars estimated using spectrophotometric method and recovery of total curcuminoids by HPLC analysis are shown in Fig. 93.

Table 94. Percentage of total curcumin, curcumin I, curcumin II, curcumin III and total curcuminoids in turmeric cultivars/varieties.

Sl. No.	Cultivar name	Total curcumin (%)	Curcumin I (%)	Curcumin II (%)	Curcumin III (%)	Curcuminoids total (%)
1	'Alleppey'	6.41	1.66	1.43	0.907	3.99
2	'Amalapuram'	3.40	1.16	0.77	0.631	2.56
3	'Amrithapani'	3.13	0.951	0.859	0.550	2.36
4	'Armoor'	2.89	0.984	0.854	0.527	2.37
5	'Jabedi'	1.71	0.773	0.555	0.026	1.35
6	'Dhagi'	1.88	0.845	0.610	0.052	1.51
7	'Katergia'	2.50	1.07	0.847	0.119	2.03
8	'Chayapasupu'	1.66	0.738	0.597	0.055	1.39
9	'Avanigadda'	1.54	0.857	0.533	0.079	1.47
10	'Dindigam'	3.40	1.14	0.984	0.604	2.72
11	'Ethamkula'	1.83	0.880	0.618	0.041	1.54
12	'GL Puram'	2.38	1.02	0.793	0.084	1.90
13	'Karhadi local'	3.21	1.00	0.935	0.558	2.49
14	'Kasturi'	2.62	0.856	0.807	0.476	2.14
15	'Kodur'	1.75	0.581	0.399	0.032	1.01
16	'Kuchipudi'	1.68	0.515	0.322	0.030	0.867
17	'Mamomthody'	4.88	1.61	1.11	1.17	3.91
18	'Muvatupuzha'	3.01	1.03	0.858	0.501	2.39
19	'Nandyal type'	1.69	0.734	0.581	0.055	1.38
20	'Rajapuri'	1.91	0.907	0.609	0.085	1.60
21	'Sugandham'	2.96	0.933	0.795	0.473	2.20
22	'Vondimita'	1.82	0.892	0.635	0.035	1.56
23	'Wynad local'	1.75	0.829	0.492	0.069	1.39
24	'Aizwal '	3.39	1.17	0.905	0.608	2.68
25	Ayur	2.75	0.972	0.821	0.049	1.84
26	'Basar Along'	5.18	1.58	1.14	1.18	3.89
27	'Gaspani '	2.29	0.793	0.656	0.375	1.82
28	'Hajo '	1.48	0.571	0.343	0.035	0.949
29	'Jorhat'	3.12	0.978	0.805	0.555	2.34
30	'Suvarna'	2.74	0.799	0.564	0.362	1.72
31	'Sudarsana'	3.83	1.16	0.653	0.711	2.52
32	'Kedaram'	7.73	1.78	1.44	1.11	4.32
33	'Suguna'	5.16	1.45	1.08	0.776	3.31
34	'Prathibha'	7.17	1.75	1.48	0.875	4.10
35	'Prabha'	5.87	1.64	1.27	0.758	3.66
36	'Alleppey Supreme'	5.34	1.65	1.35	1.15	4.16

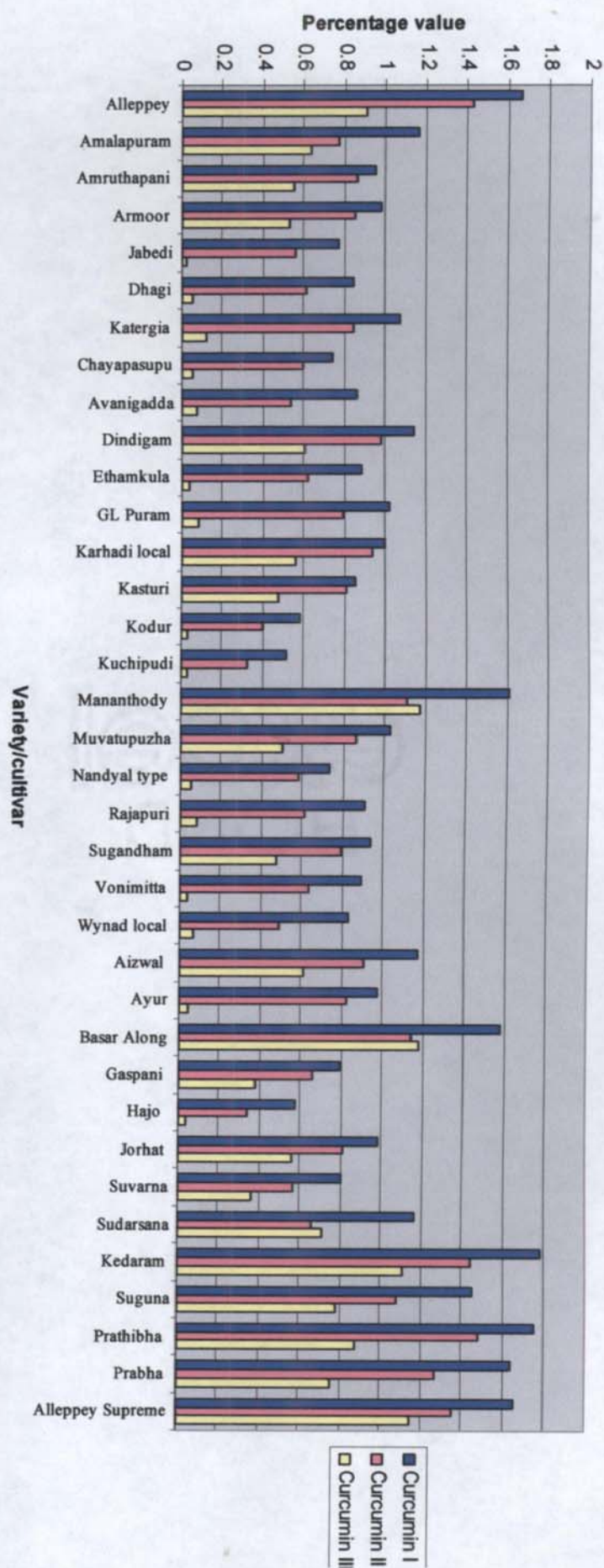


Fig. 92. Histogram showing levels of curcumin I, curcumin II and curcumin III in different turmeric varieties/cultivars studied using HPLC analysis.

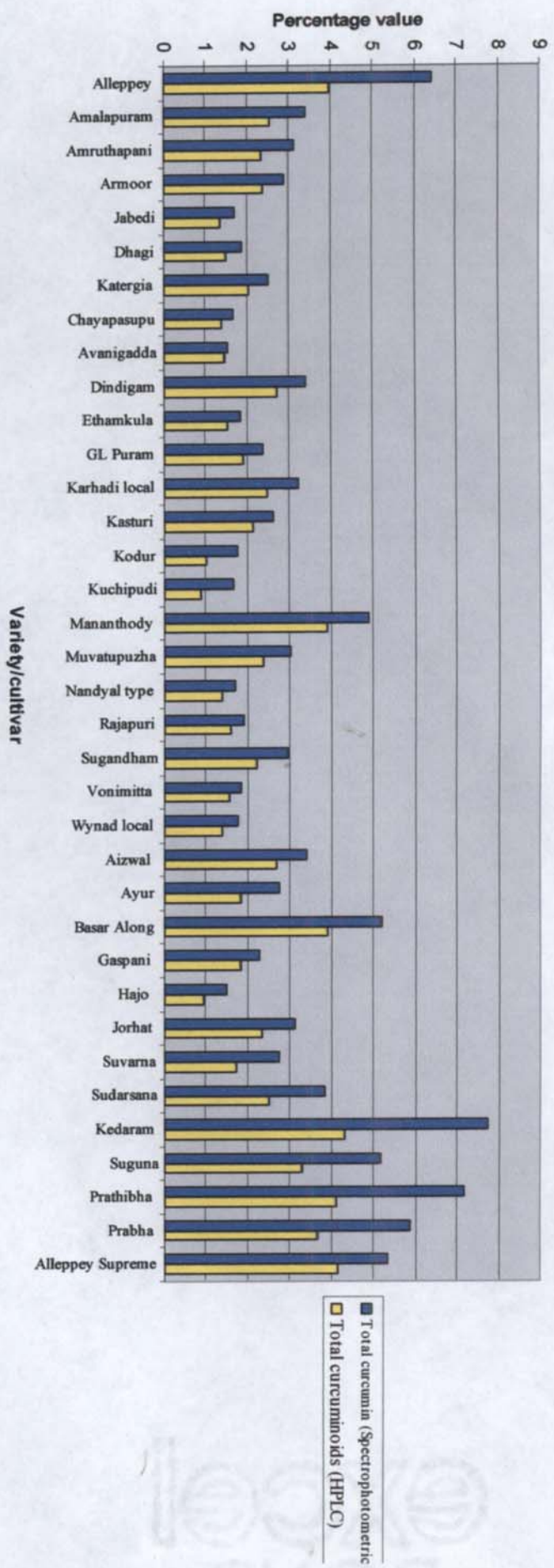


Fig. 93. Histogram showing the percentage of total curcumin in turmeric varieties/cultivars estimated using spectrophotometric method and recovery of total curcuminoids by HPLC analysis.

4.3.4. Turmerin

4.3.4. 1. *Curcuma* species

The water soluble antioxidant peptide, turmerin from fresh and dry rhizomes of 15 *Curcuma* species was estimated using Bradford' method. Both fresh mother and finger rhizomes were used besides the pooled dry rhizomes. Maximum yield for turmerin in fresh mother rhizome and dried rhizomes was recorded by *C. sylvatica* (516.47 mg % and 320.20 mg % respectively) while *C. longa* had the highest amount of turmerin in finger rhizomes (380.48 mg %). *C. amada* had minimum turmerin content in fresh mother, finger and dried rhizomes (89.32 mg %, 64.82 mg % and 62.22mg %). The amount of turmerin in fresh mother, finger rhizomes and dried rhizomes of different *Curcuma* species are given in Table. 95 & Fig. 94.

Table 95. Turmerin content in fresh mother, finger and dried whole clump of *Curcuma* species

Sl. No	Species	Fresh mother (mg %)	Fresh finger (mg %)	Dry (mg %)
1	<i>C. aeruginosa</i>	222.88	157.34	121.52
2	<i>C. aromatica</i>	350.86	176.47	202.55
3	<i>C. amada</i>	89.32	64.82	62.22
4	<i>C. caesia</i>	466.75	303.08	312.72
5	<i>C. comosa</i>	502.91	84.32	293.68
6	<i>C. decipiens</i>	238.46	159.32	118.48
7	<i>C. haritha</i>	435.17	135.28	243.03
8	<i>C. longa</i>	412.52	380.48	290.80
9	<i>C. malabarica</i>	288.83	144.15	213.07
10	<i>C. montana</i>	472.77	182.3	260.21
11	<i>C. pseudomontana</i>	451.53	369.73	280.38
12	<i>C. raktakanta</i>	479.26	206.26	269.43
13	<i>C. species</i>	286.56	192.47	162.70
14	<i>C. sylvatica</i>	516.47	317.46	320.20
15	<i>C. zedoaria</i>	197.41	209.59	142.62

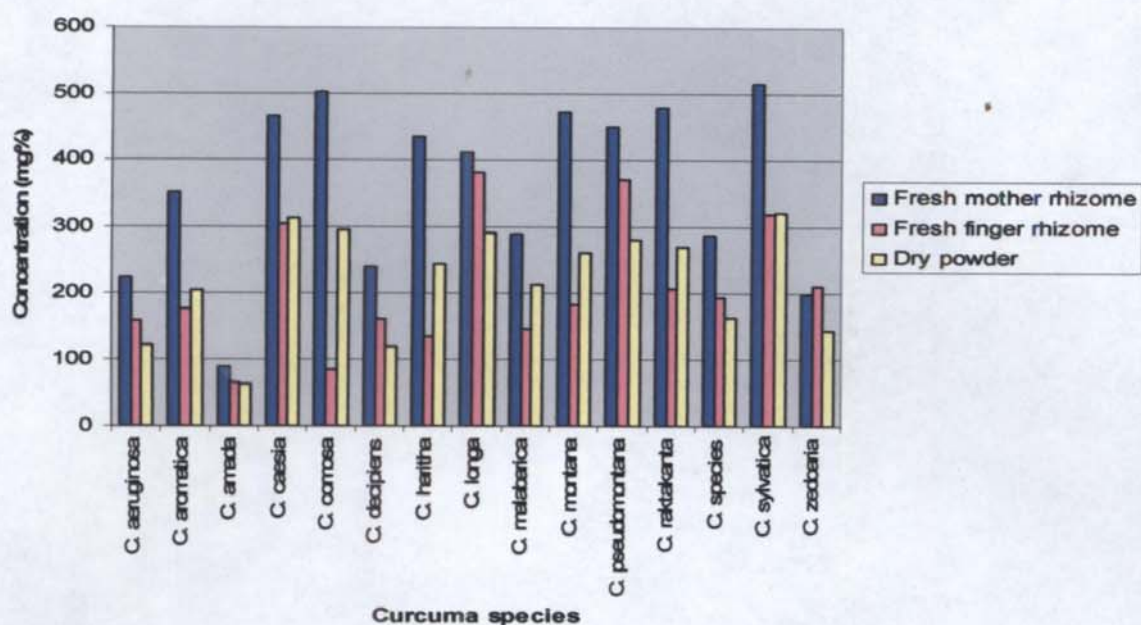


Fig. 94. Histogram showing the levels of turmerin in fresh mother rhizomes, fresh finger rhizomes and dried powder of different *Curcuma* spp.

4.3.4. 2. Turmeric cultivar/varieties

Among the fifteen turmeric cultivars/varieties used for the extraction of turmerin, the cultivar 'Rajapuri' recorded the maximum yield (318.24 mg %) for turmerin in dried turmeric powder. In all the varieties/cultivars the amount of turmerin was on par and the lowest turmerin content was recorded in the cultivar 'GL Puram' (248.36 mg %). Table 96 gives the details of turmeric cultivars studied and the turmerin content.

Table 96. Turmerin content in selected dried whole clump of turmeric cultivars/varieties

Sl. No	Variety/Cultivar	Dry powder (mg %)
1	'Alleppey'	290.80
2	'Alleppey' Supreme	292.12
3	'Amalapuram'	284.76
4	'GL Puram'	248.36
5	'Kasturi'	256.48
6	'Kedaram'	304.68
7	'Nandyal type'	289.82
8	'Prabha'	302.28
9	'Prathibha'	298.42
10	'Rajapuri'	318.24
11	'Sudarsana'	284.18
12	'Sugandham'	286.48
13	'Suguna'	289.86
14	'Suvarna'	278.92
15	'Wynad' local'	278.52

4.3.5. Rhizome protein

Biochemical characterization of *Curcuma* species and turmeric varieties/cultivars was done using HPLC analysis of rhizome proteins.

4.3.5.1. *Curcuma* species

4.3.5.1.1. Protein estimation

Among the thirteen species studied *C. comosa* showed maximum protein content in the water extract (959.1 mg %) while *C. amada* showed the lowest protein content (136.4 mg %). The salt extract of *C. pseudomontana* showed maximum protein content (946.3 mg %) and *C. amada* had the least (227.3 mg %) in this case too (Table 97 and Fig. 95).

