CYTOGENETIC (CHIAS AND RAPD) AND PHYTOCHEMICAL (GC-MS AND SEM) ASSAYS ON SOMACLONAL VARIANT OF *OCIMUM BASILICUM* L. VAR. *PURPURASCENS* BENTH.

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BOTANY

By

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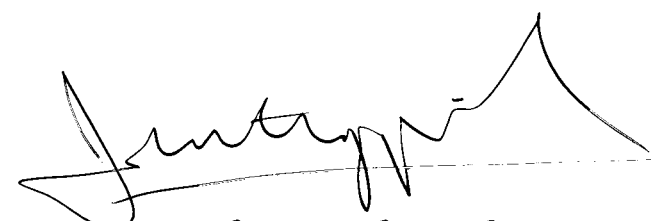
CERTIFICATE

This is to certify that this thesis entitled "Cytogenetic (CHIAS and RAPD) and Phytochemical (GC-MS and SEM) assays on Somaclonal Variant of Ocimum basilicum L. var. purpurascens Benth." is an authentic record of work carried out by Mr. Tajo Abraham, in the Department of Botany, University of Calicut, during 1998 to 2001, under my supervision and guidance and that no part thereof has been presented earlier for any other degree or diploma.



Dr. John E. Thoppil

DECLARATION

I hereby declare that the thesis entitled "Cytogenetic (CHIAS and RAPD) and Phytochemical (GC-MS and SEM) assays on Somaclonal Variant of Ocimum basilicum L. var. purpurascens Benth." submitted for the Ph. D. Degree of the University of Calicut has not been submitted for the award of any other degree or diploma and that it represents the original work carried out by me.

C. U. Campus
30-7-2001

Tajo Abraham

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CONTENTS

ABBREVIATIONS USED

MS medium: Murashige & Skoog medium

BAP: Benzyl Amino Purine

IAA: Indole 3-Acetic Acid

NAA: Naphthyl Acetic Acid

2, 4-D: 2, 4- Dichloro phenoxy acetic acid

KIN: Kinetin

CHIAS: Chromosome Image Analysis System

RAPD: Random Amplified Polymorphic DNA

CTAB method: Cetyl Trimethyl Ammonium Bromide method

EDTA: Ethylene Diamine Tetra Acetic acid

RFLP: Restriction Fragment Length Polymorphism

PCR: Polymerase Chain Reaction

GC-MS: Gas Chromatography-Mass Spectrometry

SEM: Scanning Electron Micrography

INTRODUCTION

Herbal medicines are still the mainstay of about 75-80% of the World population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. India is sitting on a gold mine of well-recorded and well-practised knowledge of traditional herbal medicine (Kamboj, 2000). We have a long history on the use of a large number of medicinal and aromatic plants for various purposes. Ancient literature, right from 'Atharva Veda', provides rich references on native plants and their properties to alleviate human sufferings and promoting a long and healthy life. Our Materia Medica is mainly based on medicinal plants of diverse origin, found all over the Indian subcontinent. A number of native plants have been used in various medicinal preparations (Rao, 1991). Though herbal drugs or phytomedicines are the very basis of traditional remedies of various cultures, their tinctures had been inducted in the allopathic medicines in the early decades, which appear to have returned in a more sophisticated form in recent years in a big way (Dev, 1997).

India is also a primary and secondary centre of origin and diversity of several medicinal and aromatic plants. The country thus serves as a 'treasure house' of diverse variability both in form and chemical constituents to facilitate selection of improved genotype for cultivation and crop improvement. We know very little about their genetic make-up, breeding behaviour, micropropagation, habitat preferences and biosynthesis of their principal chemicals (Rao, 1991). Plant improvement of clonally propagated plants utilize both the natural and induced variations through various *in vitro* and *in vivo* procedures (Skirvin, 1978). Variation has been a ubiquitous phenomenon associated with tissue culture (Carlson, 1973a; Carlson & Polacco, 1975; Green, 1977). Induced variation is considered as an alternative source to naturally occurring variability for crop improvement programmes, hybridization and recombination in plant breeding (Ansari & Siddiqui, 1995).

Somaclonal variation in yield and quality characters would be of utmost importance in crop improvement (Ahloowalia & Sherington, 1985). The present investigation involves comparison of the somatic and somaclonal genotypes of *Ocimum basilicum* L. var. *purpurascens* Benth. (Lamiaceae) with respect to improved essential oil quantity and quality. Even if the country's enriched biodiversity provides enough variability, the competent scientific force should exploit the somaclonal variants too for crop improvement.

The family Lamiaceae is a very large natural group of aromatic and medicinal plants (Morton, 1962). The typical characteristics of the family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many of the sub families particularly Nepetoideae, to which *Ocimum* belongs, are strongly aromatic due to essential oils, which consist of monoterpenes, sesquiterpenes and phenylpropanoids. The members of this genus are annual herbs, which lack a rhizome and possess only tap root system. The typical inflorescence in *Ocimum* is a thyrse composed of opposite, one to three flowered cymes (Paton *et al.,* 1999).

Ocimum is an important member of the Lamiaceae family. The name *Ocimum* is originated from the Greek word '*Okimom*' for aromatic herb (Coombes, 1995). According to Hereman (1980) the name is derived from Greek where '*Ozo*' means smell. Estimates of species number vary from 30 (Paton, 1992) to 160 (Pushpangadan & Bradu, 1995). Among these species, the most heavily used are *O. basilicum, O. americanum* and their hybrid *O.* x *citriodorum.* These species are used for essential oil production and as pot herbs (Demissew & Asfaw, 1994). The genus *Ocimum* is an important essential oil crop with around 100 tonnes of essential oil being produced throughout the World annually. About half of this is produced from *O. basilicum* and close relatives (Lawrence, 1992a). These species are also very important in indigenous systems of medicine (Keng, 1978; Gonzalez-Tejero *et al.,* 1992; Heinrich, 1992; Rivera-Nunez & Obon de Castro, 1992; Githinji & Kokwaro, 1993). India is the largest producer of basil (*O. basilicum*) oil of the World since 1996 (Varshney, 1999).

Ocimum L. is an important medicinal herb, and yet its taxonomy and nomenclature are in bit of muddle (Paton *et al.,* 1999). The most ancient books of medicines of India, namely 'Charaka Samhita' and 'Sushruta Samhita' describe the wonderful curative properties of members of Lamiaceae especially *Ocimum* L., which are attributed to their chemical principles (Gulabkunverha, 1949; Bhishagratva, 1963).

Sweet basil (*Ocimum basilicum* L.) is a perennial shrub which grows in several regions all over the World and is a widely cultivated species (Brophy & Jogia, 1986; Baritaux *et al.,* 1992; Ryding, 1994; Chowdhury, 1999). It is indigenous to lower hills of Punjab and cultivated throughout greater part of India (Lindley, 1981; Ryding, 1994). The essential oil is used in pharmaceutical industries and traditional medicines (Guenther, 1961). It is reported to have stomachic, alexipharmic, anthelmintic,

antipyretic, diaphoretic, carminative, stimulant, diuretic and expectorant properties (Kirtikar & Basu, 1975; CSIR, 1992; Sivarajan & Balachandran, 1994; Chowdhury, 1999). It also possesses insecticidal activity (Deshpande & Tipnis, 1977; Chavan & Nikam, 1982), nematicidal activity (Chatterjee *et al.,* 1982) and antimicrobial properties (Reuneni *et al.,* 1984; Prasad *et al.*, 1986; Thoppil *et al.,* 1998; Koga *et al.*, 1999). It is useful in diseases of the heart and blood, biliousness, leucoderma, itch, chronic pain in the joints, asthma, inflammations and enlarged spleen. The juice is good for toothache, earache, headache and haemorrhage. The roots are used for bowel complaints of children. The leaves with honey are useful in the treatment of croup (Chopra *et al.,* 1956; Kirtikar & Basu, 1975). The seeds are given in infusion for gonorrhoea and chronic dysentery (Chopra *et al.,* 1956; Kirtikar & Basu, 1975; Das & Agarwal, 1991). The cold infusion of seeds is used to assuage the pain of childbirth (Kirtikar & Basu, 1975; Lindley, 1981; Das & Agarwal, 1991). The seeds are used in cases of habitual constipation, piles and also in poultices for sores and sinuses (Das & Agarwal, 1991; CSIR, 1992).

Fresh herb is used as a condiment and spice (Uphof, 1968; Ryding, 1994). Basil oils are widely used in flavouring, including confectionery, baked foods, seasonings, sausages, beverages and in oral care products (Hasegawa *et al.,* 1997). It is used in perfumes, cosmetics and is a part of many fragrance compositions (Darrah, 1980; Jansen, 1981; Brophy & Jogia, 1986; Manniche, 1989; Baritaux *et al.,* 1992).

Attempts have been made in the past to distinguish between varieties of *O. basilicum*. However, there is no clear morphological discontinuity between the varieties, the widespread use of varietal names obscures important information such as variation in chemistry and chromosome number (De Baggio & Belsinger, 1996; Paton *et al.,* 1999).

Paton (1992) places *O. basilicum* in the section *Ocimum* subsect. *Ocimum* together with five endemic African species. Several varieties have been described under *O. basilicum,* among them var. *purpurascens* Benth. is with rather dense inflorescence and purplish bracts (Ryding, 1994). According to Paton and Putievsky (1996) in *O. basilicum* L. var. *purpurascens* Benth. bracts, calyces and corolla are purple or pinkish. Inflorescence is lax and often branched. Var. *thyrsiflorum* (L.) Benth. is very similar to var. *purpurascens* but differs in having a compact inflorescence with

the lateral branches growing similar to the central axis. Intermediates between var. *purpurascens* and var. *thyrsiflorum* also exist. These varieties exist as several distinct chemotypes also (Lawrence, 1992a). Eventhough different varieties of *O. basilicum* were reported (Bentham, 1832; 1848; Darrah, 1974; 1980; Paton, 1992; Ryding, 1994; Paton & Putievsky, 1996), the total number of varieties is still obscure.