Table 97. Water and salt soluble rhizome proteins from different *Curcuma* species

Sl. No	Species	Water extract (mg %)	Salt extract (mg %)
1	<i>C. aeruginosa</i>	791.9	907.9
2	<i>C. aromatica</i>	220.5	604.7
3	<i>C. amada</i>	136.4	227.3
4	<i>C. caesia</i>	939.3	928.9
5	<i>C. comosa</i>	959.1	916.3
6	<i>C. haritha</i>	866.8	928.2
7	<i>C. longa</i>	669.7	842.1
8	<i>C. malabarica</i>	842.9	941.7
9	<i>C. montana</i>	684.7	884.9
10	<i>C. pseudomontana</i>	880.2	946.3
11	<i>C. raktakanta</i>	254.9	551.5
12	<i>C. sylvatica</i>	931.2	926.9
13	<i>C. zedoaria</i>	692.7	930.3

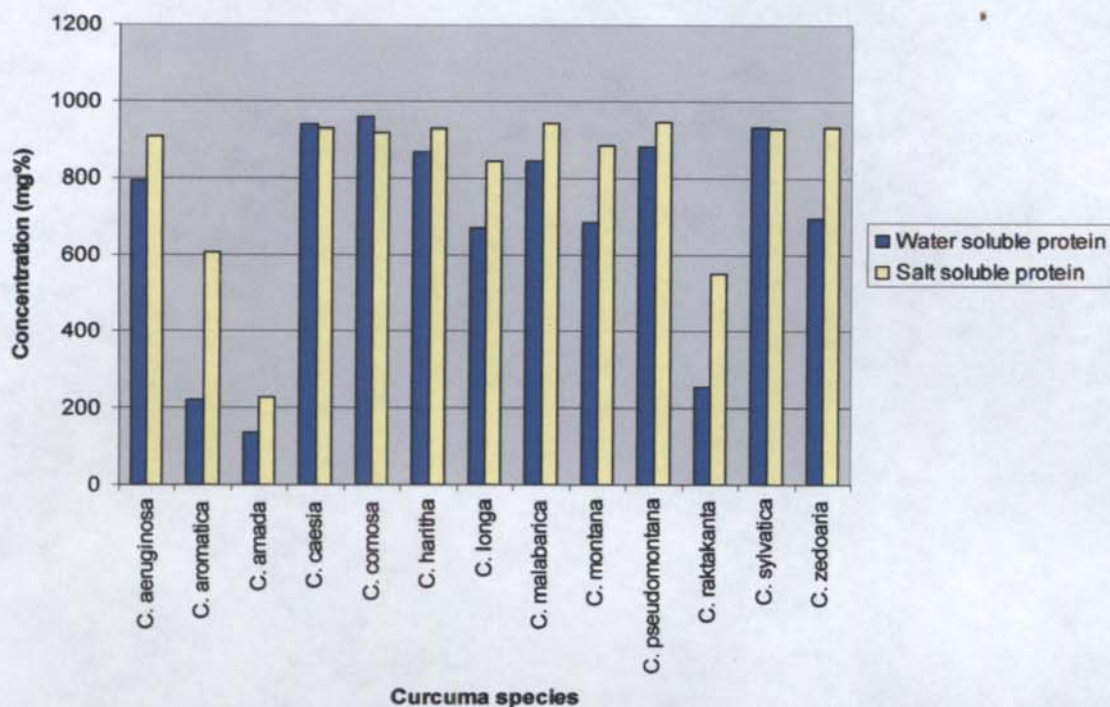


Fig. 95. Histogram showing the concentration of water soluble and salt soluble rhizome protein from different *Curcuma* species

4.3.5.1.2. HPLC analysis of water soluble *Curcuma* species rhizome proteins

Among the two wavelengths selected for the detection of rhizome proteins using anion exchange HPLC analysis 260 nm gave good response. The elution profiles of water soluble rhizome proteins obtained by AE HPLC at wavelength 260 nm in a time range of 0 – 60 min allowed all the *Curcuma* species studied to be distinguished from each other (Fig. 96).

The peaks in the water soluble rhizome protein elution profiles were scored as '1' for the presence and '0' for the absence and presented in a binary matrix format. The genetic similarity was calculated using Jaccard's similarity coefficient. The similarity matrices obtained is represented in Table 98.

Table 98. Similarity matrix obtained from the AE HPLC profiles of water soluble rhizome proteins from different *Curcuma* species using Jaccard's similarity coefficients

Row/Col	Aeruginosa	Amada	Aromatica	Caesia	Comosa	Haritha	Longa	Malabarica	Montana	Pseudomontana	Raktakanta	Sylvatica	Zedoaria
Aeruginosa	1.000000												
Amada	0.500000	1.000000											
Aromatica	0.4117647	0.4666667	1.000000										
Caesia	0.500000	0.5833333	0.600000	1.000000									
Comosa	0.4848485	0.5517241	0.5142857	0.5517241	1.000000								
Haritha	0.4516129	0.5185185	0.6060606	0.5925926	0.500000	1.000000							
Longa	0.5517241	0.560000	0.4516129	0.560000	0.5333333	0.500000	1.000000						
Malabarica	0.4666667	0.5384615	0.4375000	0.5384615	0.5161290	0.4827586	0.5925926	1.000000					
Montana	0.500000	0.500000	0.4117647	0.500000	0.6060606	0.4516129	0.5517241	0.5333333	1.000000				
Pseudomontana	0.4827586	0.560000	0.4516129	0.560000	0.4666667	0.500000	0.6153846	0.5185185	0.4827586	1.000000			
Raktakanta	0.5161290	0.5185185	0.4242424	0.5185185	0.5625000	0.4666667	0.500000	0.5517241	0.5806452	0.500000	1.000000		
Sylvatica	0.5806452	0.5185185	0.4848485	0.5185185	0.4375000	0.4666667	0.500000	0.5517241	0.5161290	0.6428571	0.4666667	1.000000	
Zedoaria	0.4666667	0.5384615	0.4375000	0.5384615	0.4516129	0.4827586	0.5925926	0.6428571	0.4666667	0.5185185	0.4827586	0.5517241	1.000000

4.3.5.1.2.1. Cluster analysis

The dendrogram constructed using UPGMA (unweighted pair-group mean arithmetic) method from the similarity matrices of genetic distances obtained from Jaccard's coefficient is given in Fig. 97. The dendrogram split at coefficient 0.56 showed six groups. The different in the groups are

- Group I - *C. aeruginosa*
- Group II - *C. amada*
- Group III - *C. longa*, *C. malabarica* and *C. zedoaria*
- Group IV - *C. pseudomontana* and *C. sylvatica*
- Group V - *C. comosa*, *C. montana* and *C. raktakanta*
- Group VI - *C. aromatica*, *C. haritha* and *C. caesia*

Among the six groups *C. malabarica* & *C. zedoaria* and *C. pseudomontana* & *C. sylvatica* showed maximum similarity (0.64) and the least similarity showed between *C. aeruginosa* and *C. aromatica* (0.41). *C. aeruginosa* and *C. amada*

formed the first and second groups in the cluster, while *C. longa*, *C. malabarica* and *C. zedoaria* formed the third group. In the dendrogram constructed based on rhizome protein profiles also *C. malabarica* and *C. zedoaria* grouped together showing maximum similarity between them. Group IV was constituted by *C. pseudomontana* and *C. sylvatica* and the members of group V and VI are the same as observed in the dendrograms constructed using molecular markers, except for *C. caesia* which grouped with *C. aromatica* and *C. haritha* in the dendrogram constructed using AE HPLC profile of rhizome proteins.

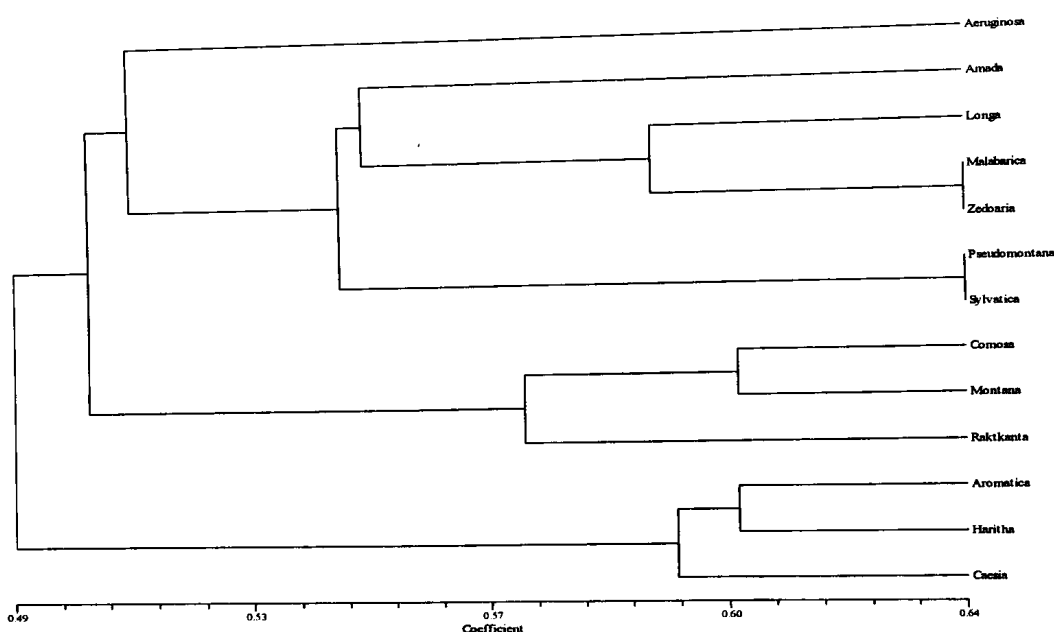


Fig. 97. UPGMA dendrogram showing genetic similarity between different *Curcuma* species calculated by AE HPLC data of water soluble rhizome proteins using Jaccard's similarity coefficient.

4.3.5.1.3. HPLC analysis of salt soluble *Curcuma* species rhizome proteins

For salt soluble proteins also the wavelength of 260 nm was found to be optimum for the detection of rhizome proteins using anion exchange HPLC (Fig. 98).

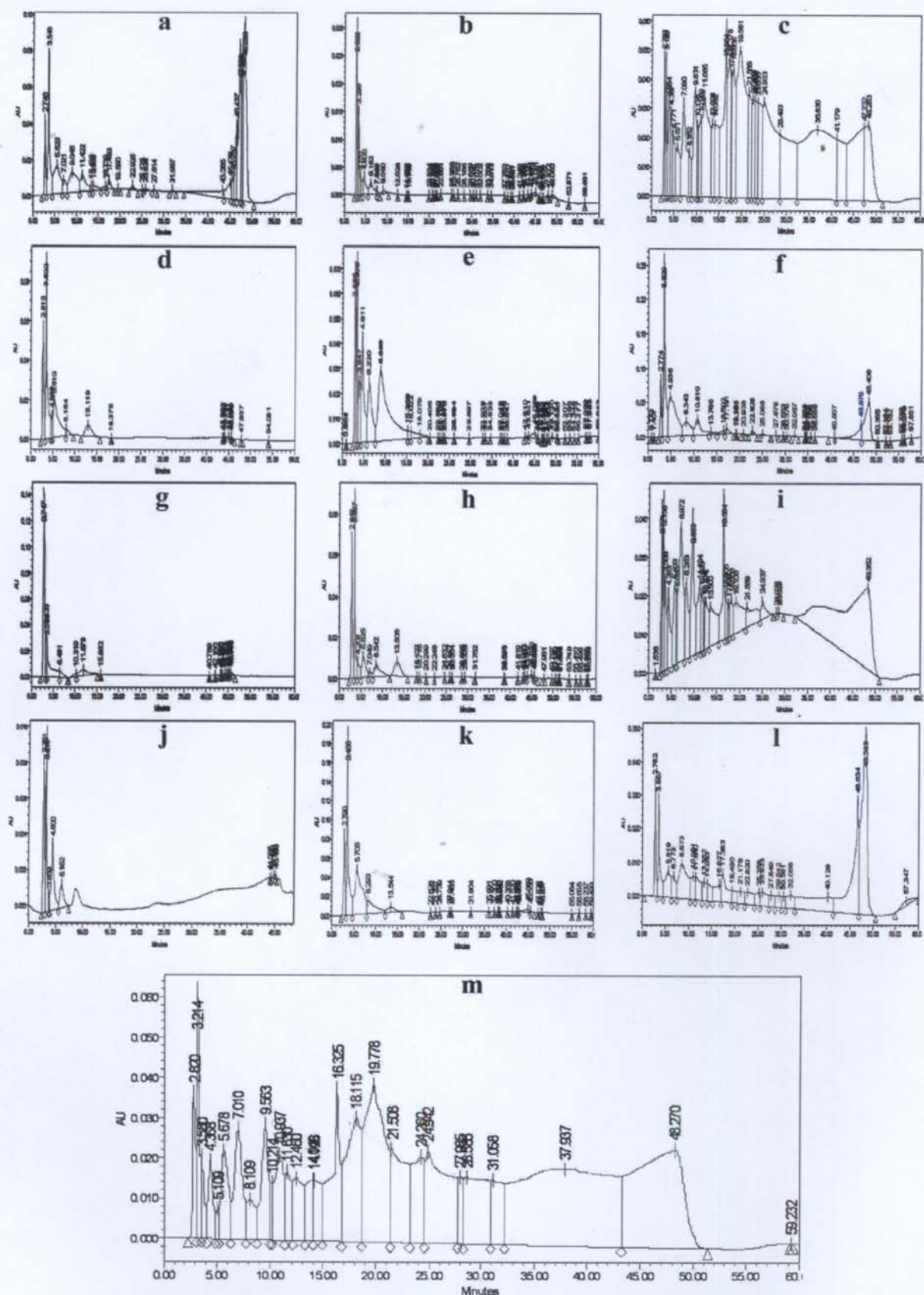


Fig. 98. AE HPLC analysis of salt soluble rhizome proteins form *Curcuma* species. a). *C. aeruginosa*, b). *C. amada*, c). *C. aromatica*, d). *C. caesia*, e). *C. comosa*, f). *C. haritha*, g). *C. longa*, h). *C. malabarica*, i). *C. montana*, j). *C. pseudomontana*, k). *C. raktakanta*, l). *C. sylvatica*, m). *C. zedoaria*.

The elution profiles of water soluble rhizome proteins obtained by AE HPLC at wavelength 260 nm in a time range of 0 – 60 min eventhough separated the different *Curcuma* species studied, no sensual pattern was observed. The similarity matrices obtained is represented in Table 99.

Table 99. Similarity matrix obtained from the AE HPLC profiles of salt soluble rhizome proteins from different *Curcuma* species using Jaccard's similarity coefficients.

Rows\Cols	Aeruginosa	Amada	Aromatica	Caesia	Comosa	Haritha	Malabarica	Montana	Pseudomontana	Raktakanta	Sylvatica	Zedoaria	Longa
Aeruginosa	1.0000000												
Amada	0.6097561	1.0000000											
Aromatica	0.3448276	0.3333333	1.0000000										
Caesia	0.5128205	0.5000000	0.3800000	1.0000000									
Comosa	0.4750000	0.5000000	0.3800000	0.6129032	1.0000000								
Haritha	0.4772727	0.4318182	0.5306122	0.5555556	0.5135135	1.0000000							
Malabarica	0.4651163	0.4878049	0.3773585	0.5428571	0.5428571	0.4634146	1.0000000						
Montana	0.3166667	0.3275862	0.2714286	0.3725490	0.4000000	0.3333333	0.3454545	1.0000000					
Pseudomontana	0.4750000	0.5000000	0.3800000	0.6129032	0.6129032	0.5135135	0.5882353	0.4000000	1.0000000				
Raktakanta	0.4523810	0.4750000	0.3653846	0.6250000	0.6250000	0.4871795	0.5135135	0.4693878	0.5757576	1.0000000			
Sylvatica	0.4318182	0.4523810	0.3518519	0.5428571	0.5428571	0.4634146	0.4871795	0.4230769	0.6875000	0.5135135	1.0000000		
Zedoaria	0.3508772	0.3888889	0.2794118	0.4166667	0.3877551	0.3454545	0.5000000	0.2753623	0.4166667	0.4000000	0.3584906	1.0000000	
Longa	0.4750000	0.5405405	0.3800000	0.6129032	0.6129032	0.5135135	0.6363636	0.3725490	0.6129032	0.5757576	0.5428571	0.4782609	1.0000000

4.3.5.1.3.1. Cluster analysis

The dendrogram constructed using UPGMA (unweighted pair-group mean arithmetic) method from the similarity matrices of genetic distances obtained from Jaccard's coefficient is given in Fig. 99. The dendrogram split at coefficient 0.52 showed five groups. The different in the groups are

- Group I - *C. aeruginosa* and *C. amada*
- Group II - *C. caesia*, *C. raktakanta*, *C. comosa*, *C. malabarica*, *C. longa*, *C. pseudomontana* and *C. sylvatica*
- Group III - *C. aromatica*, and *C. haritha*
- Group IV - *C. zedoaria*
- Group V - *C. montana*

Among the five groups in this dendrogram also *C. pseudomontana* & *C. sylvatica* showed maximum similarity (0.69) and the least similarity showed between *C. montana* and *C. zedoaria* (0.27). Another interesting pattern observed in

the dendrogram was the clustering of *C. aromatica* and *C. haritha* together as observed in the dendrograms constructed using molecular markers too.

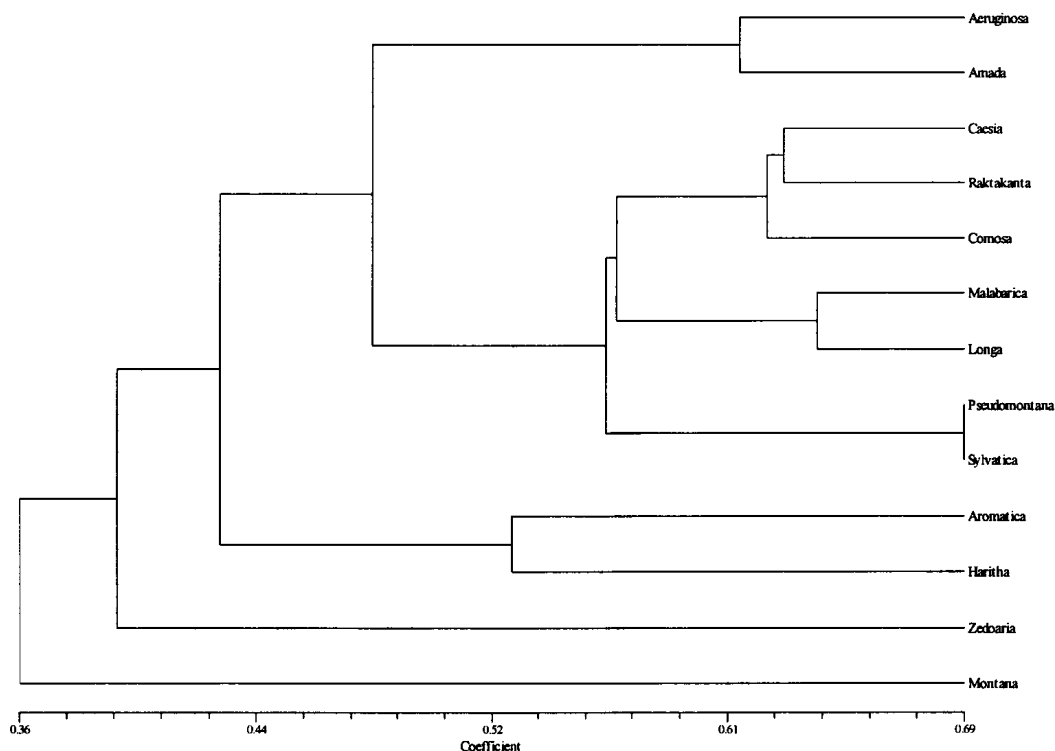


Fig. 99. UPGMA dendrogram showing genetic similarity between different *Curcuma* species calculated by AE HPLC data of salt soluble rhizome proteins using Jaccard's similarity coefficient.