O. basilicum L. var. *purpurascens* Benth. is a perennial herb growing in the southern parts of India. The plant is characterized by quadrangular, greenish pubescent and woody stem. The leaves are ovate or elliptic, acute, serrate to entire, ciliolate along the margin, gland dotted, glabrous above and softly pubescent along the mid rib and veins beneath. Inflorescence is a terminal thyrse of about 15-40 cm long. Flowers are white suffused with pink. The calyx is unequally lobed with a pinkish tinge; pubescent outer and hispid within. The corolla is unequally bilipped, upper lip is ovate or rounded in outline and four lobed; each lobe is ovate, obtuse, curved downward with a tuft of long white hairs beneath. The lower lip is oblong in outline, wavy along the margin, boat shaped, pilose outer and glabrous within. Stamens four in number, unequal in size and anthers are bright orange. Ovary is bilocular with ovules on axile placenta. Style is pinkish white with bilobed stigma. Seeds are absent.

Since the present population is sterile, they can only be propagated vegetatively, which has prevented the production of new cultivars by plant breeding. An alternative method for creating new forms of a crop plant is by selecting somaclonal variants from tissue cultured material.

The term somaclonal variation describes any variation that can arise through culture of plant cells, tissues and organs (Larkin & Scowcroft, 1981). Such variation has been observed among regenerants from a large number of species (Karp, 1991; Peschke & Phillips, 1992). Tissue culture induced genetic variation is defined as the variation that arises *de novo* during the period of dedifferentiated cell proliferation that takes place between the culture of an explant and production of regenerants (Munthali *et al.,* 1996).

Although the chromosomal constitution of certain plants seems to be highly stable *in vitro* (Sheridan, 1974), much of the variability found in tissue culture can be directly or indirectly attributed to gross chromosomal changes and chromosomal abnormalities. Chromosomal variation is a common feature of plant tissue cultures

(Patau *et al.,* 1957; Mitra *et al.,* 1960; Mitra & Steward, 1961; Patau & Das, 1961; Partenen, 1963; 1965; Melchers, 1965; Muir, 1965; Kunakh, 1974). The most common changes are increase in ploidy level (Torrey, 1959; 1961; Cooper *et al.,* 1964; Murashige & Nakano, 1966; Nishi *et al.,* 1968; Malnassey & Ellison, 1970; Kochar *et al,* 1971; Sacristan, 1971; Collins *et al.,* 1972; Kasperbauer & Collins, 1972; 1974; Geier & Kohlenbach, 1973; Maretzki & Nickell, 1973; Sharp *et al.,* 1973). Aneuploid changes, typically involving gain or loss of a few chromosomes have also been reported (De Torok & White, 1960; Muir, 1965; Halperin, 1966; Murashige & Nakano, 1966; Shimada & Tabata, 1967; Heinz *et al.*, 1969; Heinz & Mee, 1969; 1971; Norstog *et al.,* 1969; Sacristan & Melchers, 1969; Shimada *et al.,* 1969; Kao *et al.,* 1970; Sacristan & Lutz, 1970; Nickell & Maretzki, 1972; Singh *et al.,* 1972; Bayliss & Gould, 1974; Singh & Harvey, 1975). Structural changes also occur resulting in lagging chromosomes, bridges and multipolar configurations especially after repeated passages in tissue culture (Venketeswaren, 1962; 1963; Cooper *et al.,* 1964; Bayliss, 1973; 1975). A number of other mutation types that are likely to be the cause of such phenotypic variations have also been described including chromosome breakage, single base changes, changes in copy numbers of repeated sequences and alteration in DNA methylation patterns.

In this study computer aided Chromosome Image Analysis System (CHIAS) is employed for karyomorphological analysis. The conventional method of measuring and characterizing chromosome complements by visual evaluation has been subjective and imprecise by nature. These limitations may be overcome by using the CHIAS for direct quantitative assessment of each chromosome. This allows a better knowledge of their cytogenetic constitution (Fukui & Kakeda, 1990; 1994; Fukui & Iijima, 1992; Kamisugi *et al.,* 1993; Fukui & Kamisugi, 1995; Verona & Galaseo, 1995). CHIAS allows an accurate chromosome pairing mainly in those cases where the chromosome sizes are really small (Fukui & Kakeda, 1994). Semi-automatic karyotyping, including numerical data acquisition, pairing and arrangement of chromosomes were also attained, allowing a detailed construct of descriptive data (Fukui, 1988; Fukui & Iijima, 1992).

The value of tissue culture induced variation or somaclonal variation to crop improvement depends on establishing a genetic basis for this variation. Efforts to describe somaclonal variation at the molecular level have revealed the amplification of highly repetitive DNA sequences in cell cultures of different plants (Larkin & Scowcroft,

1981; Durante *et al.*, 1983; Phillips *et al.*, 1990). Recently amplification of DNA using arbitrary 10-base oligonucleotide primers has been described as a way to detect Random Amplified Polymorphic DNA (RAPD) in many eukaryotic organisms (Williams *et al.*, 1990). RAPD markers have been used to develop genetic maps (Reiter *et al.*, 1992; Chaparro *et al.*, 1994) and target genetic markers of isogenic lines (Klein-Lankhorst *et al.*, 1991).

Either RFLP (Restriction Fragment Length Polymorphism) or PCR (Polymerase Chain Reaction) based approaches can be used to identify molecular markers, but PCR represents the faster and easier alternative (Shin *et al.*, 1990). RAPD is the least expensive route to molecular marker development (Williams *et al.*, 1990; Welsh & Mc Clelland, 1990) and offers several advantages over other conventional methods. This technology is technically simple, quick to perform, yields true genetic markers and quick DNA extraction protocols are suitable for RAPD analysis (Rafalski *et al.*, 1993). Moreover this method is non-radioactive and requires only small amount of plant tissue (Chen *et al.*, 1998). RAPDs based on the amplification of multiple random segments of the genome using arbitrary primers provide high levels of polymorphisms, some of which are heritable (Lee *et al.*, 1996). Recent studies have indicated that RAPDs can provide valuable tools for genotype identification, population and pedigree analysis, phylogenetic studies, screening of segregating population of linked markers and genetic mapping (D'Ovidio *et al.*, 1990; Williams *et al.*, 1990; Welsh & Mc Clelland, 1990; Martin *et al.*, 1991; Dweikat *et al.*, 1993; Francisco-Ortega *et al.*, 1993; Szmidt & Wang, 1993; Wilkie *et al.*, 1993; Chaparro *et al.*, 1994; Rowland & Levi, 1994; Yu & Nguyen, 1994).

The RAPD technique uses the Polymerase Chain Reaction (PCR) to amplify random DNA segments using decamer oligonuleotide primers of arbitrary nucleotide sequence. This approach has several advantages compared to RFLP analysis (Williams *et al.*, 1993). Using this method polymorphisms can be revealed, since a single nucleotide change can alter the primer binding site or an insertion or deletion within the amplified region produces a band of different length. Using different primers, the possibilities of genome screening for polymorphisms are enormous (Hormaza *et al.*, 1994).

RAPD markers are useful in studies of genetic diversity but have been used only to limited extent to assess the existence of somaclonal variation in regenerants of

dicotyledonous plants (Isabel *et al.,* 1993; Munthali *et al.,* 1996) and have not yet been used to assess differences among *Ocimum* regenerants. Because some somaclonal variations are epigenetic rather than genetic in nature (Larkin & Scowcroft, 1981), analysis of regenerated plants should also include specific molecular or genetic analysis as well as gross morphological variations (Sabir *et al.,* 1992). The objective of the study presented here is to determine whether RAPD markers could be used to identify differences at the molecular level between *O. basilicum* L. var. *purpurascens* Benth. and its somaclonal variant.

O. basilicum L. is an important economic crop for their essential oils (Gulathi & Sinha, 1989). Essential oils are largely used in food, flavour, cosmetic industry (Thomas *et al.,* 1998) and in pharmaceuticals (Hocking, 1969). Primary use of the essential oils are for flavouring oral preparations, medicinally some oils are applied clinically or at home as skin irritants (Hocking, 1969) and are used in hundreds of preparations such as ointments, syrups, pills etc. (Brud & Gora, 1989). The essential oils are most widely used in aromatherapy (Roebuck, 1988).

The processing of medicinal and aromatic plants involves extraction of pharmaceutically important constituents from the essential oils of perfumery and flavour value (Kahol *et al.,* 1998). There is a great scope for utilization of indigenous essential oil plant resources. The World consumption of essential oils and perfumes is increasing at a very fast rate. In order to improve the herb yield and quality of oil, considerable efforts have been made in some important essential oil yielding plants, including *Ocimum* species (Singh, 1990).

Oils are complex mixtures and clinically they act in different complicated ways. Although we know that certain oil is beneficial in specific medical case there is no rule that similar illness in another patient can be cured with the same result. These effects are different from those achieved with simple chemicals, which are much more convenient for evaluation and used in chemotherapy. Therefore individual chemicals isolated from essential oils are more often used than the oils (Brud & Gora, 1989). So identification of trace components will definitely reveal the quality of oil. Hence Gas Chromatography (GC) or Gas Chromatogaphy-Mass Spectrometry (GC-MS) can be used to evaluate the quality of essential oils.

The present study involves GC-MS analysis of essential oils for quality characters. Mass Spectrometry (MS) differs from other common forms of spectral analysis in that the sample does not absorb radiation from the electromagnetic spectrum. MS is a destructive method of analysis, that is, the sample can not be recovered after MS analysis. MS is highly sensitive and only small quantity of sample is needed. Moreover, MS when coupled with separation technique such as GC or HPLC (High Performance Liquid Chromatography) is a highly specific way to identify organic compounds (Smith & Busch, 1999).