4.3.5.2. Turmeric cultivars/varieties

4.3.5.2.1. Protein estimation

Ten selected turmeric varieties/cultivars were used for extraction of rhizome proteins and the protein content was determined using Bradford's method. The improved variety 'Suguna' showed highest protein content in water and salt extracts while the cultivar 'Wynad' showed least protein content in water extract and the variety 'Sugandham' had the least protein content in salt extract of rhizome proteins Table 100 and fig. 100.

Table 100. Water and salt soluble rhizome proteins from different turmeric varieties/cultivars

Sl. No	Variety/Cultivar	Water extract (mg %)	Salt extract (mg %)
1	'Alleppey'	670.2	827.6
2	'Alleppey Supreme'	654.4	827.0
3	'Kedaram'	579.6	763.6
4	'Prabha'	669.7	842.1
5	'Prathibha'	511.5	690.0
6	'Rajapuri'	455.2	691.3
7	'Sudarsana'	736.2	846.7
8	'Sugandham'	236.5	456.4
9	'Suguna'	773.6	913.3
10	'Wynad'	371.0	630.9

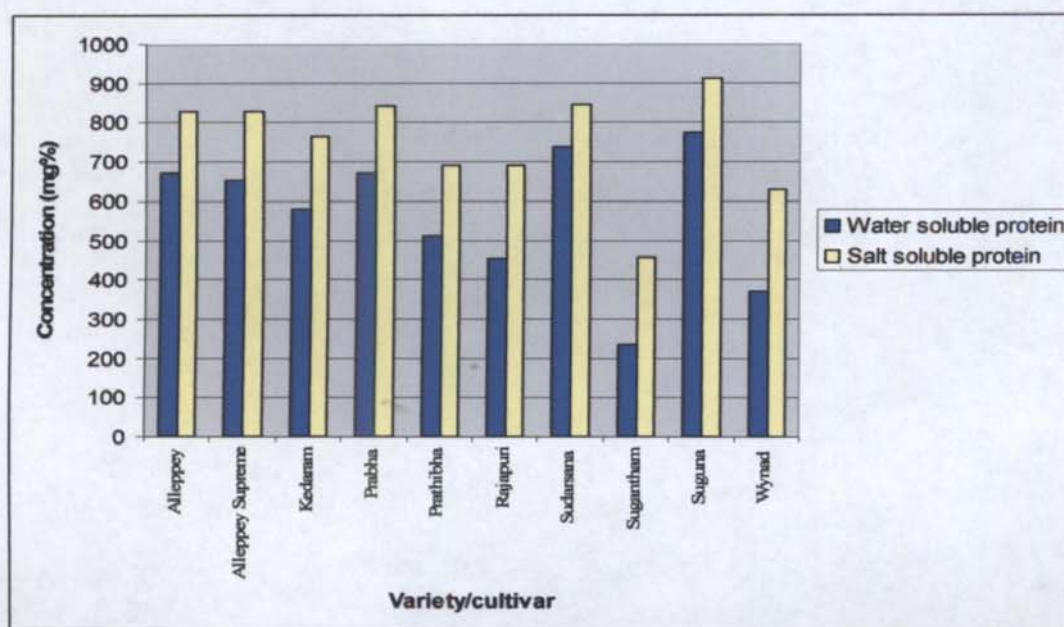


Fig. 100. Histogram showing the concentration of water soluble and salt soluble rhizome protein from different turmeric varieties/cultivars

4.3.5.2.2. HPLC analysis of water soluble proteins from turmeric varieties/cultivars

The elution profiles of water soluble rhizome proteins obtained by anion exchange HPLC at wavelength 260 nm in a time range of 0 – 60 minutes allowed the ten cultivars/varieties studied to be distinguished from each other (Fig.101).

4.3.5.2.2.1. Cluster analysis

The genetic similarity was calculated from the AE HPLC profiles of turmeric rhizome proteins using Jaccard's similarity coefficient. The similarity matrices obtained is represented in Table 101.

Table 101. Similarity matrix obtained from the AE HPLC profiles of water soluble rhizome proteins from different turmeric cultivars/varieties using Jaccard's similarity coefficients.

Rows\Cols	Alleppey	AllepSupr	Kedaram	Prabha	Prathibha	Rajapuri	Sugantam	Suguna	Sudarsana	Wynad
Alleppey	1.0000000									
AllepSupr	0.4814815	1.0000000								
Kedaram	0.6666667	0.6153846	1.0000000							
Prabha	0.5416667	0.5600000	0.5600000	1.0000000						
Prathibha	0.5769231	0.3870968	0.5357143	0.4814815	1.0000000					
Rajapuri	0.4000000	0.3750000	0.3750000	0.4137931	0.3636364	1.0000000				
Sugantam	0.2181818	0.2105263	0.2105263	0.2222222	0.2068966	0.2033898	1.0000000			
Suguna	0.3513514	0.3000000	0.3333333	0.3243243	0.2926829	0.2857143	0.1791045	1.0000000		
Sudarsana	0.4800000	0.4444444	0.4444444	0.5000000	0.4285714	0.4137931	0.2222222	0.3243243	1.0000000	
Wynad	0.5600000	0.5769231	0.5185185	0.5200000	0.5000000	0.3870968	0.2363636	0.3076923	0.5833333	1.0000000

The dendrogram constructed using UPGMA (unweighted pair-group mean arithmetic) method from the similarity matrices of genetic distances obtained from Jaccard's coefficient is given in Fig. 102. The dendrogram split at coefficient 0.55 showed six groups. The different in the groups are

- Group I - 'Alleppey', 'Kedaram' and 'Prathibha'
- Group II - 'Alleppey Supreme' and 'Prabha'
- Group III - 'Sudarsana' and 'Wynad'
- Group IV - 'Rajapuri'
- Group V - 'Suguna'
- Group VI - 'Sugandham'

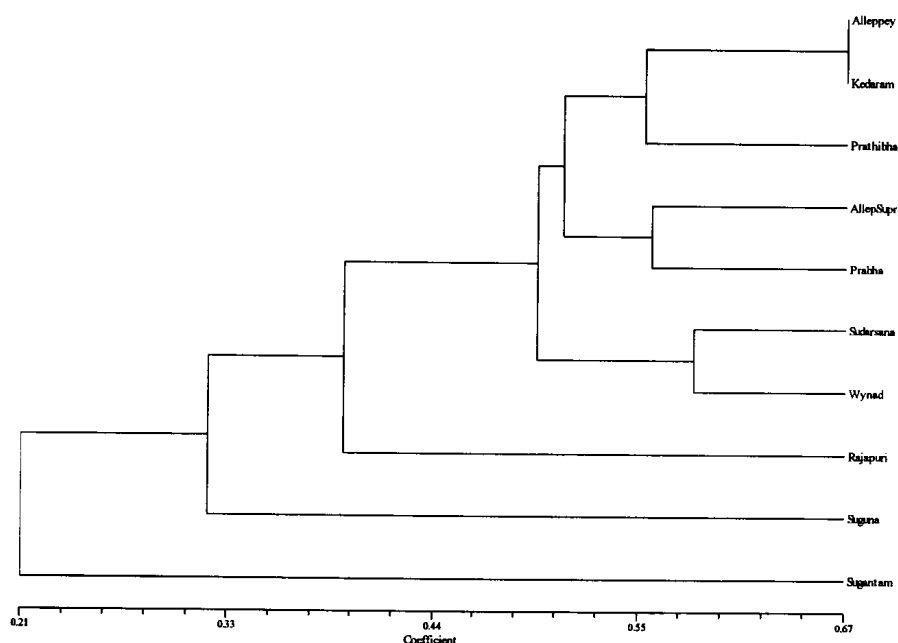


Fig. 102. UPGMA dendrogram showing genetic similarity between different turmeric varieties/cultivars calculated by AE HPLC data of water soluble rhizome proteins using Jaccard's similarity coefficient.

Among the six groups in the dendrogram 'Alleppey' and 'Kedaram' showed maximum similarity (0.67) and the least similarity showed between 'Suguna' and 'Sugandham'. The grouping of varieties/cultivars in the dendrogram constructed using AE HPLC profiles were also some what similar to the grouping of the same varieties/cultivars in the dendrograms constructed using molecular marker data.

4.3.5.2.3. HPLC analysis of salt soluble proteins from turmeric varieties/cultivars

The elution profiles of salt soluble rhizome proteins obtained by anion exchange HPLC at wavelength 260 nm in a time range of 0 – 60 minutes was failed to separate the different cultivars/varieties studied eventhough it showed the presence of peaks in the chromatogram. The proteins showed minor difference in the elution pattern and thus no further analysis was carried out for the data developed.

Discussion

Chapter 5

DISCUSSION

5. 1. MORPHOLOGICAL CHARACTERIZATION

The taxonomic history of the genus *Curcuma* gives ample evidence of several discrepancies such as improper description of the type specimens leading to difficulty in identification of specimens. Valetton (1918) thus very aptly considered the genus as ‘nomen dubium’ meaning doubtful name in English.

5.1.1. Morphological characterization of *Curcuma* species

Wide variability is observed in the fifteen *Curcuma* species studied for the conventional taxonomic and morphological features (Tables 17 & 19). No earlier reports are available for some of the morphological characters studied in the 15 *Curcuma* species. Observations on the characters such as clump weight, number of mother rhizomes, weight of mother rhizome, number of primary rhizome, weight of primary rhizome, number of secondary rhizome, weight of secondary rhizome, internodal length, number of leaves per clump, pseudostem girth are the first time report in these species. Non congruence of the morphological characters of the same species by different workers and/or over years is reported (Table 104). The variation observed for the key morphological characters of the *Curcuma* species may be due to the effect of environment in the characters selection, choose of different specimens/materials or the wrong identity of the type specimen (herbarium) used. For example, Sabu (1991) reported the rhizome colour of the *C. aromatica* as yellow to deep yellow, while in a subsequent report the same author recorded rhizome colour of *C. aromatica* as greyish yellow (Sabu *et al.*, 2006). It is also possible that the entities which now recognized as synonyms would have been considered as separate species. *C. zanthorrhiza* was misidentified in the past as *C. aromatica* and

C. zedoaria (Skornickova and Sabu, 2005). There are also reports of bract colour variation within the same species as reported in the *C. ecalcarata* (Sasikumar 2005).

Another key taxonomic character i.e. spike position in *Curcuma* is also unfortunately controversial. Roxburgh (1810) pointed out that the positional difference of spike in *Curcuma* is a matter of flowering season. Both terminal and lateral spikes are reported in the same species over seasons. Santapau (1945, 1952) reported that the spike which appears in the beginning of the season is lateral whereas the spike that appears towards the end of the season is terminal in *C. pseudomontana*. Larson and Smith (1978) also reported the limitations of solely relying on floral (spike position and colour of the coma bract) or vegetative (presence or absence of root tubers) features in discriminating *Curcuma* species. These findings thus surmise the unreliability of solely relying the conventional taxonomic tools alone in discriminating *Curcuma* species. However, these morphological tools may be useful as an adjunct to other modern molecular taxonomic tools. Beyene *et al.*, (2005) also reported that when compared with DNA fingerprinting techniques, morphological traits are relatively less reliable and efficient for precise discrimination of closely related accessions and analysis of their genetic relationships. Despite this limitation, morphological traits are useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions.

Table 104. Comparative taxonomic identity of the different *Curcuma* species

Species	Character	Reference					
		Hooker (1872–1894)	Gamble and Fischer (1956)	Velayudhan <i>et al</i> (1999)	Sabu (1991)	Sabu (2006)	Present study
<i>C. aeruginosa</i>	Spike position	...	...	Lateral	Lateral	Lateral	Lateral
	Color of the fertile bract	Lateral	...	...	Green with pink tip	Green with pink tip	Green with light purple colour at the tip
	Color of the coma bract	Pink	...	...	Pink to violet	Pink to violet. Lower one streaked violet	Pink
	Color of the corolla	Pale red	...	White	Pink	Pink	Pale yellow
	Color of the labellum	Pale yellow	...	Yellow	Yellow with deep yellow median band	Yellow with deep yellow	Yellow

					median band Present	Present
Anther spur	Present	...	Present	Present		
Leaf length (cm)	...	...		45.0 -60.0	30.0 – 40.0	68.0– 72.0
Leaf width (cm)	...	...		10.0 – 12.0	10.0- 12.0	21.0- 23.0
Leaf midrib colour	Faintly tinged purple colour fading on maturity	...	Green	Purple reddish fading on maturity	Purple patch on distal half of upper side	Purple patch on distal half of upper side
Leaf sheath colour	...	...	Dark purple	Green	Green	Purple
Leaf texture	...	...	Glabrous			Glabrous
Height of pseudostem (cm)	...	...	...	30.0-35.0	30.0 – 35.0	51.0-56.0
Pseudostem girth (cm)	...	...	...	...	...	10.0-11.0
Number of leaves per tiller	...	...	...	...	...	5.5-6.0
Number of leaves per clump	...	...	...	...	...	12.0-15.0
Number of tillers per clump	...	...	...	...	...	3.0-4.0
Clump weight (g)	--	--	...	...	...	362.0-370.0
Number of mother rhizome	...	...	...	...	...	1.0
Weight of mother rhizome (g)	...	...	...	...	...	68.0-72.0
Number of primary rhizome	...	...	...	...	...	4.0-6.0
Weight of primary rhizome (g)	...	...	...	...	...	154.0-162.0
Number of secondary rhizome	...	...	...	...	...	5.0-7.0
Weight of secondary rhizome (g)	...	...	...	...	...	128.0-132.0
Internode length (cm)	...	...	...	...	...	1.2-1.4
Rhizome colour	Verdis Green	...	Verdis Green	Blue in centre verging towards grey	Blue within	Pale blue
Aroma of rhizome	...	--	Camphoraceou s	Strongly aromatic	Strongly aromatic	Camphoraceou s
Flavour/tast e of rhizome	...	--	Bitter			Bitter
Spike position	Central	---	Lateral	Lateral / central	Lateral / central	Lateral/Termin al
Color of the fertile bract	Pale green	Pale green	...	Green	Green	Green
Color of the coma bract	Pinkish	Tinged with pink	...	Light violet	Light violet	White with pink tip
Color of the corolla	Whititsh/ lip pale yellow	...	Pale yellow	Pale yellow	Pale yellow	White/Light yellow
Color of the labellum	Pale yellow	Pale yellow	Yellow	Pale yellow with median	Pale yellow	Yellow

C. amada

					dark yellow band	with median dark yellow band	
<i>C. aromatica</i>	Anther spur	...	...	Present	Present	Present	Present
	Leaf length (cm)	...	...	...	45.0-60.0	45.0-60.0	69.0-73.0
	Leaf width (cm)	...	...	...	14.0-15.0	14.0-15.0	20.0-24.0
	Leaf midrib colour	...	...	Green	...	...	Green
	Leaf sheath colour	...	...	Green	...	...	Green
	Leaf texture	...	...	...	Upper glabrous lower hairy	Upper glabrous lower hairy	Glabrous
	Height of pseudostem (cm)	...	...	...	30.0-35.0	30.0-35.0	59.0-63.0
	Pseudostem girth (cm)	...	...	...	...	...	12.0-12.5
	Number of leaves per tiller	...	...	...	4.0-6.0	4.0-6.0	5.5-6.5
	Number of leaves per clump	...	...	...	...	...	16.0-17.0
	Number of tillers per clump	...	...	...	...	...	2.3-4.0
	Clump weight (g)	...	...	...	...	...	652.0-856.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	49.0-54.0
	Number of primary rhizome	...	...	...	...	...	7.0-9.0
	Weight of primary rhizome (g)	...	...	...	...	...	178.0-186.0
	Number of secondary rhizome	...	...	...	...	...	18.0-21.0
	Weight of secondary rhizome (g)	...	...	...	...	...	420.0-610.0
	Internode length (cm)	---	---	---	...	...	1.3-1.6
	Rhizome colour	Pale yellow	Pale yellow	Pale yellow white	Light yellow white towards periphery	Light yellow white towards periphery	Pale yellow
	Aroma of rhizome	--	...	Mango	Mango	Green mango	Mango like
	Flavour/taste of rhizome	--	...	No taste	...	...	Mango/gingery
	Spike position	Lateral	...	Lateral	Lateral	Lateral	Lateral
	Color of the fertile bract	Pale green	...	...	Pale greenish white	Pale greenish white	Green
	Color of the coma bract	less tinged with pink	...	...	Pink	Pink	Pinkish white