The amount of oil recovered from a leaf correlates well with the number of functional peltate glands on the leaf (Croteau *et al.*, 1981; Yamaura *et al.*, 1989). Reports (Keene & Wagner, 1985; Gershenzon *et al.*, 1989) suggest that terpenes are probably synthesized and accumulated in glandular trichomes. A better anatomical understanding of the secretory gland might aid in the interpretation of the synthesis and storage of the essential oil. The present work includes an attempt to describe trichome morphology of both somatic and somaclonal variant of *O. basilicum* L. var. *pupurascens* Benth. by using Scanning Electron Microscope (SEM).

As *O. basilicum* L. is a pharmaceutically and economically important crop, this study has enormous importance with the tremendous pace of progress in the crop improvement. In this work, efforts have been directed at presenting the different aspects of somaclonal variation with particular emphasis on the essential oil and the genetic principles underlying.

REVIEW OF LITERATURE

Tissue culture technique is becoming increasingly important as a tool for the clonal propagation of desirable plants, for the recovery of plants free from specific diseases and for the production of somatic hybrids. Using culture techniques it is possible to regenerate propagules with better qualities, greater vigour, higher yield and disease resistance. But somaclonal variations are frequently associated with tissue culture regenerants. A number of physiological and morphological changes have been reported in unorganized callus tissue including habituation, changes in biochemical sensitivity and requirements, alteration of growth habit and modification of cellular constituents.

The auxin requirement received considerable attention in early investigations (Gautheret, 1955a; 1955b; 1959). Fox (1963) isolated two strains of tobacco callus, one of which had no requirement for auxin and the other had no requirement for either auxin or KIN from pith explants. Forty clonal lines of tobacco, which varied in their requirement for KIN were isolated by De Marsac and Jouanneau (1972). The variation was reported to be frequent in *Citrus grandis* by Spiegel-Roy and Kochba (1975).

Plant cells in culture express biochemical sensitivities (Chaleff & Carlson, 1974). Carrot resistant to acriflavin (Blakely & Steward, 1964), tomato roots which tolerate low pyridoxine levels (Willemot & Boll, 1959), streptomycin resistant cells of haploid petunia (Binding *et al.*, 1970; Binding, 1972) and plants of tobacco (Maliga *et al.*, 1973) were reported. Cell lines with resistance to inhibitory levels of various aminoacid analogs (Zenk, 1974; Chaleff & Carlson, 1975; Palmer & Widholm, 1975) and sodium chloride (Nabors *et al.*, 1975) were also reported.

Variations in plant tissue culture also include changes in growth habit, rates, appearance and requirements. Callus cultures of *Picea glauca* (Reinert, 1956), pea (Torrey & Shigemura, 1957), tobacco (Sievert & Hildebrandt, 1965), carrot (Blakely & Steward, 1964; Muir, 1965) and wheat (Nakai & Shimada, 1975) have exhibited differences in growth habit with some clones. Nickell and Maretzki (1972) and Nickell (1973) reported differences in growth rate of yellow and white clones of sugarcane. Variability had been reported by Blakely (1965) in growth forms derived from single cells of *Haplopappus gracilis* and *Daucus carota* from the same suspension culture. Three clones of *Atropa belladonna* var. *lutea* isolated from suspension cultures differed in growth rate, nutrient requirements, cellular fine structure and chloroplast pigment content (Davey *et al.*, 1971).

Drastic colour changes are often associated with growth habit changes. Cultures of carrot (Eichenberger, 1950; Mok *et al.*, 1976; Naef & Turian, 1963; Nishi *et al.*, 1974; Sugano *et al.*, 1971), opuntia (Nickell & Tulrcke, 1959) and tobacco (De Marsac & Peaud-Lenoel, 1972) have exhibited variability in pigmentation. Other modifications in cellular constituents include changes in the levels of isozymes of sunflower (Reddy & Stahmann, 1973), *Atropa belladonna* (Simola & Sopanen, 1971; Simola, 1972) and wheat (Nakai & Shimada, 1975).

A general phenomenon of tissue culture is variation in ability to produce embryoids, organs or tissues. Only certain cells of *Cichorium endiva*, *Petroselinum hortense* (Vasil & Hildebrandt, 1966a; 1966b), crown gall infected and normal lines of tobacco (Braun, 1959; De Bouchaud, 1972) as well as sugarcane (Barba & Nickell, 1969) are capable of normal embryogenesis.

Intact plants isolated from tissue culture have been reported to be variant in many cases. Plantlets of sugarcane isolated through tissue culture techniques have shown variation in morphology, chromosome number and enzyme systems. Heinz and Mee (1969) isolated plants with heavier tillering, slower growth rate and increased erectness. Ibrahim (1969) obtained variants in carrot after high KIN treatments *in vitro*. Corduan and Spix (1974) found that altered phenotype in *Digitalis purpurea* plants regenerated from KIN containing medium differed from the parent plant for size and flower morphology.

Geranium (*Pelargonium hortorum*) plants derived from stem and anther culture varied from the parent in variegation, number of petals and stamens and phyllotaxy (Abo El-Nil & Hildebrandt, 1972). The rhizomes of some calliclones of *Coptis* were reported to produce more jatrorrhine than the parent plants (Ikuta *et al.*, 1975).

Widholm (1972a; 1972b) had succeeded in selecting both callus and intact plant lines of carrot, which were resistant to high levels of 5-methyltryptophan. Carlson (1973a; 1973b) had isolated cell and plant lines of tobacco, which were partially resistant to the disease caused by *Pseudomonas tabaci*. Streptomycin resistant tobacco plants had also been selected *in vitro* (Maliga *et al.*, 1973). Coleman (1970) reported the isolation of calliclones of sugarcane resistant to mosaic virus disease from a sensitive cultivar. Old cultures of *Petroselinum hortense* exhibited variation in the development of embryoids (Vasil & Hildebrandt, 1966b). Plantlets derived from callus

cultures of Canadian brome grass mesocotyl (Gamborg *et al.*, 1970) and *Aegilops* anthers (Kimata & Sakamoto, 1972) were completely without pigment.

Chimeral chlorophyll mutants of tobacco (Burk, 1975; Dulieu, 1971; Opatrny & Landa, 1973; 1974) and *Pelargonium* (Kameya, 1976) will segregate albino and green plantlets. *Chrysanthemum* chimeral types have also been separated into homogeneous and new chimeral combination plants in tissue culture. Cytochimeras of *Brassica oleracea* L. (Horak, 1972; Horak *et al.*, 1975) and tobacco (Burk, 1975) have yielded pure plant types of various ploidy levels.

Tobacco plants regenerated from old callus cultures have shown much variation in type (Butenko *et al.*, 1967). Syono and Furuya (1972), found that plantlets regenerated from old tissue cultures were generally weak and could not be readily cultivated into flowering specimens. Those that flowered showed many abnormalities including petaloid pistils, petals at the middle of the corolla, dehiscent corolla, anther adhered to corolla and degenerated anther. Sterility and abnormal pollen grain types were also common. Variation in leaf morphology was also observed.

Karyotype analysis of metaphase chromosomes had been used to determine rearrangements or numerical variation in the chromosomes in somaclones by many workers (Bhojwani *et al.*, 1986). Chromosomal abnormalities, especially chromosome doubling is a common feature associated with tissue culture (Morel, 1971). Calli obtained from root explants of *Zea mays* exhibited chromosomal abnormalities (Mohanti *et al.*, 1986). Ghosh and Sharma (1979) reported chromosomal variation in *Vigna* and *Pisum* cultures. This type of observation was also found in *Vicia* culture (Jha & Roy, 1982). Venketeswaren (1963) demonstrated that *Vicia faba* liquid culture cells displayed a diversity of cytological conditions and nuclear behaviour. Drazena *et al.* (1978) demonstrated the presence of an extra chromosome and various callus lines of *Allium sativum*. A high ploidy level is also typical for cell population of *Allium* callus tissue (Yamane, 1975; Sekerka, 1977a).

Cardi *et al.* (1994) reported cytological variation among leaf regenerants of three accessions of *Solanum commersonii*. Approximately 60% of the regenerated plants were diploids and 40% were tetraploids. Somaclonal variation was reported in grapevines (Bouquet *et al.*, 1982; Fallot *et al.*, 1990; Deloire & Mauro, 1991; Piven *et*

al., 1991).Somaclonal and *in vitro* mutagen induced variability was studied in grapevine and the tetraploids were isolated (Kuksova *et al.*, 1997).

Isozyme analysis has been used extensively to screen for somaclonal variation in plantlets regenerated from organogenic callus (Brettell *et al.*, 1986; Allicchio *et al.*, 1987; Wang & Holl, 1988; Noh & Minocha, 1990) and cultured immature zygotic embryos (Karp *et al.*, 1987; Ryan & Scowcroft, 1987). Eastman *et al.* (1991) surveyed somaclonal variation during spruce embryogenesis using isozyme analysis of embryogenic cultures and somatic embryos. Major *et al.* (1998) compared the isozyme and RAPD analysis to identify the variability among the clones of *Robinia pseudoacacia*.

It may be essential to carry out molecular analysis based on the detection of DNA markers to verify the genetic identity between donor plants and *in vitro* proliferated plants. RAPD markers can be used to obtain rapid information about genetic similarities in micro propagules. Al-Zahim *et al.* (1999) applied PCR based RAPD technique to detect somaclonal variation in garlic, since this method was proved to be effective in a number of other cases such as in *Lolium* (Wang *et al.*, 1993), *Triticum* (Brown *et al.*, 1993), *Picea* (Isabel *et al.*, 1993) and beet (Munthali *et al.*, 1996).