<i>C. caesia</i>	Color of the corolla	Yellow	...	Pale yellow	Pinkish white	Pinkish white	Yellow
	Color of the labellum	...	...	Yellow	Deep yellow	Deep yellow	Yellow
	Anther spur	Present		Present	Present	Present	Present
	Leaf length (cm)	...	...	...	...	40-70	74.0-76.0
	Leaf width (cm)	...	...	...	...	10-14	21.0-26.0
	Leaf midrib colour	...	...	Green	...	...	Green
	Leaf sheath colour	...	...	Green	...	...	Green
	Leaf texture	Hairy on either side	...	Hairy on either side	...	Densely pubescent below	Hairy
	Height of pseudostem (cm)	...	...	...	...	...	62.0-63.0
	Pseudostem girth (cm)	...	...	...	...	...	11.5-12.4
	Number of leaves per tiller	...	...	...	...	5-7	6.0-7.0
	Number of leaves per clump	...	...	...	...	...	18.0-19.0
	Number of tillers per clump	...	...	...	...	...	4.0-5.0
	Clump weight (g)	...	...	...	...	...	617.0-645.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	59.0-66.0
	Number of primary rhizome	...	...	...	...	...	10.0-13.0
	Weight of primary rhizome (g)	...	...	...	...	...	267.0-271.0
	Number of secondary rhizome	...	...	...	...	...	21.0-25.0
	Weight of secondary rhizome (g)	...	...	...	...	...	272.0-310.0
	Internode length (cm)	...	...	...	...	...	1.2
	Rhizome colour	Yellow	...	Pale yellow white	Yellow	Grayish yellow	Creamy white
	Aroma of rhizome	...	...	Camphoraceous	...	Aromatic	Persisting Camphoraceous
	Flavour/taste of rhizome	...	...	bitter	...	...	Bitter
	Spike position	...	...	Central/lateral	...	...	Lateral
	Color of the fertile bract	Green	...	...	...	...	Greenish purple
	Color of the coma bract	Limp red	...	...	...	...	Redish purple
	Color of the corolla	Pale yellow	...	Purple	...	...	red
	Color of the labellum	Pale yellow	...	Yellow	...	...	Yellow

<i>C. comosa</i>	Anther spur	...	...	...	...	...	Present
	Leaf length (cm)	...	...	...	...	...	59.0-62.0
	Leaf width (cm)	...	...	...	...	...	18.0-19.0
	Leaf midrib colour	Purple	...	Bright purple	...	...	Purple
	Leaf sheath colour	...	...	Purple brown	...	...	Purple
	Leaf texture	...	...	Dorsal hairy ventral glabrous	...	...	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	41.0-44.0
	Pseudostem girth (cm)	...	...	...	...	...	10.0-11.0
	Number of leaves per tiller	...	...	...	...	...	5.0-6.0
	Number of leaves per clump	...	...	...	...	...	16.0-17.0
	Number of tillers per clump	...	...	...	...	...	4.0-6.0
	Clump weight (g)	...	...	...	...	...	420.0-472.0
	Number of mother rhizome	...	...	...	...	...	1.0-2.0
	Weight of mother rhizome (g)	...	...	...	...	...	38.0-76.0
	Number of primary rhizome	...	...	...	...	...	10.0-11.0
	Weight of primary rhizome (g)	...	...	...	...	...	176.0-18.0
	Number of secondary rhizome	...	...	...	...	...	20.0-24.0
	Weight of secondary rhizome (g)	...	...	...	...	...	196.0-214.0
	Internode length (cm)	...	...	...	...	...	1.3-1.5
	Rhizome colour	Plane grey inside	...	Blue	...	...	Blue black
	Aroma of rhizome	...	...	...	...	...	Camphoraceous
	Flavour/taste of rhizome	...	...	...	...	...	Bitter
	Spike position	...	...	Lateral	...	...	Lateral
	Color of the fertile bract	...	...	...	...	...	Green
	Color of the coma bract	Bright red	...	...	...	...	White with purple tip
	Color of the corolla	Pinkish white	...	Light purple	...	...	White
	Color of the labellum	Pale yellow	...	Yellow	...	...	Yellow
	Anther spur	...	...	...	...	...	Present
	Leaf length (cm)	...	...	...	...	...	68.0-73.0
	Leaf width	...	...	...	...	...	19.0-21.0

	(cm)						
	Leaf midrib colour	...	...	Light purple green	...	...	Purple
	Leaf sheath colour	...	...	Greenish light purple	...	...	Purple
	Leaf texture	...	...	Glabrous on both sides	...	...	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	60.0-63.0
	Pseudostem girth (cm)	...	...	...	...	...	11.5-12.5
	Number of leaves per tiller	...	...	...	...	...	5.0-6.5
	Number of leaves per clump	...	...	...	...	...	13.0-14.0
	Number of tillers per clump	...	...	...	...	...	2.7-3.3
	Clump weight (g)	...	...	...	...	...	253.0-290.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	32.0-37.0
	Number of primary rhizome	...	...	...	...	...	5.0-8.0
	Weight of primary rhizome (g)	...	...	...	...	...	89.0-110.0
	Number of secondary rhizome	...	...	...	...	...	15.0-16.0
	Weight of secondary rhizome (g)	...	...	...	...	...	126.0-138.0
	Internode length (cm)	...	...	...	...	...	1.0-1.3
	Rhizome colour	Pale yellow	...	Orange yellow	...	...	Pale yellow
	Aroma of rhizome	...	...	Camphoraceous	...	...	Camphoraceous
	Flavour/taste of rhizome	...	...	Bitter	...	...	Bitter
<i>C. decipiens</i>	Spike position	...	...	central	Lateral /central	Lateral /central	Terminal
	Color of the fertile bract	...	Purple	...	Green with purple tip	Green with purple pink tip	Green
	Color of the coma bract	Purple	Purple	...	Deep purple pink	Lower green with purple tip, upper deep purple pink	Pink
	Color of the corolla	...	...	Purple spot	Deep purple	Deep purple	Creamy white
	Color of the labellum	Purple	Purple	Yellow	Yellow/purple towards base	Purple towards base	Yellow
	Anther spur	...	...	...	...	Present	Present
	Leaf length (cm)	...	...	...	...	10-30	62.0-68.0
	Leaf width	...	...	...	...	5-15	19.0-20.0

C. haritha	(cm)						
	Leaf midrib colour	...	...	Green	...	2-4	Green
	Leaf sheath colour	...	...	Greenish purple	...	...	Purple
	Leaf texture	...	...	Hairy dorsal, ventral glabrous	Glabrous	Glabrous	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	46.0-49.0
	Pseudostem girth (cm)	...	...	...	...	...	10.0-11.0
	Number of leaves per tiller	...	...	...	...	...	5.0-5.5
	Number of leaves per clump	...	...	...	...	...	9.0-10.0
	Number of tillers per clump	...	...	...	...	...	2.0-3.0
	Clump weight (g)	...	...	...	...	...	346.0-369.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	51.0-56.0
	Number of primary rhizome	...	...	...	...	...	5.0-6.0
	Weight of primary rhizome (g)	...	...	...	...	...	58.0-167.0
	Number of secondary rhizome	...	...	...	...	...	7.0-8.0
	Weight of secondary rhizome (g)	...	...	...	...	...	132.0-142.0
	Internode length (cm)	...	...	...	...	...	1.2-1.3
	Rhizome colour	...	...	White	Pale yellow white	Pale yellow white	Pale yellow
	Aroma of rhizome	...	...	Camphoraceous	...	...	Camphoraceous
	Flavour/taste of rhizome	...	...	No taste	...	...	Bitter
	Spike position	...	...	Lateral	Lateral	Lateral	Lateral
	Color of the fertile bract	...	...	...	Lower green/upper green with pink tips	Lower green/upper green with pink tips	Green
	Color of the coma bract	...	...	...	Bright pink	Bright pink	White with dark purple colour at the tip
	Color of the corolla	...	...	White	White	White	White
Color of the labellum	...	...	Yellow	Light yellow with median dark yellow band	Light yellow with median dark yellow	White with yellow lip	

							band
<i>C. longa</i>	Anther spur	...	...	...	...	Present	Present
	Leaf length (cm)	...	...	...	...	30-50	41.0-46.0
	Leaf width (cm)	...	...	...	...	10-15	15.0-16.0
	Leaf midrib colour	...	...	Light purple brown	...		Green
	Leaf sheath colour	...	...	Light purple brown	...	Green with light pink dots	Green
	Leaf texture	...	...	Dorsal glabrous/ventral hairy	Upper and lower pubescent	Upper and lower pubescent	Hairy
	Height of pseudostem (cm)	...	...	...	...	30	29.0-35.0
	Pseudostem girth (cm)	...	...	...	...	...	8.5-9.6
	Number of leaves per tiller	...	...	...	...	...	5.0-5.5
	Number of leaves per clump	...	...	...	...	...	9.0-12.0
	Number of tillers per clump	...	...	...	...	4-6	2.0-4.0
	Clump weight (g)	...	...	...	...	...	269.0-294.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	30.0-34.0
	Number of primary rhizome	...	...	...	...	...	3.0-4.0
	Weight of primary rhizome (g)	...	...	...	...	...	98.0-104.0
	Number of secondary rhizome	...	...	...	...	...	7.0-8.0
	Weight of secondary rhizome (g)	...	...	...	...	...	132.0-152.0
	Internode length (cm)	...	...	...	...	...	1.1-1.2
	Rhizome colour	...	...	White	Yellowish grey	Yellowish grey	Creamy white
	Aroma of rhizome	...	...	Camphoraceous	Non aromatic	Non aromatic	Persisting Camphoraceous
	Flavour/taste of rhizome	...	...	Slight bitter	...	...	Bitter
	Spike position	...	...	central	central	central	Terminal
	Color of the fertile bract	Pale green	Pale green	...	Pale green	Pale green	Green
	Color of the coma bract	Tinged with pink	Tinged with pink	...	White	White	White, White with pink, green & purple colour at the tip
	Color of the corolla			White	White	White	White
	Color of the	Pale yellow	Yellow	Yellow	light yellow	Light	White with

labellum				with median dark yellow band at centre	yellow with median dark yellow band at centre	yellow lip
Anther spur	...	...	...	...	Present	Present
Leaf length (cm)	...	...	...	...	45-60	69.0-72.0
Leaf width (cm)	...	...	...	...	15-20	19.0-21.0
Leaf midrib colour	...	...	Green	...	...	Green
Leaf sheath colour	...	...	Green	...	...	Green
Leaf texture	...	...	Glabrous	...	Glabrous	Glabrous
Height of pseudostem (cm)	...	...	...	...	...	50.0-56.0
Pseudostem girth (cm)	...	...	...	...	...	9.0-12.0
Number of leaves per tiller	...	...	...	...	...	5.0-7.0
Number of leaves per clump	...	...	...	...	...	11.0-14.0
Number of tillers per clump	...	...	...	...	4-6	4.0
Clump weight (g)	...	...	...	...	...	446.0-470.0
Number of mother rhizome	...	...	...	...	...	1.0
Weight of mother rhizome (g)	...	...	...	...	...	64.0-68.0
Number of primary rhizome	...	...	...	...	...	7.0-9.0
Weight of primary rhizome (g)	...	...	...	...	...	182.0-212.0
Number of secondary rhizome	...	...	...	...	...	22.0-26.0
Weight of secondary rhizome (g)	...	...	...	...	...	186.0-194.0
Internode length (cm)	...	...	...	...	...	1.1-1.3
Rhizome colour	Bright yellow	Bright yellow	Light orange yellow to orange yellow	Deep orange yellow	Deep orange yellow	Deep orange yellow
Aroma of rhizome	...	...	Camphor /turmeric	Strongly aromatic	Strongly aromatic	Camphoraceous
Flavour/taste of rhizome	...	...	Slight bitter	...	...	Bitter
Spike position	...	...	Lateral	...	...	Lateral
Color of the fertile bract	...	...	...	...	...	Green with light Purple colour at the tip
Color of the coma bract	...	...	...	...	...	Purple
Color of the	...	...	White purple	...	...	Pink

C. malabarica

<i>C. pseudomontana</i>	Anther spur	...	...	...	...	Present	Present
	Leaf length (cm)	...	...	...	...	...	60.5-63.0
	Leaf width (cm)	...	...	...	...	...	23.0-24.0
	Leaf midrib colour	...	...	Green	...	...	Green
	Leaf sheath colour	...	...	Green	...	...	Purple
	Leaf texture	...	...	Glabrous	...	Lower surface pubescent	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	68.0-75.0
	Pseudostem girth (cm)	...	...	...	...	...	11.5-12.5
	Number of leaves per tiller	...	...	...	...	...	5.5-6.0
	Number of leaves per clump	...	...	...	...	...	12.0-13.0
	Number of tillers per clump	...	...	...	...	...	2.3-3.0
	Clump weight (g)	...	...	...	...	...	349.0-405.0
	Number of mother rhizome	...	...	...	...	...	1.0-2.0
	Weight of mother rhizome (g)	...	...	...	...	...	39.0-62.0
	Number of primary rhizome	...	...	...	...	...	6.0-8.0
	Weight of primary rhizome (g)	...	...	...	...	...	123.0-146.0
	Number of secondary rhizome	...	...	...	...	...	22.0-25.0
	Weight of secondary rhizome (g)	...	...	...	...	...	182.0-196.0
	Internode length (cm)	...	...	...	...	...	1.2-1.3
	Rhizome colour	Pale yellow	Pale yellow	Pale yellow	Light orange yellow	Light orange yellow	Pale yellow with light blue out of circle
	Aroma of rhizome	...	...	Camphoraceous	...	...	Camphoraceous
	Flavour/taste of rhizome	...	...	Bitter	...	...	Bitter
	Spike position	...	...	Central	Lateral / terminal	Lateral/terminal	Lateral/Terminal
	Color of the fertile bract	...	Green with pink tip	...	Green with purple tinge/purple	Green with purple tinge/purple	Greenish purple
	Color of the coma bract	...	Purple	...	Bright pink	Bright pink	Dark purple
	Color of the corolla	...	...	...	Yellowish white	Yellowish white	Creamy white
	Color of the labellum	...	Yellow	Bright yellow	Bright yellow	Bright yellow	Yellow
	Anther spur	...	...	...	...	Present	Present

<i>C. raktakanta</i>	Leaf length (cm)	...	...	...	...	40-50	69.0-84.0
	Leaf width (cm)	...	...	...	...	6-9	23.0-25.0
	Leaf midrib colour	...	...	Green			Purple
	Leaf sheath colour	...	...	Purple	Green purple white	Green to purple to violetish	Purple
	Leaf texture	...	...	Hairy	Upper surface pubescent	Upper surface pubescent	Hairy
	Height of pseudostem (cm)	...	...	...	...	35	64.0-79.0
	Pseudostem girth (cm)	...	...	...	...	...	10.0-11.0
	Number of leaves per tiller	...	...	...	...	6-7	5.0-5.5
	Number of leaves per clump	...	...	...	...	...	10.0-11.0
	Number of tillers per clump	...	...	...	...	...	2.0-3.0
	Clump weight (g)	...	...	...	...	...	103.0-110.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	31.0-34.0
	Number of primary rhizome	...	...	...	...	...	3.0-4.0
	Weight of primary rhizome (g)	...	...	...	...	...	62.0-73.0
	Number of secondary rhizome	...	...	...	...	...	0
	Weight of secondary rhizome (g)	...	...	...	...	...	0
	Internode length (cm)	...	...	...	...	...	0.9-1.0
	Rhizome colour	...	...	White	Yellow centre, white towards periphery	Yellow centre, white towards periphery	Pale yellow
	Aroma of rhizome	...	...	Camphoraceous	Pleasantly aromatic	Pleasantly aromatic	Camphoraceous
	Flavour/taste of rhizome	...	...	No taste	...	...	Bitter
	Spike position	...	...	Lateral	Lateral	Lateral	Lateral
	Color of the fertile bract	...	...	...	Green with pink tip	Green with pink tip	Green with light purple
	Color of the coma bract	...	...	...	...	Deep pink	Purple
	Color of the corolla	...	...	White, very light purple	Light pink	Light pink	White
		...	...				
	Color of the labellum			Yellow	Yellow with median dark yellow band	Yellow with median dark yellow	White with yellow lip

						band	
<i>C. sylvatica</i>	Anther spur	...	...	...	...	Present	Present
	Leaf length (cm)	...	...	...	...	35-45	68.0-78.0
	Leaf width (cm)	...	...	...	...	10-12	19.5-21.0
	Leaf midrib colour	...	...	Green	Deep pink	Deep pink	Green
	Leaf sheath colour	...	...	Dark purple brown	Reddish purple	Reddish purple	Purple
	Leaf texture	...	...	Glabrous	Glabrous above pubescent below	Glabrous above pubescent below	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	59.0-65.0
	Pseudostem girth (cm)	...	...	...	...	...	9.8-10.5
	Number of leaves per tiller	...	...	...	...	4-6	6.0-6.5
	Number of leaves per clump	...	...	...	...	...	11.0-12.0
	Number of tillers per clump	...	...	...	...	...	3.0-4.0
	Clump weight (g)	...	...	...	...	...	428.0-473.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	39.0-42.0
	Number of primary rhizome	...	...	...	...	...	10.0-12.0
	Weight of primary rhizome (g)	...	...	...	...	...	172.0-189.0
	Number of secondary rhizome	...	...	...	...	...	26.0-28.0
	Weight of secondary rhizome (g)	...	...	...	...	...	209.0-241.0
	Internode length (cm)	...	...	...	...	...	1.2-1.3
	Rhizome colour	...	...	Pale yellow	Green yellow inside, white towards periphery	Grayish to yellowish inside, whitish towards periphery	Pale yellow with bluish green outer ring
	Aroma of rhizome	...	...	Camphoraceous	...	Aromatic	Camphoraceous
	Flavour/taste of rhizome	...	...	Slight bitter	...	...	Bitter
	Spike position	...	...	Lateral	...	...	Lateral
	Color of the fertile bract	...	...	...	...	...	Greenish purple
	Color of the coma bract	...	...	...	...	...	Dark purple
	Color of the corolla	...	...	White	...	...	Creamy white