Dolezel and Novak (1985) and Novak *et al.* (1986) had previously shown changes in chromosome number in garlic callus culture. Piccioni *et al.* (1997) estimated somaclonal variation in alfalfa by RAPD finger printing. Variation in clonally propagated *Angelica* plants was analyzed using RFLP and RAPD by Watanabe *et al.* (1998). Yamazaki *et al.* (1993; 1994; 1995) elucidated the molecular genetic relations of *Lupinus* and *Glycyrrhiza* plants by RFLP and RAPD. The study of Hashmi *et al.* (1997) demonstrated the feasibility of using RAPD markers to identify somaclonal variants of peach and provides evidence for the existence of genetic differences among these variants.

The RAPD method has been used to determine the genotype of microspore derived embryos in *Brassica* (Horn & Rafalski, 1992), the genetic variation among androgenic monoploids of diploid *Solanum* species (Singsit & Ozias-Akins, 1993), the genetic composition of anther derived *Hordeum bulbosum* plants (Gudu *et al.*, 1993) and potato (Veilleux *et al.*, 1995). Wallner *et al.* (1996) applied RAPD technique to

compare the field grown and *in vitro* micropropagated plants of *Achillea* species, while Shoyamma *et al.* (1997) used the RAPD to assess the micropropagated clones in *Panax notoginseng*. Similarly from the conservation point of view, Parani *et al.* (1997) used the RAPD to compare the mother plant and *in vitro* grown progenies of *Piper longum* to maintain the fidelity of elite genotype that were to be conserved. The microspore origin of anther culture derived plants of flax was determined by using randomly amplified polymorphic DNA (Chen *et al.*, 1998).

Among the tree species RAPD analysis has recently been applied for the estimation of somaclonal variation in Norway spruce (Heinze & Schmidt, 1995; Fourre *et al.*, 1997), *Picea mariana* (Isabel *et al.*, 1993), white spruce (Isabel *et al.*, 1996), poplar (Rani *et al.*, 1995), and peach (Hashmi *et al.*, 1997). Rival *et al.* (1998) employed RAPD analysis to investigate the genetic fidelity of culture derived regenerants of oil palm. The genetic stability of *in vitro* propagated pines has been reported by Goto *et al.* (1998). Munthali *et al.* (1996) have applied RAPD technology to analyze somaclones of the dicotyledonous crop plant, fodder beet. Mezencev *et al.* (1997) used RAPD markers to detect genetic change in protoplast derived rice plants. The extent of somaclonal variation at the DNA level has been assessed in tissue cultures and primary regenerants of rice (Yang *et al.*, 1999).

Many species of *Ocimum* serve as a natural source of various essential oils. Particular attention should be given to basil oil since it is widely used in pharmaceutical and perfumery industries. The chemical composition of *O. basilicum* has been the subject of considerable study (Lawrence 1978; 80; 82; 86a; 86b; 88; 89; 90; 92a; 92b; 98). There are distinct variations in the major components within the species (Peter & Remy, 1978).

The chemistry of European sweet basil oil was investigated more than a century ago by Bonastre (1831), later by Dupont and Guerlain (1897; 1898) and by Bertram and Walbaun (1897). In 1961, Guenther reported that there were four chemical varieties of basil oil. Pogany (1967) analyzed essential oil of *O. basilicum* using GC-MS.

The essential oil constituents of *O. basilicum* were analyzed using infrared spectrum (Lawrence *et al.*, 1971). They identified about 66 constituents from the oil. In 1972, Lawrence *et al.* for the first time isolated trans-ocimene oxide, germacrene D, caryophyllene oxide and T-cadinol from the oil of *O. basilicum* of Thai origin. Georgiev

and Genov (1973) analyzed Bulgarian sweet basil oil constituents. In the same year Zola and Garnero (1973) compared the chemical composition of Italian, French and Moroccan basil oils. In 1974, Karawaya *et al.* compared the chemical composition of *O. basilicum* oil with that of *O. rubrum* oil of Egyptian origin. Terhune *et al.* (1974) used MS, NMR and hydrogenation studies to structurally elucidate 1-epibicyclosesqui phellandrene as a trace component in basil oil. Skrubis and Markakis (1976) studied the variation in chemical composition of essential oil of basil with photoperiods. Using the GC-MS, Masada (1976) reported the oil constituents of *O. basilicum*. Lang and Horster (1977) reported the isolation of chemical components from tissue cultures of *O. basilicum*. In 1977, Conan examined the chemical composition of three different commercial types of basil oils.

Peter and Remy (1978) reported the chemical composition of a number of commercial basil oils. A description and history of the production of basil oil in Thailand was presented by Pichitakul and Chomchalow (1978). In 1980, Lawrence *et al.* reported that the chemical composition and morphological characteristics of *O. basilicum* were very variable. Huang *et al.* (1981) identified different components from the basil oil of Chinese origin. Fleisher (1981) examined the essential oil of two different strains of *O. basilicum* raised in Israel. Two years later, Cheng and Liu (1983) compared the chemical composition of the leaf, stem and flower oils of *O. basilicum* and *O. basilicum* var. *minimum* L. Modawi *et al.* (1984) found that the essential oil of two strains of *O. basilicum* var. *thyrsiflorum* collected from Sudan contained same compounds but their proportion varied. In 1985, Lemberkovics and Verzar-Petri used GC to analyze the essential oil sample of basil.

In 1977, Gulati examined the chemical composition of Indian *O. basilicum* oil, which was rich in methylchavicol. Takahashi *et al.* (1979) characterized a trace amount of mint sulphide in a sample of basil oil. The chemical composition of the oils of *O. basilicum* obtained from 26 different seed sources had been reported by Lawrence *et al.* (1980). Randriamiharisoa and Gaydou (1985) examined a variety of oils of *O. basilicum*. In 1985, Tapanes *et al.* analyzed the chemical composition of lab distilled *O. basilicum* oil obtained from plant materials raised in an experimental garden in Havana. Brophy and Jogia (1986) reported the analysis of two oils of *O. basilicum* produced from plant material grown locally in Fiji. In the same year Srinivas (1986) reported the oil composition of basil flowers. In 1986b, Lawrence analyzed the steam-distilled oil of *O. basilicum* using GC. Randriamiharisoa *et al.*

(1986) examined the chemical composition of 28 different samples of Madagascan basil oil using GC and GC-MS. In 1986, Kartnig and Simon compared the variation in linalool, methyl chavicol and eugenol from the oils of seven accessions of *O. basilicum* harvested at three different times.

In 1987, Boniface *et al.* used discriminant analysis to differentiate between basil oils of different origins. Berrada *et al.* (1987) used retention time data to examine the composition of lab-distilled oil of *O. basilicum* obtained from plant material found growing wild in Morocco. Ekundayo *et al.* (1987) used GC-MS to analyze the chemical composition of two basil oils produced from *O. basilicum* growing at two locations in Nigeria. Murugesan and Damodaran (1987) reported a chemotype of *O. basilicum*, the oil of which was rich in linalool and virtually devoid of 1, 8-cineole and eugenol. Nykanen and Nykanen (1987) examined the variability in the chemical composition of basil oil produced from fresh and dried plants. Using GC-MS Tsai and Sheen (1987) compared the chemical composition of the oil obtained from leaves, flowers and stem of *O. basilicum*. In 1988, Chien described a computer data base assisted method of analysis and its use in determining the chemical composition of basil oil. In the same year Lawrence (1988) reported the chemical composition of a number of commercial sample of methyl chavicol rich basil oils. Lawrence (1989) reported the results of the chemical composition of more than 200 analyses of basil oils produced from plants cultivated in North America.

In 1989, Akgul examined the chemical composition of oil of *O. basilicum* produced from dried leaves obtained from plant material that was cultivated in Erzurum region (E. Tukey). Tateo (1989), Tateo and Verdio (1989) and Tateo *et al.* (1989) analyzed a number of different basil oils and extracts. Gaydou *et al.* (1989) described the sequiterpene hydrocarbon composition of Madagascan basil oil. The composition of the essential oil of basil grown under various conditions in Finland was analyzed by capillary gas chromatography and mass spectrometry and two chemotypes were identified. The essential oil of one chemotype was high in linalol and estragole whereas, the oil of the other was high in linalool and eugenol (Nykanen, 1989). Skaltsa and Philianos (1986) isolated and identified cafeic acid and aesculin from alcoholic extracts of the leaves of sweet basil. The ether extract of the leaves of *O. basilicum* contains p-coumaric acid and aesculin (Skaltsa & Philianos, 1990). Gulati and Sinha (1989) studied chemical composition of three varieties of *O. basilicum* such

as *O. basilicum* (methyl cinnamate type), *O. basilicum* (methyl chavicol type) and *O. basilicum* (eugenol type).

An year later Manninen *et al.* (1990) compared the major components of two basil chemotypes, which were analyzed by capillary gas chromatography. Capillary GC analysis of the essential oil of *O. basilicum* var. *canum* showed that it belongs to the methyl cinnamate type. Analysis proved that it contains 46-72% methyl cinnamate (Fun & Svendsen, 1990). In 1991, Mariani *et al.* compared the head space volatiles of three Italian cultivars of basil using GC-MS. Sheen *et al.* (1991) used preference ranking to evaluate the aroma of the oil obtained from various parts of a single cultivar of basil grown in Taiwan. The volatile components obtained by steam distillation of *O. basilicum* var. *hispidum* were identified and the main component was dihydrotagetone (82%) (Ruberto *et al.*, 1991). Singh and Bordoloi (1991) identified methyl cinnamate as the major essential oil component in *O. basilicum.* In 1991, Schubert and Mosandl determined the enantiomeric distribution of linalool in two commercial samples of sweet basil oil. Bobin *et al.* (1991) examined the changes in the aroma of basil leaves harvested at an optimum time and stored over various periods of time. In 1992, Fleisher and Fleisher analyzed the chemical composition of methyl cinnamate/linalool rich basil oil produced from plant grown in Israel. Baritaux *et al.* (1992) studied the effects of drying and storage of the plant materials of *O. basilicum* on essential oil composition. The principal components found in the essential oil were methyl chavicol, eugenol, linalool and 1,8-cineole. The content of methyl chavicol and eugenol was found to be decreased after drying and storage. Hussain *et al.* (1990) and Lemberkovics *et al.* (1993) reported the presence of monoterpenes in *O. basilicum* oils.