<i>C. zedoaria</i>	Color of the labellum	...	...	Yellow	...	...	Yellow
	Anther spur	...	...	...	...	...	Present
	Leaf length (cm)	...	...	...	...	...	58.0-63.0
	Leaf width (cm)	...	...	...	...	...	22.0-23.0
	Leaf midrib colour	...	...	Light purple brown	...	...	Purple
	Leaf sheath colour	...	...	Green	...	...	Purple
	Leaf texture	...	...	Glabrous	...	...	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	60.0-67.0
	Pseudostem girth (cm)	...	...	...	...	...	10.0-12.0
	Number of leaves per tiller	...	...	...	...	...	5.0-5.5
	Number of leaves per clump	...	...	...	...	...	10.0-13.0
	Number of tillers per clump	...	...	...	...	...	2.3-3.0
	Clump weight (g)	...	...	...	...	...	370.0-397.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	42.0-46.0
	Number of primary rhizome	...	...	...	...	...	4.0-7.0
	Weight of primary rhizome (g)	...	...	...	...	...	128.0-134.0
	Number of secondary rhizome	...	...	...	...	...	10.0-12.0
	Weight of secondary rhizome (g)	...	...	...	...	...	190.0-218.0
	Internode length (cm)	...	...	...	...	...	1.1-1.2
	Rhizome colour	...	...	White	...	...	Lemon yellow
	Aroma of rhizome	...	...	Mango	...	...	Camphoraceous
	Flavour/taste of rhizome	...	...	Slight bitter	...	...	Bitter
	Spike position	...	...	Lateral	Lateral	...	Lateral
	Color of the fertile bract	...	Green tinged with red	...	Green with purple margin	...	Green with light purple colour at tip
	Color of the coma bract	Bright red	Crimson/purple	...	Pink	...	Purple
	Color of the corolla	White	...	Light purple	White with pink tinge	...	Pink
	Color of the labellum	Pale yellow	Yellow	Yellow	Pale yellow with deep yellow band	...	Yellow
	Anther spur	...	...	...	...	...	Present

<i>Curcuma</i> species	Leaf length (cm)	...	...	...	...	...	81.0-86.5
	Leaf width (cm)	...	...	...	...	...	23.0-25.0
	Leaf midrib colour	Purple	...	Light purple green	Purple on upper side, fading by maturity	...	Purple
	Leaf sheath colour	...	...	Green	...	...	Green
	Leaf texture	...	...	Glabrous	...	...	Glabrous
	Height of pseudostem (cm)	...	...	...	...	...	73.5-75
	Pseudostem girth (cm)	...	...	...	...	...	11.0-13.6
	Number of leaves per tiller	...	...	...	...	...	6.0-7.0
	Number of leaves per clump	...	...	...	...	...	13.0-14.0
	Number of tillers per clump	...	...	...	...	...	3.0-4.0
	Clump weight (g)	...	...	...	...	...	295.0-343.0
	Number of mother rhizome	...	...	...	...	...	1.0
	Weight of mother rhizome (g)	...	...	...	...	...	161.0-171.0
	Number of primary rhizome	...	...	...	...	...	4.0-5.0
	Weight of primary rhizome (g)	...	...	...	...	...	120.0-138.0
	Number of secondary rhizome	...	...	...	...	...	1.0-3.0
	Weight of secondary rhizome (g)	...	...	...	...	...	10.0-29.0
	Internode length (cm)	...	...	...	...	...	1.7-1.8
	Rhizome colour	Pale yellow/white	...	Light orange yellow	Yellow to deep yellow	...	Orange Yellow
	Aroma of rhizome	...	...	Camphoraceous	Camphoraceous	...	Camphoraceous
	Flavour/taste of rhizome	...	...	Bitter	...	...	Bitter
	Spike position	...	...	...	...	...	Lateral
	Color of the fertile bract	...	...	...	...	...	Green
	Color of the coma bract	...	...	...	...	...	Greenish white light purple
	Color of the corolla	...	...	...	...	...	White
	Color of the labellum	...	...	...	...	...	White with yellow lip
	Anther spur	...	...	...	...	...	Present
	Leaf length (cm)	...	...	...	...	...	42.3-45.5
	Leaf width	...	...	...	...	...	18.8-19.6

(cm)							
Leaf midrib colour	...	...	...	...	...	...	Green
Leaf sheath colour	...	...	...	...	...	...	Green
Leaf texture	...	...	...	...	...	...	Hairy
Height of pseudostem (cm)	...	...	...	...	...	...	31.0-33.5
Pseudostem girth (cm)	...	...	...	...	...	...	10.0-11.0
Number of leaves per tiller	...	...	...	...	...	...	7.0-9.5
Number of leaves per clump	...	...	...	...	...	...	11.0-18.0
Number of tillers per clump	...	...	...	...	...	...	2.0-3.0
Clump weight (g)	...	...	...	...	...	...	167.0-176.0
Number of mother rhizome	...	...	...	...	...	...	1.0
Weight of mother rhizome (g)	...	...	...	...	...	...	59.0-64.0
Number of primary rhizome	...	...	...	...	...	...	3.0-4.0
Weight of primary rhizome (g)	...	...	...	...	...	...	63.0-71.0
Number of secondary rhizome	...	...	...	...	...	...	3-4
Weight of secondary rhizome (g)	...	...	...	...	...	...	32-41
Internode length (cm)	...	...	...	...	...	...	1.2-1.4
Rhizome colour	...	...	...	...	...	...	Creamy white
Aroma of rhizome	...	...	...	...	...	...	Camphoraceous
Flavour/taste of rhizome	...	...	...	...	...	...	Bitter

5.1.1.1. Cluster analysis

Grouping of the *Curcuma* species studied was done based on UPGMA analysis using Jaccard's similarity coefficients. The UPGMA dendrogram split at a Jaccard's similarity index of 0.44 and 0.52 in the two dendrograms constructed using all the morphological characters and key characters alone (Figs. 14 & 15), forming seven groups each. In the dendrograms, *C. zedoaria* and *C. malabarica* showed maximum similarity (1.00) suggesting the mistaken identity of the entity maintained in the genebank. In the first dendrogram (Fig. 14), three species viz, *C. haritha*, *Curcuma* species and *C. decipiens* formed individual group eventhough

these species shared many salient characters with the other species studied in the dendrogram. This incongruity is also seen in other groups in the dendrogram too. Highly unstable morphological characters studied such as fresh clump weight, weight of mother, primary and secondary rhizomes, number of leaves per clump and spike position might be the reason for this anomaly.

Velayudhan *et al.*, (1996 & 1999) also reported the anomalies in grouping of 31 *Curcuma* species using morphological and taxonomic traits and clustering of different taxonomic groups of *Curcuma* in the same cluster.

However, in the second dendrogram (Fig. 15) constructed using only the key characters, clustering of the species was more sensual and thus indicating the reliability of using the qualitative aerial (leaf mid rib colour, colour of leaf sheath & leaf texture), rhizome (rhizome colour, aroma of rhizome & rhizome flavour/taste) and floral characters (colour of the fertile bract, colour of the corolla, colour of the labellum and anther spur) for grouping of *Curcuma* species rather than using more quantitative characters. For example, the species such as *C. aromatica* and *C. haritha* both sharing similar qualitative rhizome traits and leaf texture grouped together. Similarly the *C. malabarica* and *C. zedoaria* also formed a single group.

Nevertheless, the role of some of the quantitative characters such as the number of mother rhizomes per clump, number of primary and secondary rhizomes can not be ignored while characterizing the *Curcuma* species.

5.1.2. Characterization of turmeric varieties/cultivars

Intraspecific variation for bract colour is observed in *C. longa* accessions studied. The colour of the coma bract varied from white ('Alleppey'), white with pink ('Jorhat'), white with purple colour at the tip ('Karhadi local') to white with green tip ('Kuchipudi') (Fig. 10). *C. longa*, the extensively cultivated species of the genus, has undergone selection by mankind for yield and other quality traits over time and space. Bract colour may be governed by genes having pleiotropic effects controlling yield and quality traits. Conscious selection for quality and yield would have thus affected the bract colour indirectly, resulting in varying bract colour.

Velayudhan *et al.* (1999) also reported coma bract colour variation in 21 morphotypes of turmeric studied. The disparity of coma bract colour in different morphotypes ranged from white with pink tip, whitish green to white.

Good variability for the various morphological and yield characters is reported in the turmeric varieties/cultivars. Intraspecific variation for yield and quality traits in *C. longa* is well documented. Among the seven aerial morphological characters studied, high variability was observed for leaf length followed by pseudostem height, number of leaves per tiller, leaf width, number of tillers per clump and pseudostem girth. For rhizome characters maximum variability was observed for clump weight, number of secondary rhizomes, weight of secondary rhizomes, weight of mother rhizomes, weight of primary rhizomes, number of primary rhizomes, internode length, dry recovery and number of mother rhizomes. Many authors have reported good variability for yield, yield attributes and quality traits in *C. longa* (Sinkar *et al.*, 2005; Singh *et al.*, 2003; Chandra *et al.*, 1999; Sheshagiri and Uthaiiah (1994); Jalgaonkar, 1990; Pujari *et al.*, 1987; Philip and Nair, 1986 & 1983). In the present study the improved varieties such as 'Prabha', 'Prathibha', 'Alleppey Supreme', 'Kedaram' and 'Sudarsana' showed a stable performance for total clump weight (fresh yield), weight of mother rhizomes, weight of primary rhizome and weight of secondary rhizomes studied over the years, while 'Suvarna', 'Sugandham' and 'Suguna' showed a wide range for these characters.

5.1.2.1. Cluster analysis

A UPGMA dendrogram is constructed using only above ground and underground characters of turmeric cultivars/varieties studied, since they were shy to flower and some of the cultivars did not flower at all during the study period. The accessions studied clustered in eleven groups (Fig. 16). Two distinct patterns of grouping could be deciphered from the dendrogram. Among the eight improved varieties studied, except 'Suguna', 'Sugandham' and 'Suvarna' clustered with the popular cultivars like 'Alleppey', 'GL Puram', 'Katergia' and 'Wynad'. The clustering together of improved varieties are as expected since selection for yield and/or quality has been one of the major crop improvement strategies in this clonally propagated crop and these improved varieties are highly selected genotypes, sharing

many genes in common. Currently there are 25 improved varieties in turmeric and all of them are bred for high quality and high yield (Sasikumar, 2007).

The clustering pattern of the accessions in the second group of the dendrogram could be observed more sensual from a geographical origin of the cultivars. The cultivar 'Aizwal' was the only exception in this group. Similar situation is also observed in groups IV, VII, IX and X too. Rao *et al.* (2005) reported the discrepancy of clustering pattern of turmeric cultivars. The authors reported that cultivars collected from same source are distributed in different clusters while cultivars from different geographical regions are grouped in the same cluster. Another possible reason for the exception could be the introduction of turmeric clones to different parts of the country by formal and informal means and their recollection as distinct cultivars at a later time.

5.1.3. Characterization of open pollinated progenies of turmeric

In general the true turmeric seedling progenies registered a wide range for the morphological characters studied indicating wider spectrum of variability in the true turmeric seedlings. Thus the open pollinated progenies of the turmeric may meaningfully be exploited for the varietal improvement programme.

Turmeric, though a triploid set seeds and seedling progeny selection has become a regular crop improvement strategy in this crop (Nambiar *et al.*, 1982; Sasikumar 2005). 'IISR 'Prabha'' and 'IISR Prathibha' are improved turmeric varieties evolved by exploiting the variability of the true seedling progenies (Sasikumar *et al.*, 1996).

5.1.3.1. Cluster analysis

The clustering pattern of the seedling progenies and their parents based on UPGMA analysis did not reveal any specific pattern. Four groups were discernable in the dendrogram. However, the OP progenies together formed separate groups in the dendrogram and the mother lines clustered separately in different groups. Though the progenies clustered separately from their mother lines, the percentage similarity of the progenies was well within the similarity of their parents as observed

using Jaccard's similarity coefficients. An elaborate study using more number of offsprings per mother may be ideal in this context.

5.2. MOLECULAR CHARACTERIZATION

Molecular characterization of *Curcuma* is in a nascent stage now. Relying heavily on morphological characters alone in species delimitation has its own constraints in the genus as reported earlier (Sasikumar; 2005, Syamkumar and Sasikumar, 2007) and also observed in the present study (Section.5.1). Thus, molecular marker analysis has much significance in the ongoing context of taxonomic revision of the genus. Molecular characterization of germplasm of horticultural crops is very much in vogue now (Raina *et al.*, 2001; Talhinhos *et al.*, 2003; Jatoi *et al.*, 2006; Sheela *et al.*, 2006; Techaprasan *et al.*, 2006; Palai *et al.*, 2007; Sarwat *et al.*, 2008).

5.2.1. Isolation of genomic DNA from fresh leaves

The prerequisite for any molecular marker analysis is the good quality genomic DNA. The major restraint to the application of molecular markers in plants is the extraction of good quality genomic DNA with good yield (Dixit, 1998). Plant tissues contain high levels of polysaccharides, polyphenols, proteins and alkaloids which co precipitate with DNA preparations and make them averse to PCR amplification and restriction digestion. Since the biochemical constituents of plant tissues vary considerably, each plant tissue requires a specific protocol for isolation of genomic DNA (Khanuja *et al.*, 1999). Thus the original CTAB protocol by Doyle & Doyle (1987) was modified and used for the extraction of DNA from fresh leaves of the different *Curcuma* species, turmeric varieties/cultivars and the open pollinated progenies. Good quality DNA with high yield was obtained using the protocol and the DNA yield ranged from 93 $\mu\text{g g}^{-1}$ (*C. zedoaria*) to 153 $\mu\text{g g}^{-1}$ (*C. aromatica*^b) in fifteen *Curcuma* species, 95 $\mu\text{g g}^{-1}$ ('Nandyal type') to 149 $\mu\text{g g}^{-1}$ ('Sudarsana') in thirty six turmeric varieties/cultivars and 106 $\mu\text{g g}^{-1}$ ('OP Avanigadda') to 158 $\mu\text{g g}^{-1}$ ('OP Alleppey') in twelve open pollinated progenies of turmeric (Tables. 34-36).

5.2.2. Isolation of DNA from fresh rhizomes

Turmeric (*Curcuma longa* L), a rhizomatous spices used worldwide in alternative systems of medicine. The whole plant is aromatic, but it is the

underground rhizomes, fresh or processed, that is the valuable commodity of commerce. Molecular marker technology based on the traded produce (rhizome) is important in turmeric for identifying varieties/accessions, germplasm organization, identifying market samples, and gene cloning (Syamkumar *et al.*, 2003). Further, preserving leaf material for a long time is difficult and the leaf itself may not be available during the off season. Thus a protocol to isolate and amplify DNA from recalcitrant tissue like rhizome is very pertinent albeit cumbersome. The DNA isolated from fresh matured rhizomes of other plants tissue by the original CTAB method of Doyle and Doyle (1987) was usually found to be contaminated with high levels of proteins and polysaccharides making it opaque and difficult to dissolve in nuclease free water or TE buffer. The presence of essential oils, polysaccharides, polyphenols, alkaloids, and other secondary metabolites in turmeric rhizomes poses further problem in DNA isolation and PCR amplification (Syamkumar *et al.*, 2003). The original protocol was modified by precipitating in ethanol instead of isopropanol and by increasing concentrations to 4% CTAB, 2 M NaCl, 0.3% β -mercaptoethanol, and 1% PVP in the present study. Increased concentrations of CTAB, PVP, β -mercaptoethanol, and NaCl helped to remove polyphenols, polysaccharides, and alkaloids from recalcitrant sample (Smith *et al.*, 1991; Fang *et al.*, 1992; Bult *et al.*, 1992; Rogers and Bendich, 1985). The modified CTAB method developed for isolating PCR amplifiable DNA from fresh mature rhizomes of turmeric yielded 10.75 – 14.44 $\mu\text{g g}^{-1}$ DNA with a purity ranging from 1.83-1.87(A_{260}/A_{280}).