Gupta (1994) examined the main components of the oils of an original accession, self-crosses and F_1-F_4 crosses. Riaz *et al.* (1994) analyzed oils from five different methyl chavicol rich cultivars of *O. basilicum* that were produced from plants grown in five different locations in Pakistan. In 1995, Retamer *et al.* analyzed five samples of basil oil produced from plants grown in Sante Fe (Argentina). DiCesare *et al.* (1995) used GC-MS to analyze aroma isolate from basil grown in Italy. Hethelyi *et al.* (1995) analyzed essential oil composition of three cultivars of basil grown in Greece. In 1995, Nianga *et al.* analyzed oils produced from basil grown in a variety of locations of different climate in Guinea. The chemical composition of *O. basilicum* var. *glabratum* produced from Turkey has been reported (Perez-Alonso *et al.*, 1995).

By using GC and GC-MS, Chalchat *et al.* (1996) determined two chemotypes of *O. basilicum* from Mali. A comparison on major essential oil components of oils produced from eight varieties of *O. basilicum* was made by Thoppil (1996). Also in 1996, Marotti *et al.* compared the composition of the oils produced from Italian cultivars of basil. The microwave assisted hydrodistillation was used by Thach *et al.* (1996) as a lab technique to isolate oil from basil grown in Vietnam. Lemberkovics *et al.* (1996) found that the essential oil content of basil grown in Hungary reached a maximum at the full flowering stage. Essential oil from nine accessions of *O. basilicum* var. *basilicum*, three accession of *O. basilicum* var. *purpurascens*, one accession of intermediate between var. *basilicum* and var. *purpurascens* and one accession of *O. basilicum* var. *difforme* and two Dark Opal cultivars were analyzed by Grayer *et al.* (1996). A volatile concentrate of basil that was produced by simultaneous distillation and extraction was analyzed by GC and GC-MS (Venskutonis *et al.*, 1996). In 1996, Mizrahi *et al.* compared the composition of oils of Argentinean cultivars of *O. basilicum* with those of imported cultivars. Lachowicz *et al.* (1996) compared the composition of oils produced from frozen fresh leaves and dried leaves of three Australian basil cultivars. Chemical composition of the essential oil of *O. basilicum* var. *purpurascens* from tissue cultured plants have been reported (Gupta, 1996).

Lachowicz *et al.* (1997) compared the oil yield, composition and supercritical extract composition of five cultivars of basil grown in Australia. In 1997, Pasquier and Chalchat analyzed basil oil produced from plant types of various origins. Chalchat *et al.* (1997) also analyzed three oils of *O. basilicum* produced from plants grown in three different locations in Benin. Pallado *et al.* (1997) described analysis of supercritical fluid CO_2 extract of ground basil leaves using GC-MS and compared it with a steam distilled oil and a petroleum ether soxhlet extract of the same collection of basil leaves. Ravid *et al.* (1997) examined the distribution of linalool enantiomers in basil oil using chiral GC analysis. During 1997, Hasegawa *et al.* used GC-MS for component identification of oils produced from nine different cultivars of basil grown in the Philippines. The water produced during the steam distillation of basil in India was analyzed by Machale *et al.* (1997). The air dried ground leaves of commercially available *O. basilicum* were subjected to supercritical CO_2 extraction by D'Alpaos *et al.* (1997). Nacar and Tansi (1997) determined essential oil yields and components of different basil cultivars grown in the Mediterranean regions of Turkey.

The composition of essential oil of *O. basilicum* was described by GC and GC-MS (Baratta *et al.*, 1998). The major component identified was estragole (86.1%). Silva *et al.* (1998) investigated the essential oils from the leaves and inflorescence of *O. basilicum* var. *purpurascens*. The main constituent was found to be linalool. Essential oils from leaves of *O. basilicum* collected from different areas of Benin were analyzed by GC-MS and two chemotypes were identified (Moudachirou *et al.*, 1999). The effect of drying methods on the relative abundance of major flavour volatiles, rehydration rate, colour and structural integrity of volatile components of *O. basilicum* was evaluated by Yousif *et al.* (1999). Eunjoo and Woo (1999a) studied the composition and content of essential oil in hydroponically grown basil at different seasons. They also studied the effect of magnesium on the content and composition of essential oil of basil cultivars grown in hydroponics. (Eunjoo & Woo, 1999b). In a study Chalchat *et al.* (1999) described the chemical composition of essential oils from 24 samples of *O. basilicum* and 53 components were identified. In 1999 Johnson *et al.* examined the effect of UV-B treatment on the essential oil composition of sweet basil.

The effect of different harvesting times on essential oil yields and components of different basil cultivars grown in Cukurova region (Turkey) was reported by Nacar and Tansi (2000). Tansi and Nacar (1999; 2000) analyzed essential oil components in leaves and flowers of *O. basilicum* var. *citriodorum* by GC. Vieira and Simon (2000) reported chemical characteristics of basil found in the markets and used in traditional medicine in Brazil. The effect of drying on the concentration of the volatile compounds of *O. basilicum* was also reported (DiCesare et al., 2000).

Labiates carry a great diversity of epidermal hairs, many of which are non-glandular (El-Gazzar & Watson, 1970; Werker *et al.*, 1985b). Variation in morphology of glandular trichomes within Labiatae had been reported (Bruni & Modenesi, 1983; Venkatachalam *et al.*, 1984; Werker *et al.*, 1985b; Dudai *et al.*, 1988), but most were either capitate or peltate (Amelunxen, 1964; 1965).

Morphology and distribution of trichomes in different parts of different species of *Teucrium* were studied by Maleci and Servettaz (1991); Maleci *et al.*, (1992; 1995) and Servettaz *et al.* (1992; 1994). Ultrastructure of glandular trichomes of *Teucrium scorodonia* L. have been reported (Sevinate-Pinto & Antunes, 1991). The trichome morphology and phytochemical characters of *Thymus* were studied by Maleci *et al.*,

(1997a; 1999). Modenesi *et al.* (1984) described development and secretion of clubbed trichomes in *Thymus vulgaris*. Various aspects of glandular hair development and essential oil secretion in *Origanum* species have been discussed (Bosabalidis & Tsekos, 1982a; 1982b; 1984). Werker *et al.* (1985a) reported types and distribution of glandular hairs in four chemotypes of *Origanum vulgare* L. The morphology and distribution of trichomes in *Stachys germanica* were published by Falciani *et al.* (1995). Analysis of trichome in *Rosmarinus officinalis* was made by Maleci *et al.* (1997b).

Numerous kinds of hairs on the surface of leaves of different *Ocimum* had been described (Blaque, 1923; Guillaumin, 1930; Dro & Hefendehl, 1973; Gupta & Bhambie, 1979). Foliar appendages of *Ocimum gratissimum* were described by Colson *et al.* (1991). The taxonomical significance of the epidermal characters in the genus *Ocimum* have also been reported (Olowokudejo & Sheteolu, 1988). In 1993, Werker reviewed different types of glandular trichomes in Lamiaceae. In the same year Werker *et al.* (1993) reported the correlation between development of glandular hairs and the oil secreted by hairs in *O. basilicum.*

Previous reports on chromosome number and essential oil composition of *O. basilicum* L. are presented in the table A and table B respectively.

Table. A. PREVIOUS REPORTS ON CHROMOSOME COUNTS OF *OCIMUM BASILICUM* L.

Name of the Taxa	Chromosome No.	Authority
Ocimum basilicum L.	2n=48	Vaarama, 1947
	,,	Morton, 1962
	,,	Mehra & Gill, 1972
var. *thyrsiflorum*	,,	
var. *purpurascens*	,,	
var. *glabratum*	,,	
var. *minimum*	,,	Pushpangadan *et al.*, 1975
O. basilicum L.	,,	Sanjappa, 1979
var. *pilosum*	2n=68	Cherian & Kuriachan, 1981
var. *glabratum*	2n=52	
var. *thyrsiflorum*	,,	Krishnappa & Basavaraj, 1982
O. basilicum L.	2n=48	
var. *purpurascens*	,,	
var. *minima*	,,	Pushpangadan & Sobti, 1982
O. basilicum L.	,,	Sobti & Pushpangadan, 1982
	2n=52	
	2n=48	Singh & Sharma, 1983
	,,	Ma *et al.*, 1984
	2n=30	Vembu, 1984

O. basilicum	2n=52	
	2n=48	Singh, 1985;,1987
var. *glabratum*	,,	
var. *pilosum*	,,	
var. *purpurascens*	,,	
var. *thyrsiflorum*	,,	Thoppil & Jose, 1994
O.basilicum L.	2n=56	
	2n=52	
	2n=72	
	2n=74	
var. *purpurascens*	2n=50	
	2n=52	
var. *difforme*	2n=53	
	2n=52	Paton & Putievsky, 1996

Table. B. PREVIOUS REPORTS ON CHEMICAL COMPONENTS IN *OCIMUM BASILICUM* L.