5.2.3. Molecular characterization of the *Curcuma* species using RAPD/ISSR markers

Two dominant molecular markers viz. RAPD and ISSR were used for the molecular characterization of fifteen *Curcuma* spp. besides the 18S ribosomal RNA sequence analysis. Eventhough the dominant markers have limitation in identifying the dominant homozygotes from heterozygotes, co migration of non homologous bands and artifactual resolution (Clark and Lanigan, 1993; Karp *et al.*, 1996; Vekemans *et al.*, 2002), several reasons have been given to justify the use of dominant markers for genetic diversity analysis rather than sampling sequence or morphological characters. First, in contrast to single gene analysis, dominant markers scan multiple loci from across the different genomes (El - Rabey *et al.*,

2002) and are therefore robust to problems that may affect single coalescent gene such as introgression lineage sorting and unrecognized paralogy (Doyle, 1992, Maddison, 1997). Second, insufficient sequence variation have been found in the genes previously sampled for the study group (Pelzer *et al.*, 2003, Koopman 2005). Third, the sequence data sampled may have been insufficient to resolve the phylogentic relationship among the study group (Huang *et al.*, 2002, Mort *et al.*, 2003). Fourth, sampling dominant markers may be more efficient than sampling other characters (Demeke *et al.*, 1992; Bakkeren *et al.*, 2000). Fifth, dominant markers have been more informative and reliable than morphological characters (Kardolus *et al.*, 1998).

The genetic diversity of the germplasm can be estimated by different methods. When two genotypes are compared using the data generated from any dominant marker based on the presence (1) or absence (0) of bands, the following situation occurs $a = 1,1$; $b = 1,0$; $c = 0$; $d = 0,0$. In the present study we have used three types of similarity coefficients for grouping the genotypes. The Jaccard's and Sorensen Dice coefficients are more or less similar except for the double weight given for the positive co occurrence (a) in Sorensen – Dice coefficient, while in Jaccard's a single weight is given for positive co occurrence. But in Simple Matching coefficients stress is given for the negative co occurrence (d). Thus the choice of similarity coefficients should be based on some criteria because few structural changes of more differentiated groups can alter the relationship between cultivars with high genetic similarity (Duarte *et al.*, 1999; Simmons *et al.*, 2007). Duarte *et al.*(1999) further reported that the cluster results with Jaccard's and Sorensen – Dice were identical but the latter is having higher projection efficiency in the two dimensional space (smaller distortion and stress, higher correlation)and thus can be considered as the most adequate coefficient for genetic diversity analysis using RAPD makers. Landry and Lapoint (1996) suggested that the Dice or Jaccard coefficients might be preferable to the Simple Match coefficient when using RAPD analysis to compare groups of distantly related taxa. Hallden *et al.* (1994) considered the Simple Match coefficient to be the more appropriate measure of similarity when closely related taxa are considered. When rather distinct individuals are compared and the shared absence of a band does not contribute to similarity of individuals, the Jaccard coefficient of similarity is preferable to the Dice coefficient. Kosman and

Leonard (2005) reported that there is no justification for giving more weight to the shared presence of bands when comparing individuals from different taxa and the more weightage given to the Dice coefficient method over Jaccard's coefficient. Keeping these observations in mind three different similarity coefficients viz. Jaccard's, Sorensen – Dice and Simple Matching methods were used for the analysis of molecular data derived from RAPD and ISSR markers in the fifteen *Curcuma* species, thirty six turmeric cultivars/varieties and twelve open pollinated progenies studied.

A common pattern of clustering was evident in all the three dendrograms constructed using the data derived from RAPD markers in the fifteen *Curcuma* species. In total, seven groups were observed in the *Curcuma* species studied using RAPD markers by Jaccard's and Sorensen Dice coefficients. In the ISSR dendrogram the grouping pattern was slightly different from that of RAPD. This may be due to the lesser number of amplicons produced by the primers studied are used for calculating the similarity indices and subsequent construction of dendrograms. The number of ISSR primers used for the characterisation of the *Curcuma* species might not have been sufficient to scan the entire genome, leading to the difference. The dendrogram constructed based on the combined dominant markers did not vary much from the individual dendrograms. In the combined RAPD/ISSR dendrograms the grouping was similar to the RAPD dendrogram in two of the coefficient used viz. Sorensen Dice and Jaccard's coefficients. The clustering of the fifteen *Curcuma* species using the Simple Matching coefficient however, showed a little difference in the grouping pattern. The entity, *C. aeruginosa*, the lone number of the group II (Sorensen Dice and Jaccard's coefficients) grouped with *C. caesia* in the dendrogram constructed using Simple Matching coefficient. This difference in the grouping of species in the dendrograms can be considered as a reflection of the percentage similarity between any two entities in a dendrogram constructed based on any one dominant marker at a time. For example, *C. caesia* and *C. aeruginosa* had very high similarities (0.76) in the ISSR dendrogram and it is reflected in the combined dendrogram too. Further, a combined genetic diversity estimates based on several molecular markers cover more genomic regions than a single marker alone. Hence the combined use of more than one dominant molecular

marker will be more desirable for the genetic diversity analysis and germplasm characterisation.

In all the dendrograms constructed using separate or combined molecular markers, irrespective of the grouping procedure adopted, the species *C. montana* and *C. raktakanta* showed the highest similarity (Group I). Syamkumar and Sasikumar (2007) based on combined RAPD/ISSR molecular characterization of 15 Indian *Curcuma* species also observed that the separate species status given to these two entities need revisiting. In all the dendrograms, except the RAPD dendrogram constructed using Sorensen – Dice coefficients the entities *C. malabarica* and *C. zedoaria* showed maximum similarity between the two. A similar pattern of grouping was observed in the dendrogram constructed using the key morphological characters as well. These findings warrant that further studies are required to delimit their status as separate species. For example, *C. zanthorrhiza* was misidentified in the past as *C. aromatica* and *C. zedoaria* based on morphological traits. (Skornickova and Sabu, 2005).

C. decipiens, *C. longa* and the entity *Curcuma* species also grouped singly in all the dendrograms (Tables 43 & 52).

A comparison of the dendrograms constructed based on the key morphological characters (Jaccard's similarity coefficients) and the combine RAPD and IISR marker (Sorensen Dice and Jaccard's coefficients) showed some non congruence though there was some similarity also between the two dendrograms. In the group I of the dendrogram constructed using the key morphological characters and combined molecular markers (RAPD/ISSR), species such as *C. montana* and *C. comosa* clustered together though there were additional entities as well in the group. In the group II of both the dendrograms (morphological and molecular) *C. malabarica* and *C. zedoaria* clustered together. However, in the morphological grouping there were two additional entities namely *C. pseudomontana* and *C. sylvatica* in this group. *C. aromatica* and *C. haritha* formed a single group in the dendrograms using morphological and molecular markers. The entity *Curcuma* species was the lone member in the group VII of the dendrogram constructed using

molecular markers whereas this entity clustered with *C. longa* in the dendrograms using morphological characters.

The non congruence or congruence between the morphological and molecular grouping of the germplasm can be interpreted as a degree of correspondence between the molecular markers and the morphological traits in question. Arunachalam *et al.* (2005) reported that morphological variation due to the physical environment may not be detectable by molecular markers.

5.2.4. Molecular characterization of the *Curcuma* species using 18Sr RNA sequence analysis

The genus *Curcuma* originated in the Indo- Malayan Region (Purseglove, 1981) has got a Pan Asian distribution. Further, the genus is represented by synonymous entities over different countries. It has now been established that the Chinese species *C. albicoma* and *C. chuanyujin* are synonyms of *C. sichuanensis* and *C. kwangsiensis*, respectively. The Chinese species *C. wenyujin* is now recognized as a synonym of *C. aromatica*. *C. phaeocaulis* was misidentified in the past as *C. zedoaria*, *C. caesia* and *C. aeruginosa* in China. *C. kwangsiensis* var. *puberula* and var. *affinis* are not accepted and the identity of the Taiwan species *C. viridiflora* remains suspicious (Sasikumar, 2005; Liu and Wu, 1999).

Single nucleotide polymorphisms (SNP) represent the most widespread type of sequence variation in genomes, yet they have only emerged recently as valuable genetic markers for revealing the evolutionary history of populations. Single nucleotide polymorphisms are ideal for analyses of speciation and historical demography, especially in the light of recent theory suggesting that many unlinked nuclear loci are needed to estimate population genetic parameters with statistical confidence (Brumfield *et al.*, 2003). SNPs are recently used for authentication and identification of *Curcuma* species used as drugs in Chinese medicine (Cao *et al.*, 2001; Sasaki *et al.*, 2002).

Comparative sequence analysis of the 18S rRNA regions of three Indian *Curcuma* species namely *C. aromatica*, *C. longa* and *C. aeruginosa* revealed its perfect sequence homology with the same Chinese species in these regions

indicating that the entities recognized as *C. aromatica*, *C. longa* and *C. aeruginosa* in India and China are the same. (Fig. 40) However, there was no sequence homology with the Chinese population of *C. zedoaria* and the Indian population of *C. zedoaria*, thereby suggesting that the entity recognized as *C. zedoaria* in India and China are not the same. However, the entity recognized as *C. zedoaria* in Japan showed perfect homology for the 18S rRNA regions with the Indian *C. zedoaria* population. This implies that the entity recognized as *C. zedoaria* in India and Japan is the same while *C. zedoaria* of China is distinct from the Indian and Japanese populations of *C. zedoaria*. Based on the *trnK* gene analysis it is already reported that the Japanese *C. zedoaria* and the Chinese *C. zedoaria* are not the same (Sasaki *et al.*, 2002).

The Indian *C. zedoaria* which is now recognized as a synonym of *C. zanthorrhiza* based on the key taxonomic (morphological) features (Skornickova and Sabu, 2005) showed complete sequence homology with the *C. zanthorrhiza* of China in the region thereby justifying the reports of Skornickova and Sabu (2005). In all the *Curcuma* species reported so far, only one SNP was observed at 234th position in the entire 1800 bp sequence of 18S rRNA studied. However in the present study a new base transition at 526th position (A – G) is observed in the *C. caesia* from India. In addition to the SNP at 234th position (thymine) *C. decipiens* and the entity *Curcuma* species showed three more transitions at 223, 238 and 1490 (C – T) and a transversion (T – A) at 1500 position (Fig. 40).

5.2.5. Molecular characterization of the turmeric cultivars/varieties using RAPD/ISSR markers

Most of the improved varieties studied clustered distinctly from the land races/cultivars. Further, most of the land varieties, collected mainly based on vernacular names, from one geographical region clustered together along with few released varieties which were evolved through germplasm selection of material collected from the same region. Similar grouping pattern of turmeric varieties is reported earlier (Nayak *et al.*, 2006). The improved varieties of turmeric such as ‘Kedaram’, ‘Alleppey Supreme’, ‘Prabha’ and ‘Prathibha’ clustered together with the local cultivar ‘Alleppey’ in all the dendrograms (Group I). In this group ‘Alleppey Supreme’ and ‘Prathibha’ showed maximum similarity between them

followed by 'Prabha' & 'Prathibha' and 'Prabha' & 'Kedaram'. The varieties 'Alleppey Supreme' and 'Kedaram' are clonal /germplasm selections of turmeric collected from Kerala. 'Prabha' and 'Prathibha' are varieties evolved through true turmeric seedling selections from mother clones of unknown identity. The original germplasm of 'Kedaram' is collected from Thodupuzha, Kerala, a location within the home turf of the famous Alleppey Finger Turmeric (AFT). 'Prabha' and 'Prathibha' are seedling (true turmeric seedlings) of unknown mother parentage, developed from bulk seedlings raised from open pollinated seedlings collected from different turmeric clones (Sasikumar *et al.*, 1996).

In the dendrogram constructed using the morphological characters too the grouping was almost akin to the grouping derived from the RAPD/IISR dendrogram except for the inclusion of the cultivars/varieties such as 'Sudarsana', 'Katergia', 'GL Puram' and 'Wynad' in group I.

Twelve collections from the Andhra Pradesh and one collection from Kerala formed the second group in the cluster. In this group all the accessions showed more than 90% similarity between them (Fig. 63). The entries 'Katergia' and 'Dhagi' showed maximum similarity in this group. 'Jabedi' and 'GL Puram' in this group showed 92% similarity between them. 'Chayapasupu' and 'Avanigadda' showed a similarity of 95% between them followed by 'Kodur' and 'Kuchipudi' (94%). 'Rajapuri' and 'Vondimitta' shared 95% similarity between them, while 'Vondimitta' showed maximum similarity with 'Wynad' (94%). 'Rajapuri' and 'Vondimitta' are collections from nearby regions (Andhra Pradesh and Maharashtra), though 'Wynad' is a collection from a distant area of Kerala. The two released varieties 'Suguna' and 'Sudarsana' grouping with 'Basar Aalong' and 'Mamomthody' formed the third group of the cluster. 'Mamomthody' & 'Basar Aalong' in this group shared 78 % similarity between them while the two released varieties showed only 66% similarity between them. 'Suguna' and 'Sudarsana', evolved through selection of germplasm collected from North East India showed maximum similarity with 'Basar Aalong' of this group collected from Arunachal Pradesh. Six collections from Andhra Pradesh, two collections each from Assam & Kerala, one collection each from Mizoram and Nagaland besides two improved varieties ('Suvarna' and 'Sugandham') formed the last group of the cluster. Three

collections from Andhra Pradesh ('Amalapuram', 'Amrithapani' & 'Armoor') were most similar among the thirty six samples studied and they shared more than 99% similarity between them. The other two members in this group Karhadi and 'Dindigam' showed 90% similarity between them while the improved variety 'Suvarna' showed maximum similarity (95%) with 'Jorhat' collected from Assam. The collections from Nagaland and Assam showed maximum similarity between them (92%), however, 'Aizwal' collected from Mizoram had maximum similarity with 'Ayur' collected from Kerala (90%) and they grouped together.

In all the dendrograms constructed using separate or combined molecular markers, irrespective of the grouping procedure adopted, the varieties such as 'Prathibha' and 'Alleppey supreme' clustered together and showed maximum similarity within the group. Similarly in all the dendrograms the cultivars 'Amrithapani' & 'Armoor' and /or 'Amalapuram', 'Amrithapani' & 'Armoor' clustered together showing maximum similarity. This suggest that these cultivars are genetically very similar or got collected as distinct accessions based on vernacular names (Table 66).

Thus the genetic diversity analysis of the turmeric cultivars and varieties underscore the earlier finding of Shamina *et al.*(1998) based on isozyme polymorphism that cultivars collected from the same geographical region based on vernacular names will not be genetically distinct notwithstanding good genetic variability in this clonally propagated crop. The enigma of clustering of few accessions from distant geographical localities, such as 'Wynad' in group II, 'Mamomthody' in group III and few other collections in group IV together might be also due to the spread of the cultivars along with the movement of settlers throughout the length and breadth of the country and mixing up of the introduced material with the local races and recollecting of the material under a 'wrong' identity.

In order to have a varietal discrimination technique based on rhizome DNA, twelve turmeric varieties/cultivars were studied. The dendrogram constructed using the Jaccard's, Sorensen Dice and Simple Matching similarity coefficients yielded three distinct groups. The improved variety 'Suvarna' formed a lone group in the

dendrogram (Group I). Popular cultivars such as 'Jabedi', 'Amrithapani', 'Armoor', 'Katergia', 'Dhagi' and 'Amalapuram' formed the II group. In this group too, 'Amrithapani' and 'Armoor' had maximum similarity as observed in all the dendrograms constructed using the molecular markers (Fig.63). The third group in the dendrogram was constituted by the two improved varieties viz. 'Sudarsana' and 'Suguna'. These two varieties are having maximum rhizome morphology too. In an earlier study using isozymes makers also these two varieties formed a single cluster in the dendrogram (Shamina *et al.*, 1998). 'Prabha' and 'Prathibha' along with the popular cultivar 'Alleppey' constituted the IV cluster in the dendrogram. Similar grouping pattern was observed in the dendrogram constructed using molecular markers derived from leaf DNA as well.

Turmeric is a rhizome propagated crop. All the varieties/cultivars are not easily discernable based on aerial and/or rhizome morphology. This handicap could give rise to unscrupulous seed trade practice in this crop. A protocol based on rhizome DNA for varietal identification could be thus more relevant in this scenario. Similar observation was reported earlier in ginger and turmeric (Syamkumar *et al.*, 2003).

5.2.6. Molecular characterization of the open pollinated progenies of turmeric using RAPD/ISSR markers

In all the dendrograms constructed using RAPD and RAPD plus ISSR markers a similar grouping pattern of the OP lines and their mother lines studied was evident irrespective of the similarity coefficients used. Three groups were observed in all the dendrograms. The accessions which showed the maximum and minimum similarity coefficients in all the dendrograms were also the same except for the least similar accessions in group II and group III (Table 85). While in the ISSR dendrograms the grouping pattern was slightly different and five different groups were formed by the OP lines and their mother parents. A definite pattern or clustering of the OP lines could not be observed in the present case. Perhaps the very few number of progenies studied may be the reason for this behaviour. The morphological characterization also showed the similar trend.

5.3. BIOCHEMICAL CHARACTERIZATION

5.3.1. Essential oil

Curcumin, essential oil and oleoresin are the three most important biochemical constituents in *Curcuma*. *C. longa* is considered as the richest source for curcumin (2-8%), oleoresin (7-15%) and essential oil (2-5%). However, the present study revealed that *C. aromatica* is a better source of rhizome and leaf oil than the cultivated species, *C. longa* (Table 86). The rhizomes and leaves of *C. aromatica*, which is devoid of any varieties at present are characterized by high camphoraceous aroma (volatile compounds) probably an indication of the high essential oil content in it. The species can be cultivated even in marginal lands with minimum care. Thus *C. aromatica* can be a better choice for bioprospecting of *Curcuma* essential oil which has immense potential in perfumery, aromatherapy etc.

A perusal of the literature reveals lack of coherence in the essential oil profile of the same species using the same plant parts. Even the same authors have reported different values for essential oil profile from a single species or same plant parts in different works. This anomaly may be due to the effect of environments, varieties, maturity variations, analytical methodology or even in incorrect taxonomic identity of the specimens used (Sasikumar, 2005).

The yield of rhizome essential oil in the cultivars of *C. longa* from India is in the range of 2 – 5 % (Sasikumar, 2001). However, Pothitirat (2006) reported an essential oil content ranging from $7.00 \pm 0.00\%$ to $8.20 \pm 1.66\%$ in Thailand turmeric. In the present study the highest rhizome oil is observed in the improved variety 'Prathibha' (7.33%). The leaf oil content in the cultivars varied from 0.6 – 1.3 %. Maximum leaf oil yield was observed in the cultivars 'Jabedi' (1.3%) followed by 'Rajapuri' (1.2%). Improved turmeric varieties such as 'Prathibha' are known for high curcumin and essential oil contents (Sasikumar *et al.*, 1996).

Leaf oil of turmeric has relevance in aromatherapy (Sasikumar, 2005), perfumery, cosmetics and soap industry (Ramachandraiah *et al.*, 1998; Sirirugsa, 1997).

Usually the turmeric crop is harvested when the aerial portion turns yellow and this plant part generally goes as agricultural waste. This crop residue which generally goes unutilized can be a potential source for extracting turmeric oil. From the present study it is observed that the fully matured turmeric leaves contains upto 1.3% of essential oil which can be used for the extraction of essential oil thus adding value to the waste.

5.3.1.1. GC MS analysis of essential oil

The essential oil of *Curcuma* contains a variety of molecules credited with very many pharmacological properties (Sasikumar, 2005). In the present study, it is observed that myrcene as the major compound in *C. amada*. About 74.97 % of the total volume of the fresh rhizome oil was constituted by this molecule. Singh *et al.* (2003) also reported 80.54% of myrcene in *C. amada* rhizome oil. Myrcene is a very potent analgesic substance and might be an alternative to the already available analgesic drugs. The antimutagenic effect of myrcene is also evaluated in mammalian cells *in vitro* (Kauderer *et al.*, 1991). Myrcene is also reported to have potent molluscicidal activity against *Bulinus truncates* and have important application in combating schistosomiasis (Laholu, 2003). Since *C. amada* is rich in myrcene, this species can effectively be exploited for the isolation of this pharmacologically important molecule besides the already existing culinary use of the species. *C. amada* is also widely used in traditional medicine for sprains, bruises, treatment of various intestinal diseases, fever, jaundice etc (Jain, 1995). Another beneficial use of *C. amada* oil is in pest control (Singh *et al.*, 2002).

Species such as *C. haritha*, *C. pseudomontana* and *C. raktakanta* recorded ar-turmerone as the major essential oil component in the GC MS analysis. Among the three species *C. pseudomontana* recorded the highest percentage (63.46%) of the compound. Lee (2006) reported that ar-turmerone is a potent inhibitor of collagen induced platelet activation. It inhibits the arachidonic acid induced platelet aggregation suggesting that ar-turmerone may have an effect on cyclooxygenase pathway in platelets. ar-turmerone is also reported have repellency to *Tribolium castaneum* Hbst. (Chander *et al.*, 1992; Chowdhury *et al.*, 2000). The fresh rhizome essential oil analysis of this three species is the first report and the GC MS profile of *C. pseudomontana* is new to the science too! Thus the above said species can

effectively be utilized for the extraction of the pharmacologically active compounds like ar turmerone. Cultivation of these species can be easily undertaken even in marginal soils.

Curcuma aromatica is a chemotype of L - camphor among the eleven species studied as it contains 22.24 % of camphor in the total volume of the essential oil. Choudhuri *et al.* (1996) also reported highest percentage of camphor (25.6%) in *C. aromatica*. Shi *et al.* (2002) reported that *C. aromatica* oil inhibits hepatoma in mice by inhibiting the expressions of Cyclin D and proliferative cell nuclear antigen and as a result inhibits the growth of hepatic carcinoma. *C. aromatica* essential oil also cause complete mycelial growth inhibition of *Colletotrichum falcatum* and have good insecticidal action against *Odontotermes obesus* Rhamb, a pest of sugarcane (Singh *et al.*, 2002).

The rhizome oil of *C. malabarica* and *C. zedoaria* recorded highest amount of xanthorrhizol in the fresh rhizome oil. Antimetastatic potential of xanthorrhizol for mouse lung metastasis was reported by Choi *et al.* (2005). The expression levels of cyclooxygenase 2, matrix metalloproteinases 9 (MME 9) and phosphorylated extracellular signal regulated kinase (ERK) were also attenuated by xanthorrhizol. Xanthorrhizol is also having antibacterial, contractile activity of uterine smooth muscles, anti candidal, induction of cell death in oral squamous carcinoma cells, neuroprotective, induction of apoptosis, and attenuation of cisplatin induced nephrotoxicity and antimicrobial activities (Kim *et al.*; Monter *et al.*, 1999; Rukayadi *et al.*, 2006).

C. caesia recorded 1,8 - cineole as the majour compound in the essential oil (25.60%). *Curcuma caesia* is used as a carminative and for treatment of rheumatic pains, headache besides its known use as a stimulant (Rahaman and Yusuf, 1996). The essential oil also possesses antibacterial and anti fungal properties (Rahaman and Yusuf, 1996). *C. comosa*, *C. montana* and *C. sylvatica* showed highest amount of epicurzerenone in fresh rhizome oil. The essential oil profile of *C. comosa* and *C. montana* are new reports.

The cultivated varieties of *C. longa* gave a different profile for the leaf essential oil studied. Varieties/ cultivars like 'Alleppey', 'Kedaram', 'Prabha' and 'Sugandham' recorded highest percentage of α - terpinolene (10.63 - 18.79%) whereas α phellandrene was the highest observed compound in 'Alleppey Supreme'. 'Suguna' and 'Sudarsana' recorded highest amount of β - sesquiphellandrene. In case of 'Rajapuri' and 'Wynad', zingiberene was the highest observed compound. The variety 'Prathibha' showed a highest amount of *P* - cymene. Bioassay of essential oil indicated that *C. longa* as good antifungal material and cause complete mycelial growth inhibition of *Colletotrichum falcatum*, *Fusarium moniliforme*, *Curvularia pallescens*, *Aspergillus niger*, *Fusarium oxysporium* (Singh *et al.*, 2002). Essential oil of turmeric is known to have

5.3.2. Oleoresin

Turmeric oleoresin is being used increasingly by the processed food industries in the West to impart colour and aroma to foods and beverages. Oleoresin is a mixture of compounds namely curcumin, volatile oil and other active ingredients, non volatile fatty and resinous material extractable by solvents, used singly, in sequence or in combination (Sasikumar, 2001). The oleoresin yield of the cultivars/ varieties was in the range of 10.4 to 15.2%. The highest amount of oleoresin content was observed in the variety 'Prathibha' (15.2%) followed by 'Alleppey Supreme' (15.0%) and the lowest was in the cultivar 'Hajo'. It is also observed that the varieties which recorded high oleoresin content are also rich in curcumin. These varieties are bred for high yield and good quality (Sasikumar, 1996 & 2005).

5.3.3. Curcumin

In future, turmeric may be more of a medicinal crop than a spice. The relative proportion of the curcuminoids in the total curcumin is believed to be pharmacologically important (Verghese, 1999). Individual turmeric varieties/cultivars rich in one or other of these curcuminoids may fetch a premium price. Among the different species studied, detectable total curcumin content was observed in only six species in which *C. longa* recorded the maximum curcumin content. In the different turmeric varieties/cultivars studied, the total curcumin

content ranged from 1.9 – 6.5 % and the released varieties showed higher levels of total curcumin.

5.3.3.1. HPLC analysis of curcuminoids

Usually ethanol is used as the mobile phase in HPLC analysis of curcuminoids. Tonnesen and Karlsen (1983) who first isolated the three curcuminoids (curcumin, demethoxy curcumin and bisdemethoxy curcumin) using HPLC in a Nucleosil NH₂ column stationary phase, used ethanol at a flow rate of 1.2 ml as mobile phase. In addition to ethanol various solvents such as methanol, acetonitrile & tetrahydrofuran; stationary phase (C₁₈ and NH₂) and different methods (isocratic and gradient elution) are used for the analysis of curcuminoids (Khurana and Ho 1988; Cooper *et al.* (1994). Jayaprakasha *et al.* (2002) reported a validated gradient HPLC method with solvent mixture of methanol, acetic acid and acetonitrile, with a run time of 20 min for separation of curcuminoids. In the present study we have used chloroform:ethanol (80:20) as the mobile phase, a Nucleosil NH₂ column as the stationary phase and obtained four peaks instead of three reported earlier. Separation of individual curcuminoids in the HPLC system is known to be influenced by the mobile phase (Jayaprakasha *et al.*, 2002; Marsin *et al.*, 1993; Taylor and McDowell, 1992; Rouseff, 1988).

The *Curcuma* species so far analysed for curcuminoids using HPLC are *C. longa*, *C. xanthorrhiza*, *C. mangga*, *C. heyneana*, *C. aeruginosa* and *C. soloensis* (Jayaprakasha *et al.*, 2005; Bos *et al.*, 2007). In the present study we analysed curcuminoids from six *Curcuma* species viz, *C. comosa*, *C. longa*, *C. malabarica*, *C. montana*, *C. raktakanta* and *C. zedoaria* using the newly developed HPLC method. The curciminoid content ranged from 0.04% to 4.0%. The highest percentage of curcuminoids was detected in *C. longa* and the lowest was in *C. raktakanta*. Except for *C. longa* all other species studied are the new reports.

Variation in curcumin yield in spectrophotometric method and HPLC method is known. Usually, the HPLC method yields about 60% of the yield of the spectrophotometric method (Verghese, 1999). This may be due to the presence of other compounds absorbing in the region of 420-430 nm influencing the results in spectrophotometric analysis. The difference may also be due to the difference in

intensities of three curcuminoids in organic solvents. At fixed excitation and emission wavelengths (420-470 nm) the relative fluorescence intensities of curcumin, demethoxycurcumin and bis demethoxycurcumin in ethanol are 1:2.2:10.4 (Tonnesen and Karlsen, 1983).

In the present study among the thirty six varieties/ cultivars studied, maximum curcuminoid content was observed in the variety 'Kedaram' (4.3%). This variety also showed the highest amount of curcumin I (1.5). Variety 'Prathibha' showed the highest amount of curcumin II whereas the cultivar 'Basar Along' and 'Mamomthody' recorded maximum curcumin III content (1.2 % each). The yield of total curcuminoids in the *Curcuma* species and turmeric varietal/cultivars studied was also low as compared to the spectrophotometric value

5.3.4. Turmerin

Turmerin is low molecular weight (5 KDa) water soluble anti oxidant peptide first isolated from turmeric powder by Srinivas *et al.* (1995). This molecule is credited with many medicinal properties such as antimutagenic, antivenomous and DNA protectant activity against ultraviolet radiation, lipid peroxides and fuel smoke condensate (Srinivas *et al.*, 1995). Though the author reported a turmerin level of 100 mg% in *C. longa*, up to 320 mg% turmerin is recoverable from turmeric powder (Srinivas, Personal communication) and there is a US patent on this compound. In the present study among the fifteen *Curcuma* species studied, *C. sylvatica* (516.5 mg%) followed by *C. comosa* (502.9 mg%) showed highest amount of turmerin in fresh mother rhizomes, while *C. longa* (380.5 mg%) followed by *C. pseudomontana* (369.7 mg %) had high turmerin in fresh finger rhizomes. Among the different parts of the rhizome studied fresh mother rhizomes were the better source for this molecule. As compared to the fresh material, the dry rhizome yielded low levels of turmerin though the superiority of the species *C. sylvatica* and *C. comosa* was evident here also. However *C. caesia* recorded the second highest amount of turmerin in dry rhizomes. Thus for bioprospecting of turmerin these species may be ideal.

Turmerin levels within selected cultivars of *C. longa* showed a variation from 248.32 ('GL Puram') to 318.24 mg %('Rajapuri'). 'Rajapuri' turmeric is one of the highly priced commodity in the domestic market.

5.3.5. HPLC analysis of rhizome proteins

The biochemical method (AE HPLC) developed for the characterisation *Curcuma* species and turmeric varieties using rhizome proteins well discriminated the entities studied. The thirteen *Curcuma* species studied clustered into six different groups based on the elution pattern of the water soluble rhizome proteins in HPLC (Fig. 96). Even though the salt soluble proteins showed variation in the elution profile and placed the *Curcuma* species studied in different groups, the grouping pattern was not meaningful. In case of the turmeric varieties/cultivars studied, the AE HPLC profiles of water soluble proteins placed the entities in six groups and well separated these closely related entities in the dendrogram (Fig.101). Due to its high reproducibility and resolution efficiency, AE-HPLC analysis proteins has proved to be a powerful tool for the identification and characterization of cultivars, landraces and androgenetic lines in other horticultural crops such as *Solanum melongena* L., *Lycopersicon esculentum* Mill., *Phaseolus vulgaris* L. and *Allium cepa* L. (Mennella *et al.*, 1999, 2001, 2003 and 2005). The salt soluble proteins did not show any variation in their profiles in different turmeric varieties/cultivars studied. The same handicap of salt soluble seed proteins in the identification onion land races is reported by Mennella *et al.* (2005).

The seed/rhizome proteins studied are reported to be independent of environmental growth conditions. The advantages of seed/rhizome storage protein analysis, compared with the morpho-physiological characterization based on traits recorded in field are the weak or null effect of the environmental factors on their expression, the effective discrimination among genotypes, the low cost and time-saving peculiarities with respect to agronomic description of the plants (Mennella *et al.*, 2005). In addition to discriminating the closely related turmeric varieties/cultivars these profiles could allow the identification of biochemical markers linked to useful agronomical traits.

Summary and Conclusion

Chapter 6

SUMMARY AND CONCLUSION

Curcuma is now gaining global importance as a mighty cure to combat a variety of ailments as the genus is credited with molecules having anti-inflammatory, hypocholesteremic, choleric, antimicrobial, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic, anticancerous properties and insect repellent activity besides of course its known use as a spice. *Curcuma* is represented by eighty species globally and in India there are about forty species though some of these species are now turning out as synonymous entities. Species diversity and cultivar diversity of *C. longa* is one of the highests in India. Correct taxonomic identity of the specimen is one of the foremost requirements in effective bioprospecting of the genus. Unfortunately taxonomic confusion galore in the genus, globally. Global taxonomic revision of the genus is now in the offing. Traditional taxonomic tools alone is proving inadequate in resolving the taxonomic confusion existing in the genus as some of the key traditional taxonomic tools such as spike position and bract colour are beset with controversy. Molecular tools can be used as an adjunct in this context. Biochemical studies would give further impetus to the identity of the specimens. The present study is thus an attempt at morphological, molecular and biochemical characterization of fifteen Indian *Curcuma* species, thirty six turmeric varieties/cultivars and twelve open pollinated progenies of turmeric as a prelude to the global taxonomic revision of the genus.