Name of the Taxa	Parts used	Chemical component	Authority
Ocimum basilicum L.	Herb	Methyl chavicol Linalool	Hirschosohn, 1893.
	,,	Methyl chavicol Linalool	Dupont & Guerlain, 1896.
	,,	α-pinene 1,8-cineole Camphor Methyl chavicol	Bertram & Walbaum, 1897.
	,,	Methyl chavicol	Laloue, 1912.
	,,	Methyl chavicol	Pigulevskii, 1916.
	,,	Methyl chavicol 1, 8-cineole Eugenol Trans- ocimene	Gildemeister & Hoffmann, 1922.
	,,	Methyl chavicol Linalool 1, 8-cineole Eugenol	Nelson, 1935.
	,,	Methyl chavicol Linalool 1,8-cineole Eugenol Methyl cinnamate	Bonaccorsi, 1936.
	,,	Methyl cinnamate	Sorgonna-Luisi, 1936.
	,,	Methyl cinnamate	Rakshit, 1938.
	,,	Eugenol Camphor	Markov, 1940.
	,,	Linalool Methyl cinnamate	Nigam & Dutt, 1946.

O. basilicum L.	Herb	Methyl chavicol Linalool Eugenol	Finney & Warriner, 1947.
	,,	Methyl chavicol Linalool	Khorana & Vangikar, 1950
	,,	Methyl chavicol	Naves, 1950.
	,,	Linalool	Brown & Matthews, 1952/53.
	,,	Methyl chavicol Linalool Methyl cinnamate Trans -ocimene	Dinghra *et al.*, 1954.
	,,	Linalool Methyl cinnamate Linalyl acetate	Handa *et al.*, 1955.
	,,	Linalool Linalyl acetate	Handa *et al.*, 1957.
	,,	Methyl chavicol Linalool 1, 8-cineole Camphor	Pogany *et al.*, 1957.
	,,	Methyl chavicol Linalool Linalyl acetate	La Bruto & Calvarano, 1959.
	,,	Methyl chavicol Linalool	Sacco, 1960.
	,,	Linalool	Hafez *et al.*, 1964.
	,,	Methyl chavicol Linalool 1, 8-cineole Eugenol Geraniol Myrcene p-cymene	Ivanov *et al.*, 1964.

Ocimum basilicum L.	Herb	Methyl chavicol Linalool 1, 8-cineole Eugenol Methyl cinnamate Geraniol α-terpineol Citronellol Safrol Citral	Horhammer *et al.*, 1964.
	,,	Methyl chavicol Linalool 1, 8-cineole Eugenol Geraniol Myrcene α-terpineol p-cymene	Tchorbadziev *et al.*, 1964/65.
	,,	α-pinene Camphene β-pinene Myrcene Limonene Ocimene 1, 8-cineole α-terpineol Linalool Camphor Methyl chavicol p-methoxy-benzaldehyde Bornyl acetate Methyl eugenol p-methoxy-cinnamaldehyde	Pogany, 1967.
	,,	Linalool 1, 8-cineole Trans-ocimene Camphor α-terpineol α-pinene Camphene β-pinene Limonene Bornyl acetate Methyl eugenol	Pogany *et al.*, 1968.
	,,	Methyl chavicol Linalool 1, 8-cineole Methyl cinnamate Trans- ocimene	Nigam *et al.*, 1970.

O. basilicum L.	Herb	Geraniol α-pinene p-cymene Limonene Borneol β-farnesene Nonanal Methyl heptanone	Nigam *et al.*, 1970.
	,,	α-pinene (0.4%) Camphene (0.1%) δ-3-carene (0.2%) Myrcene (0.1%) α-terpinene (0.1%) Limonene (0.2%) 1,8-cineole+Cis-ocimene (2.4%) γ-terpinene (0.1%) 3-octanone+Trans-ocimene (1.0%) Fenchyl acetate (0.6%) Copaene (0.2%) Linalool+Camphor (0.7%) Linalyl acetate (0.1%) Fenchyl alcohol (0.5%) Caryophyllene + Terpinen-4 ol (2.4%) Methyl chavicol (87.8%) α-terpineol (0.1%) Citronellol (0.3%) Methyl eugenol (1.6%) Eugenol (0.5%)	Lawrence *et al.*, 1971.
	,,	Methyl chavicol (88.2%) Trans-ocimene oxide Germacrene D Caryophyllene oxide T-cadinol	Lawrence *et al.*, 1972.
	,,	Linalool (50-63%) Bornyl acetate (4-10%) Geraniol (6-8%) Methyl chavicol (5%) Nerol (3%) Eugenenol (3%)	Georgiev & Genov, 1973.
	,,	α-pinene (0.11-0.35%) Camphene (0.02-0.09%) β-pinene (0.21-0.67%) δ-3-carene (0.06-0.33%) Myrcene (0.16-0.68%) α-terpinene (tr.)	Zola & Garnero, 1973.

Ocimum basilicum L.	Herb	Limonene (0.12-0.26%) 1, 8-cineole + Cis-ocimene (2.70-8.10%) p-cymene (tr.) Terpinolene (0.07-0.22%) Cis-allo-ocimene (tr.) Menthone (tr.) Fenchyl acetate (0.07-0.41%) Linalool (39.10-43.80%) Caryophyllene+ Terpinen-4 ol (0.80-1.00%) Methyl chavicol (2.60-31.80%) Geraniol (0.05-0.39%) Methyl eugenol (0.07-0.80%) Methyl cinnamate (0.16-0.50%) Eugenol (3.40-19.20%)	Zola & Garnero, 1973.
	,,	α-pinene (1.32%) β-pinene (0.80%) Ocimene (7.44%) Linalool (37.60%) Linalyl acetate (2.40%) α-terpineol (0.50%) Methyl chavicol (33.80%) Benzyl acetate (1.63%) Phenyl ethyl alcohol (1.36%) Nerolidol (0.16%) Farnesol (0.70%) Geranyl acetate (0.84%) Eugenol (1.02%) Isoeugenol (0.75%)	Karawaya *et al.*, 1974.
	,,	α-pinene (0.07-0.29%) β-pinene (0.19-0.37%) β-myrcene (0.52-1.24%) D-3-carene (1.96-2.87%) β-phellandrene (0.26-0.46%) Linalool (80.70-87.30%) Linalyl acetate (0.86-2.48%) Valencene (1.06-2.01%)	Skrubis & Markakis, 1976.

Ocimum basilicum L.	Herb	α-pinene Camphene β-pinene Myrcene Limonene 1,8-cineole Trans-ocimene 3-octanone p-cymene Camphor Linalool Bornyl acetate Caryophyllene Terpinen -4 ol Methyl chavicol α-terpineol Citronellol Eugenol Methyl cinnamate	Masada, 1976.
	,,	α-pinene (0.11-0.47%) Camphene (0.02-0.10%) β-pinene (0.07-0.83%) Myrcene (0.12-0.80%) Limonene (2.04-9.34%) Cis-ocimene (0.03-2.52%) p-cymene (0.05-0.16%) Cis-3-hexanol (0.02-0.08%) Fenchyl acetate (0.09-0.55%) Camphor (0.37-1.43%) Linalool (1.16-54.37%) Fenchyl alcohol (1.20-9.51%) Methyl chavicol (2.38-85.76%) α-terpineol (0.83-1.90%) Citronellol (0.65-3.69%) Geraniol (0.03-0.38%) Methyl cinnamate (0.05-0.34%) Eugenol (0.74-12.19%)	Conan, 1977.
	,,	α-pinene (0.04%) Camphene (tr.) β-pinene (tr-0.02%) Myrcene (0-0.11%) p-cymene (tr.) 1, 8-cineole (0-0.06%) Terpinolene (0-0.74%) Linalool (10.15-28.22%)	Gulati, 1977.

O. basilicum L.	Herb	Citronellal (tr.) Camphor (0-0.03%) Borneol (0-tr.) Nerol+Geranial (0-0.20%) Geraniol (0-0.02%) Neral (0-0.02%) Anethole (0-0.19%) Methyl chavicol (68.22-86.72%) Isobornyl acetate (0-tr.) Thymol (0-tr.) Eugenol (0-0.15%) Methyl eugenol (0-0.05%) Isoeugenol (0.04-0.43%) Methyl isoeugenol (0-0.43%) Aceto eugenol (0-tr.) β-caryophyllene (0.02%) α-humulene+ Farnesene (tr.-0.29%) γ-cadinene (0-tr.)	Gulati, 1977.
	,,	Linalool Borneol Thymol Eugenol	Lang & Horster, 1977.
	,,	Cis-ocimene+1,8-cineole (tr-13.60%) Linalool (0.20-75.40%) Methyl chavicol (0.30-88.60%) Methyl cinnamate (tr-15.50%) Eugenol (tr-11.20%)	Lawrence *et al.*, 1980
	,,	Methyl chavicol Linalool 1, 8-cineole Ocimene Linalyl acetate Eugenol 1-epibicyclo- sesquiphellandrene Menthol Menthone Cyclohexanol Cyclohexanone Myrcenol Nerol	Huang *et al.*, 1981.

O. basilicum L.	Herb	Linalool (40.5%) Methyl chavicol (28.90%) Eugenol (10.2%) 1,8-cineole (8.5%) Monoterpene hydrocarbon (2.0%)	Fleisher, 1981.
	Leaf	α-pinene (0.28%) Camphene (0.01%) β-pinene (1.18%) Sabinene (0.33%) Myrcene (1.17%) α-terpinene (0.04%) Limonene (0.69%) β-phellandrene (0.10%) 1,8-cineole (8.05%) γ-terpinene (0.37%) Ocimene (4.02%) p-cymene (0.07%) Terpinolene (0.09%) Citronellal (0.01%) Linalool (6.78%) Methyl thymol (0.44%) Caryophyllene (6.06%) Methyl chavicol (35.03%) Citronellyl acetate (0.14%) Neral (0.03%) α-terpineol (0.12%) α-terpinyl acetate (1.15%) Geranial (0.72%) Methyl eugenol (30.53%) Eugenol (0.35%)	
var. *minimum*	,,	α-pinene (0.22%) β-pinene (0.27%) Camphene (0.01%) Sabinene (0.01%) Myrcene (0.57%) α-terpinene (0.01%) Limonene (0.46%) β-phellandrene (0.01%) 1,8-cineole (2.00%) γ-terpinene (0.18%) Ocimene (1.56%) p-cymene (0.01%) Terpinolene (0.04%) Linalool (0.74%) Methyl thymol (0.14%) Caryophyllene (5.85%) Methyl chavicol (17.72%) Citronellyl acetate (0.07%) Neral (0.35%) α-terpineol (0.15%) α-terpinyl acetate (1.12%) Geranial (0.88%) Methyl eugenol (64.25%) Eugenol (0.44%)	Cheng & Liu, 1983.

O. basilicum L. var. *thyrsiflorum*	Herb	Methyl chavicol (40%) Linalool (40%) Eugenol+1, 8-cineole (20%)	Modawi *et al.*, 1984.
O. basilicum L.	,,	α-bergamotene α-cedrene γ-muurolene γ-cadinene α-bisabolol	Lemberkovics & Verzar-Petri, 1985.
	,,	α-pinene (0.04-0.28%) Camphene (0.01-0.04%) β-pinene (0.14-0.45%) δ-3-carene (0.07-0.15%) Myrcene (0.06-0.15%) Limonene (0.21-0.32%) 1, 8-cineole (2.55-4.43%) (Z)-ocimene (0.03-0.07%) (E)-ocimene (0.58-1.17%) α-fenchyl acetate (0.21-0.45%) Linalool (1.12-2.72%) α-fenchyl alcohol (0.01-1.34%) β-caryophyllene (0.09-0.95%) Borneol (0.01-0.30%) Methyl chavicol (81.6-86.2%) Geraniol (0.01-1.46%) Methyl eugenol (0.91-3.99%)	Randriamiharisoa & Gaydou, 1985.
	,,	α-pinene (0.44%) Camphene (0.08%) β-pinene (1.60%) Myrcene (1.03%) 1, 8-cineole+limonene (15.52%) Trans-ocimene (1.61%) Cis-ocimene (0.20%) Camphor (0.88%) Linalool (10.03%) Methyl chavicol (56.58%) α-terpinyl acetate (0.65%) α-terpineol (2.39%) Borneol (0.30%) α-humulene (0.44%)	Tapanes *et al.*, 1985.

O. basilicum L.	Herb	Myrcene (tr.) α-terpinene+ 1,8-cineole (tr.) Trans-ocimene+ Terpinolene(tr.) Fenchone (tr.) α-fenchyl acetate (tr.) α-fenchyl alcohol (tr.) α-cubebene (tr.) Linalool (22.3%) β-elemene+ β-caryophyllene+ Aromadendrene (4.5%) Methyl chavicol (tr.) δ-terpineol (tr.) α-humulene (tr.) α-terpineol (tr.) Bicyclogermacrene (tr.) γ-cadinene (tr.) (Z)-methyl cinnamate (4.5%) Methyl eugenol (24.7%) (E)-methyl cinnamate (23.6%) Eugenol (3.2%)	Brophy & Jogia, 1986.
	Flower	1,8-cineole (3.62-5.91%) Linalool (62.31-62.37%) Eugenol (7.76-8.62%) Methyl chavicol (26.79%) (Z)-methyl cinnamate (5.68%) (E)-methyl cinnamate (22.54%)	Srinivas, 1986.
	Herb	α-pinene (0.04-0.28%) Camphene (tr.-4.0%) β-pinene (0.14-0.45%) Sabinene (0.07-0.17%) δ-3-carene (0.07-0.17%) Myrcene (0.06-0.17%) Limonene (tr.-0.32%) 1,8-cineole (2.55-4.43%) (Z)-ocimene (tr.-0.08%) (E)-ocimene (0.58-1.25%) p-cymene (tr.-0.05%) Terpinolene (tr.-0.05%) (Z)-allo ocimene (tr.-0.05%) Fenchone (tr.-0.22%) α-fenchyl acetate (0.18-0.45%)	Randriamiharisoa *et al.*1986.

O. basilicum L.	Herb	α-thujone (0.07-0.22%) β-caryophyllene (0-1.13%) β-elemene (0-0.93%) Bornyl acetate (0-0.67%) Borneol (tr.-4.35%) Methyl chavicol (73.9-87.3%) Terpinen-4 ol (0.11-0.30%) Geranyl acetate (0.22-0.44%) Geraniol (tr.-1.63%) Methyl eugenol (0.87-4.16%) Anisaldehyde (tr-0.10%) Eugenol (0.35-0.58%) p-methoxy-cinnamaldehyde (0.05-0.42%) Linalool (0.97-2.72%) Camphor (tr.-0.37%) α-fenchyl alcohol (tr.-1.34%)	Randriamiharisoa *et al.*1986.
	,,	Linalool (62.2-65.1%) Methyl chavicol (3.8-4.7%) Eugenol (2.0-2.6%)	Kartnig & Simon, 1986.
	,,	1, 8- cineole Cis- ocimene Linalool Methyl chavicol Methyl cinnamate Eugenol	Boniface, 1987.
	,,	1, 8-cineole (3.2%) Camphor (1.1%) Linalool (34.2%) Methyl chavicol (0.8%) Eugenol (2.0%) Trans-methyl cinnamate (38.5%) Thymol (1.2%)	Berrada *et al.*, 1987.
	,,	α-pinene (0.16%) β-pinene(0.36%) Sabinene (0.15%) Myrcene (0.11%) Limonene (0.09%) 1,8-cineole (3.09%)	Ekundayo *et al.*, 1987.

O. basilicum L.	Herb	5-methyl-2-heptanone (0.01%) p-cymene (0.01%) 1-octen-3 ol (0.06%) Linalool (0.33%) Octanol (0.11%) Terpinen-4 ol (0.05%) α-guaiene (0.30%) β-caryophyllene (0.89%) Methyl chavicol (84.09%) α-terpineol (0.30%) α-humulene (0.54%) γ-guaiene (0.43%) β-bisabolene (1.72%) Geranyl acetate (tr.) Calamenene (0.02%) Geraniol (0.41%) Caryophyllene oxide (0.54%) Methyl eugenol (0.02%) Anisaldehyde (0.14%) Nerolidol (tr.) Spathulenol (0.04%) Eugenol (0.03%) Thymol (0.07%) β-eudesmol (tr.) p-methoxy-cinnamaldehyde (0.67%) p-methoxycinnamyl alcohol (0.54%)	Ekundayo *et al.*, 1987.
	,,	Linalool	Murugesan & Damodaran, 1987.
	,,	1,8-cineole (2.30-8.6%) Linalool (2.60-56.2%) α-fenchyl alcohol (0-1.1%) Methyl chavicol (0-77.1%) Chavicol (0.1-1.2%) (Z)-methyl cinnamate (0-1.7%) Eugenol (0-41.0%) (E)-methyl cinnamate (0-12.0%) Methyl eugenol (0.04-1.1%) β-elemene (0-0.4%) β-caryophyllene (0-0.9%) α-bergamotene (0-0.6%) Germacrene D (0.1-1.1%) T-cadinene (0.1-0.8%) δ-cadinol (0.3-5.4%)	Nykanen & Nykanen, 1987.

O. basilicum L.	Leaf	α-pinene (0.13%) Camphene (0.35%) Sabinene (0.22%) Myrcene (0.13%) Limonene (0.02%) α-phellandrene (0.08%) 1, 8- cineole (5.47%) (Z)-β-ocimene (1.33%) Hexanol (tr.) 3-hexenol (0.03%) 3-octanol (0.02%) 6-methyl-3-heptanone (0.17%) Sabinene hydrate (0.01%) Terpinolene (tr.) (Z)-allo-ocimene (0.02%) Linalool (0.37%) β-copaene (0.01%) β-elemene (0.90%) Methyl chavicol (84.69%) β-selinene (0.54%) α-terpineol (0.90%) α-cubebene (0.66%) Germacrene B (0.01%) β-gurjunene (0.08%) (E)-anethole (0.01%) Methyl eugenol (2.44%) β-himachalene (0.13%) Viridifloral (0.02%) β-patchoulene (0.09%) Cedrol (0.02%)	Tsai & Sheen, 1987.
	Herb	α-thujene (0.14%) Benzaldehyde (0.11%) α-pinene (0.36%) Camphene (0.02%) β-pinene (0.26%) 3-hexenyl acetate (0.02%) Benzyl alcohol (0.63%) p-cymene (0.65%) 1,8- cineole (5.24%) Limonene (3.61%) Benzyl formate (0.18%) Linalool oxide (0.32%) Fenchone (0.14%) Linalool (1.34%) α-fenchyl alcohol (0.17%) Camphor (0.50%) Borneol (0.09%) p-cymen-8 ol (0.16%) Estragole (83.01%) Octyl acetate (0.06%) α-fenchyl acetate (0.29%) Anisic aldehyde (0.06%) Methoxy cymene (0.55%) Isobornyl acetate (0.07%) Methyl eugenol (0.11%)	Chien, 1988.