6.1. MORPHOLOGICAL CHARACTERIZATION

6.1.1. *Curcuma* species

Fifteen *Curcuma* species, thirty six turmeric varieties/cultivars and twelve open pollinated progenies of turmeric are characterized based on twentyeight common morphological traits besides twenty key morphological characters. Dendrograms constructed based on Jaccards similarity coefficients thrown out a total of seven groups each in the two dendrograms constructed based on the key morphological characters and the twentyeight common morphological characters. Maximum similarity was observed between *C. malabarica* and *C. zedoaria* in both the dendrograms. However, there was some absurdity in the grouping pattern of species in the dendrogram constructed using the twentyeight common morphological characters. The characters like leaf mid rib colour, colour of leaf sheath, leaf texture, rhizome colour, aroma of rhizome, rhizome flavour/taste, number of mother rhizomes per clump, number of primary and secondary rhizomes, colour of the fertile bract, colour of the corolla, colour of the labellum and anther spur are found more reliable morphological characters in the characterization of the *Curcuma* because these key morphological traits produced a sensual grouping pattern in the dendrograms as observed in the grouping together of species such as *C. aromatica* & *C. haritha* and *C. malabarica* & *C. zedoaria* together in the dendrogram, which occur in common habitat and share many similar features

6.1.2. Turmeric varieties/cultivars and open pollinated progenies

Good infraspecific variation was observed in the thirty six turmeric varieties/cultivars studied using different morphological traits. High variability for both morphological and yield traits could be observed. The aerial morphological characters such as leaf length followed by pseudostem height and number of leaves per tiller showed a highest variation while for the rhizome characters maximum variability was observed for clump weight followed by number of secondary rhizomes, weight of secondary rhizomes, weight of mother rhizomes, weight of primary rhizomes, number of primary rhizomes, internode length, dry recovery and number of mother rhizomes. The improved varieties such as 'Prabha', 'Prathibha', 'Alleppey Supreme', 'Kedaram' and 'Sudarsana' showed a stable performance for these characters over the years but the other three improved varieties viz. 'Suvarna',

‘Sugantham’ and ‘Suguna’ showed a wide variation for these characters over years. The coma bract colour was also found to vary among the turmeric cultivars. The colour varied from white (‘Alleppey’), white with pink (‘Jorhat’), white with purple colour at the tip (‘Karhadi local’) to white with green tip (‘Kuchipudi’).

The turmeric varieties/cultivars are clustered in eleven groups in the dendrogram constructed using different morphological characters using Jaccard’s similarity coefficients. The eight improved varieties barring ‘Suguna’, ‘Sugantham’ and ‘Suvarna’ clustered together in the dendrogram. The clustering of improved varieties are as expected since selection for yield and/or quality has been one of the major crop improvement strategies in this clonally propagated crop and thus these varieties would have many genes in common resulting in their together grouping. Another grouping pattern discernable was on the basis of the geographical origin of the cultivars. In general the cultivars from same geographical region grouped together with few exceptions. One possible reason for the exception could be the introduction of turmeric clones to different parts of the country by formal and informal means and their recollection as distinct cultivars at a later date.

The open pollinated progenies of turmeric showed a wide variability for the different morphological characters studied suggesting the release of the locked up variability in the true turmeric seedlings. No specific clustering pattern was observed in the dendrogram constructed using different morphological characters studied. The OP progenies and their parents clustered in four different groups; the progenies and mother parents forming different groups.

6.2. MOLECULAR CHARACTERIZATION

Good quality DNA with high yield is a pre requisite of molecular marker analysis. DNA was isolated from fresh leaves of different *Curcuma* species, turmeric varieties/cultivars and open pollinated progenies of turmeric by using a modified CTAB protocol. The DNA yield ranged from 93 $\mu\text{g g}^{-1}$ (*C. zedoaria*) to 153 $\mu\text{g g}^{-1}$ (OP ‘Alleppey’) among the different *Curcuma* species, turmeric varieties/cultivars and open pollinated progenies of turmeric studied.

For developing a molecular marker based technology for the identification of the seed rhizome, another modified CTAB protocol was developed to isolate good quality DNA from fresh matures rhizomes of turmeric. The yield of the DNA from fresh mature rhizomes ranged from 10.75 – 14.44 $\mu\text{g g}^{-1}$ DNA with a purity ranged from 1.83-1.87.

Two dominant molecular markers viz. RAPD and ISSR were used for the molecular characterization of fifteen *Curcuma* species, thirty six turmeric varieties/cultivars and twelve open pollinated progenies of turmeric besides the 18 S rRNA sequence analysis of *Curcuma* species.

6.2.1. *Curcuma* species

Fourty eight random decamer primers (RAPD) produced a total of four hundred and fourty nine scorable bands in the 15 *Curcuma* species studied, out of which four hundred and nine were polymorphic. The dendrograms constructed using three different similarity coefficients viz. Jaccards, Sorensen - Dice and Simple Matching coefficients produced a total of seven groups in the *Curcuma* species studied using the RAPD markers with maximum similarity between *C. montana* and *C. raktakanta*. Eight ISSR primers produced a total of ninety one scorable bands in the 15 *Curcuma* species studied and 95.6% of the markers were polymorphic. In all the three similarity matrices produced by the ISSR markers, *C. zedoaria* & *C. malabarica* showed maximum similarity followed by *C. raktakanta* & *C. montana*. The fifteen *Curcuma* species studied were placed in six different groups in all the dendrograms. Fourty eight RAPD and eight ISSR primers together produced a total of five hundred and fourty markers among the fifteen *Curcuma* species studied, out of which four hundred and ninety six (91.85%) were polymorphic. In all the dendrograms constructed using separate or combined molecular markers, irrespective of the grouping procedure adopted, the species *C. montana* and *C. raktakanta* showed the highest similarity followed by *C. malabarica* and *C. zedoaria*. Since a combined genetic diversity estimate based on several molecular markers cover more genomic regions than a single marker alone, more than one dominant molecular marker will be more desirable for the genetic diversity analysis

and germplasm characterization. Thus the present findings demand a relook into the separate status accorded to the above four species, at present.

For the characterisation of *Curcuma* species, the 18 S rRNA regions of 15 Indian *Curcuma* species were amplified using specific primers. An 1800 bp product obtained digested with four restriction enzymes placed them in two groups. Further, using the three internal primers designed, the 18 S rRNA region was amplified and the products (620 – 654 bp) were sequenced using forward and reverse primers. The sequence analysis revealed that species namely *C. aromatica*, *C. longa* and *C. aeruginosa* have the perfect sequence homology with the same Chinese species for these regions indicating that the entities recognized as *C. aromatica*, *C. longa* and *C. aeruginosa* in India and China may be the same. However, the Indian population of *C. zedoaria* showed sequence homology with the Japanese *C. zedoaria* population for the region suggesting that the entity recognized as *C. zedoaria* in India and China may be different. Except for the four species viz. *C. aromatica*, *C. longa*, *C. aeruginosa* and *C. zedoaria* all the eleven species studied were new to the science. So far only one SNP was reported at 234th position in the entire 1800 bp sequence of 18 S rRNA of the different *Curcuma* species investigated. However, in the present study a new base transition at 526th position (A – G) is observed in *C. caesia* from India. In addition to the SNP at 234th position (thymine), *C. decipiens* and the entity *Curcuma* species showed three more transitions at 223, 238 and 1490 (C – T) and a transversion (T – A) at 1500 position.

6.2.2. Turmeric varieties/cultivars and open pollinated progenies

Forty one random decamer primers were used for the molecular characterisation of 36 cultivars/varieties of turmeric produced a total of 498 scorable bands out of which 394 (79.1%) were polymorphic. The percentage polymorphism ranged from a maximum of 95% showed by OPB 08 to a minimum of 54 % showed by OPJ 10. Three different similarity coefficients viz. Jaccards, Sorensen - Dice and Simple Matching coefficients used for the molecular marker analysis produced a total of seven groups in the cultivars/varieties of turmeric studied using RAPD markers with maximum similarity between the cultivar “Armour” and “Amrithapani” and these two cultivars were unable to be separated by the 41

random decamer primers used. Fifteen ISSR primers used for characterising the 36 turmeric cultivars/varieties produced a total of 160 scorable bands in which 118 were polymorphic constituting 73.8 % of the total markers produced. No single primer yielded 100% polymorphic bands in the turmeric cultivars/varieties studied. Cultivars 'Amalapuram' and 'Amrithapani' followed by 'Amalapuram', 'Amrithapani' and 'Armoor' showed maximum similarity in the dendrograms constructed using ISSR markers. Three different similarity coefficients viz. Jaccards, Sorensen - Dice and Simple Matching coefficients placed the 36 turmeric varieties/cultivars in four groups irrespective of the molecular marker studied. In all the dendrograms constructed using separate or combined molecular markers, irrespective of the grouping procedure adopted, the varieties such as 'Prathibha' and 'Alleppey Supreme' clustered together and showed maximum similarity within the group. Similarly in all the dendrograms the cultivars 'Amrithapani' & 'Armoor' and/or 'Amalapuram', 'Amrithapani' & 'Armoor' clustered together showing maximum similarity. This suggest that these cultivars are genetically very similar or got collected as distinct accessions based on vernacular names.

Fifty two random decamer primers produced a total of 553 scorable bands in the 12 open pollinated progenies studied out of which 328 (59.31%) were polymorphic. The percentage of polymorphism ranged from a maximum of 90.9% percent showed by primers OPC 09 & OPC 20 to a minimum of 11.1 % showed by primer OPD 07. Twelve ISSR primers produced a total of 109 scorable bands in the 12 open pollinated progenies studied out of which 63 (58.76%) were polymorphic. The percentage of polymorphism ranged from a maximum of 80.0 % showed by primer ISSR 11 to a minimum of 40.0 % revealed by primer ISSR 04. A total of 662 bands were produced by the combined RAPD and ISSR markers and 391 (59.06 %) were polymorphic among the 12 open pollinated progenies and their parents studied. In all the UPGMA dendrograms constructed using the genetic similarities obtained from Jaccards, Sorensen- Dice and Simple Matching coefficients based on RAPD and ISSR markers, the clustering pattern of OP progenies and parents were similar and formed three groups in the dendrogram and a definite pattern of clustering of the OP lines could not be observed in the present case. This may be due to very few numbers of progenies used for molecular characterisation.

Turmeric is a vegetatively propagated crop, rhizomes are used as planting material. All the varieties/cultivars are not easily discriminated based on aerial and/or rhizome morphology. This could lead to unscrupulous seed trade practice in this crop. A protocol based on rhizome DNA for varietal identification could be thus more relevant. In order to have a varietal discrimination technique based on rhizome DNA, twelve turmeric varieties/cultivars were studied and the dendrogram constructed using the Jaccard's, Sorensen Dice and Simple Matching similarity coefficients well separated the genotypes studied. The grouping pattern of entities studied was similar to the grouping observed in the dendrogram constructed using the leaf DNA.

6.3. BIOCHEMICAL CHARACTERIZATION

6.3.1. Essential oil

Highest essential oil percentage in fresh rhizomes was observed in *C. aromatica* (4.8 %) followed by *C. caesia* and *C. sylvatica* (3.6 %) and the lowest percentage in *C. haritha* (2.4 %) among the *Curcuma* species studied. The essential oil yield from fresh matured leaves ranged from 0.90 (*C. zedoaria*) to 1.6 % (*C. aromatica*). Since *C. aromatica* contains highest percentage essential oil in both rhizomes and leaves this species can be a better choice for bioprospecting of *Curcuma* essential oil as it can be cultivated even in marginal lands with minimum care.

Essential oil from dried rhizomes and fresh matures leaves of the turmeric cultivars/varieties ranged from 4.67 to 7.67 %. Cultivars namely 'Gaspani', 'Vondimitta' and 'Jabedi' recorded lowest oil yield while 'Rajapuri' and 'Ayur' recorded maximum oil yield. Most of the cultivars/varieties recorded one percent fresh leaf essential oil. The highest percentage of fresh leaf oil was recorded in cultivar 'Jabedi' (1.3 %) and the minimum was recorded in the variety 'Sudarsana' (0.6 %).

The eleven *Curcuma* species used for the GCMS analysis of fresh rhizome oil generated six chemotypes based on the presence of highest observed compound in the rhizome oil. Three species namely *C. haritha*, *C. pseudomontana* and *C.*

raktakanta showed highest percentage of ar turmerone, while *C. comosa*, *C. montana* and *C. sylvatica* showed epicurzerenone as the major compound. *C. zedoaria* and *C. malabarica* recorded highest amount of xanthorrhizol in the fresh rhizome oil as the major compounds. *C. amada*, *C. aromatica* and *C. caesia* are the specific chemotypes identified for myrcene, camphor and 1,8 cineole, respectively.

A total of 48 compounds were identified in the fresh leaf oil of the ten varieties/cultivars of turmeric studied using GCMS. Based on the highest observed compound in the GC MS profile, the varieties were placed into five different groups. In six of the samples studied, monoterpene hydrocarbons α phellandrene, ('Alleppey' Supreme), α terpinolene ('Alleppey', 'Kedaram', 'Prabha' & 'Sugandham') and *para* cymene ('Prathibha')) constituted the major compounds while the sesquiterpene hydrocarbons, zingiberene ('Rajapuri' & 'Wynad') and β sesquiphellandrene ('Suguna' & 'Sudarsana') were the major volatile oil compounds in the rest. This study gives an idea about the different *Curcuma* species and varieties/cultivars that can be used for the extraction of these pharmacologically active compounds.

6.3.2. Oleoresin

Oleoresin content in the different turmeric varieties/cultivars ranged from 10.4 % ('Hajo') to 15.2 % ('Prathibha').

The content of curcumin, the most treasured molecule in the genus studied, ranged from 0.069 to 6.41 %. Maximum curcumin content was recorded in *C. longa* (6.41 %) and a minimum in *C. raktakanta* (0.069 %). In the 36 turmeric varieties/cultivars studied, the percentage curcumin content ranged from 1.9 % ('Vondimitta') to 6.5 % ('Prathibha').

6.3.3. Curcumin and curcuminoids

Among the six *Curcuma* species profiled for the curcuminoids level, *C. longa* recorded highest percentage of all the three curcuminoids. Except *C. longa*, all the other species profiled for the curcuminoids content were the first time report. The amount of total curcuminoids separated using HPLC ranged from 0.04 (*C.*

raktakanta) to 4.0 % (*C. longa*) among the six *Curcuma* species studied. In the 36 turmeric varieties/cultivars profiled using HPLC for the curcuminoids content, the percentage of total curcuminoids ranged from 0.87 ('Kuchipudi') to 4.32 % ('Kedaram'). The varieties/cultivars varied considerably for the three curcuminoids content. The level of curcumin I was found to be maximum in the improved variety 'Kedaram' (1.78 %) and minimum in cultivar 'Kuchipudi' (0.51 %). Variety 'Prathibha' showed highest content of curcumin II (1.48 %) and 'Kuchipudi' (0.32 %) had the lowest content of curcumin II. The cultivar 'Basar Along' had the highest content of curcumin III (1.18 %) while the least curcumin III content was recorded in 'Jabedi' (0.026 %). The improved varieties such as 'Kedaram' and 'Prathibha' were the rich source of curcumin I and curcumin II while 'Basar Along' was the cultivar having maximum content of curcumin III. This study would help the tailor made production of these molecules more viable.

6.3.4. Turmerin

Turmerin content in the fresh/dry rhizomes and mother/finger rhizomes of the 15 *Curcuma* species studied varied significantly. Maximum yield for turmerin in fresh mother rhizome was recorded in *C. sylvatica* while *C. longa* recorded maximum turmerin yield from finger rhizomes. *C. amada* had minimum turmerin content in fresh mother, finger and dried rhizomes.

6.3.5. HPLC analysis of rhizome proteins

AE HPLC analysis of rhizome proteins (water extractable fraction) studied in the thirteen *Curcuma* species and ten turmeric genotypes (eight improved varieties and two popular cultivars) revealed parity in clustering pattern with the molecular data. The salt soluble fraction of rhizome proteins did not give any clear pattern of grouping of the species or cultivars.

EPILOGUE

In nutshell, the study on morphological, molecular and biochemical characterisation of fifteen *Curcuma* species, thirty six turmeric varieties/cultivars threw out following conclusions:

1. All the grouping patterns (morphological, molecular and biochemical) revealed that the entities recognized now as *C. zedoaria* and *C. malabarica* in India may be synonyms as these two species showed very high similarity between them. Similarly the separate species status given to *C. raktakanta* and *C. montana* also warrants revisiting as these entities also clustered together in all the dendrograms.
2. The entity recognized as *C. zedoaria* in India and Japan may be one and the same and distinct from the Chinese population of *C. zedoaria* as revealed by the 18S rRNA studies.
3. Three turmeric cultivars namely 'Amalapuram', 'Amrithapani' and 'Armoor', all from Andhra Pradesh known by vernacular identity, showed very high similarity among them. This implies that the cultivars recognized as 'Amalapuram', 'Amrithapani' and 'Armoor' may not be genetically distinct.
4. For bioprospecting of pharmacologically important constituents/molecules the species such as *C. aromatica* (essential oil), *C. longa* var. 'Prathibha' (oleoresin), *C. longa* var. 'Kedaram' (curcumin I), *C. longa* var. 'Prathibha' (curcumin II), *C. longa* var. 'Basar Along' (curcumin III), and *C. sylvatica* for turmerin are found ideal.

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