O. basilicum L.	Herb	β-elemene (0.09%) α-bergamotene (0.45%) elemol (0.14%) Bulnesol (0.45%) γ-eudesmol (0.10%)	Chien, 1988.
	,,	α-pinene (tr.-0.52%) Camphene (tr.-0.10%) β-pinene (tr.-0.79%) Sabinene (tr.-0.34%) Myrcene (tr.-0.71%) α-terpinene (tr.-0.24%) Limonene (0.06-2.08%) 1,8-cineole (0.56-6.01%) Cis-ocimene (tr.-0.09%) γ-terpinene (tr.-0.07%) Trans- ocimene (0.07-1.81%) p-cymene (tr.-0.11%) Terpinolene (tr.-0.11%) Hexanol (tr.-0.03%) Cis-3-hexenol (tr.-0.13%) Trans-2-hexenol (tr.-0.05%) Cis-linalool oxide (tr.-0.15%) 1-octen-3 ol (tr.-0.03%) Trans-linalool oxide (tr.-0.45%) α-fenchyl acetate (tr.-0.01%) Copaene (tr.-0.36%) Camphor (tr.-0.50%) Linalool (0.70-6.33%) α-fenchyl alcohol (0.13-0.76%) Bornyl acetate (0.20-1.13%) β-elemene (0.10-0.56%) Caryophyllene+ Terpinen-4 ol (0.24-6.18%) Methyl chavicol (75.06-87.35%) α-terpineol (tr.-0.93%) Germacrene D (tr.-0.42%) Nerol (tr.-0.06%) Geraniol (tr.-0.24%) Caryophyllene oxide (tr.-0.01%) Cis-methyl cinnamate (tr.-0.05%)	Lawrence, 1988.

O. basilicum L.	Herb	Methyl eugenol (1.04-2.66%) Trans-methyl cinnamate (tr.-0.15%) Eugenol (tr.-1.18%) 10-epi(a)-cadinol (0.09-1.25%) Chavicol (tr.-0.21%)	Lawrence, 1988.
	,,	α-pinene (0.14%) Camphene (0.08%) β-pinene (0.26%) Limonene (0.29%) 1, 8-cineole+Cis-ocimene (3.00%) p-cymene (0.14%) γ-terpinene (0.45%) α-fenchyl acetate (0.17%) Camphor (0.10%) Linalool (45.70%) α-fenchyl alcohol (3.64%) β-caryophyllene (2.87%) Terpinen-4 ol (1.65%) α-terpineol (1.96%) Methyl chavicol (2.70%) α-terpinyl acetate (0.03%) Citronellol (1.53%) Geraniol (1.25%) Methyl eugenol (9.57%) Methyl cinnamate (1.98%) Eugenol (13.4%) Isoeugenol (2.04)	Akgul, 1989.
	,,	α-cubebene (0.14%) Bicycloelemene (0.10%) β-bisabolene (0.29%) (E)-α- bisabolene (0.75%) α-copaene (0.36%) α-bourbonene (0.17%) β- bourbonene (0.05%) α-gurjunene (0.52%) (E)-α-bergamotene (0.51%) (Z)-α-bergamotene (0.35%) β-elemene (18.0%) β-caryophyllene (34.6%) α-guaiene (1.10%) Scapanene (0.23%) α-elemene (0.32%) Allo-aromadendrene (0.31%) Eremophillene (0.44%) α-humulene (4.67%) γ-muurolene (1.89%)	Gaydou *et al.*, 1989.

O. basilicum L.	Herb	α-amorphene (2.10%) Viridiflorene (0.63%) Germacrene D (2.77%) β-selinene (5.83%) α-selinene (1.04%) Bicyclogermacrene (3.35%) (Z)-α-bisabolene (1.01%) (E,E)-α-farnesene (0.15%) δ-cadinene (7.70%) γ-cadinene (3.02%) Cubenene (0.17%) α-cadinene (0.32%) (Z)-calamenene (0.46%) (E)-calamenene (0.13%) α-calacorene (0.03%) β- calacorene (0.02%) δ-or γ- calacorene (0.04%)	Gaydou *et al.*, 1989.
	"	Limonene Myrcene E-ocimene β-pinene Sabinene γ-terpinene Terpinolene E-α-bergamotene β-bisabolene α-bulnesene γ-cadinene δ-cadinene β-caryophyllene β-elemene β-farnesene Germacrene D Germacrene B α-guaiene α-humulene γ-muurolene β-selinene Borneol Fenchol Geraniol Linalool E-linalool oxide E-sabinene hydrate α-terpineol 4-terpineol δ-cadinol β-selinenol 1,8-cineole Camphor 1-cyclohexylidene-propan-2-one Fenchone	Nykanen, 1989.

O. basilicum L.	Herb	Chavicol Eugenol Anethole Estragole Eugenol methyl ether Isobornyl acetate Methyl E-cinnamate Methyl Z-cinnamate Octyl acetate α-terpinyl acetate	Nykanen, 1989.
var. *canum*	,,	α-pinene (0.5%) β-pinene (1.0%) Camphor (0.2%) Sabinene (0.4%) Myrcene (1.0%) α-phellandrene (tr.) α-terpinene (0.1%) p-cymene (tr.) Limonene (0.4%) Cis-β-ocimene (tr.) Trans- β- ocimene(tr.) γ-terpinene (0.1%) Terpinolene (0.1%) 1,8-cineole (7.0%) Trans-sabinene hydrate (0.1%) Linalool (6.6%) α-fenchol (tr.) δ-terpineol (0.3%) Terpinen-4 ol (0.2%) α-terpineol (1.2%) Citronellol (tr.) Geraniol (0.5%) Borneol (0.1%) Isoborneol (tr.) Citronellal (tr.) Geranial (tr.) Camphor (1.8%) Fenchone (tr.) Piperitenone (tr.) Piperitenone epoxide (0.1%) Fenchyl acetate (0.6%) Bornyl acetate (1.4%) Geranyl acetate (0.5%) α-copaene (0.1%) β-elemene (0.8%) β-bourbonene (0.1%) β-caryophyllene (0.1%) α-bergamotene (2.6%) α-humulene (0.4%) Germacrene D (1.0%) γ-cadinene (1.9%) E-nerolidol (0.3%)	Fun & Svendsen, 1990

O. basilicum L. var. *canum*	Herb	β-caryophyllene epoxide (0.1%) Cubenol (0.9%) T-cadinol (5.3%) α-cadinol (0.5%) α- bisabolol (0.3%) Eugenol (tr.) Methyl eugenol (0.1%) Estragole (1.2%) Cis-methyl cinnamate (8.2%) Trans-methyl cinnamate (45.6%) Octen-3 ol (tr.)	Fun & Svendsen, 1990
O. basilicum L.	Leaf	α-pinene (0.13%) Myrcene (1.00%) 1,8-cineole (0.67%) *p-cymene* (1.24%) Methyl heptenone (0.73%) Linalool (32.10%) Methyl chavicol (0.53%) α-terpineol (0.70%) Geranyl acetate (0.98%) *Geraniol* (0.07%) Methyl eugenol (0.05%) (E)-methyl cinnamate (0.41%) Eugenol (22.50%) Isoeugenol (tr.)	Bobin, *et al.* 1991.
cv. 'Genovese'	Herb	α-pinene (0.01%) β-pinene (0.21%) Myrcene (0.55%) Limonene (0.01%) 1, 8-cineole (8.27%) (Z)-β-ocimene (0.03%) γ-terpinene (1.11%) Terpinolene (0.04%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.35%) 1-octen-3 ol (0.93%) Linalool (45.67%) Bornyl acetate (1.30%) β-caryophyllene (14.37%) Borneol (0.17%) α-terpineol (0.65%) Eugenol (7.13%)	
cv. 'Napoletano'	,,	α-pinene (0.01%) β-pinene (0.02%) Myrcene (0.02%) Limonene (0.01%) 1, 8-cineole (3.26%)	Mariani *et al.*, 1991.

O. basilicum L. cv. "Napoletano'	Herb	(Z)-β-ocimene (0.02%) 1-octen-3 ol (0.01%) Linalool (63.09%) Bornyl acetate (0.01%) β-caryophyllene (0.69%) Methyl chavicol (30.27%)	
cv. 'Greco'	,,	α-pinene (0.04%) β-pinene (0.01%) Myrcene (0.05%) 1, 8-cineole (26.80%) γ-terpinene (0.04%) Terpinolene (0.11%) (Z)-3-hexenyl acetate (0.01%) (Z)-3-hexenol (0.02%) 1-octen-3 ol (0.12%) Linalool (44.06%) Bornyl acetate (tr.) β-caryophyllene (14.29%) Methyl eugenol (6.85%) Eugenol (6.15%)	Mariani *et al.*, 1991.
var. *hispidum*	Leaf	Ethanol (0.10%) 2-methyl propanal (0.9%) 2-methyl propan-1 ol (0.06%) Isobutyl formate (0.06%) Myrcene (0.63%) Limonene (0.16%) Dihydrotagetone (82.27%) Cis-linalol oxide (tr.) Ipsenone (0.17%) Trans-linalol oxide (tr.) Linalol (0.62%) Cis-tagetone (0.49%) Trans-tagetone (0.15%) Terpinen-4 ol (0.17%) α-terpineol (0.73%) Elsholtzione (0.41%) β-caryophyllene (0.14%) α-humulene (0.54%) Cis-β-bisabolene (0.15%) Nerolidol (0.28%) Curcumene (0.35%)	Ruberto *et al.*, 1991.
O. basilicum L.	Whole Plant	α-pinene (0.13%) β-pinene (0.31%) Sabinene (0.24%) Myrcene (0.11%) Limonene (0.02%) β-phellandrene (0.06%) 1, 8-cineole (6.09%) (Z)-β-ocimene (2.20%) Hexanol (0.004%)	Sheen *et al.*, 1991.

O. basilicum L.	Whole Plant	(Z)-3-hexenol (0.06%) 3-octanol (0.02%) 1-octen-3 ol (0.22%) Sabinene hydrate (0.01%) Elixene (0.04%) α-copaene (0.01%) Linalool (1.67%) Trans-α-bergamotene (0.006%) β-elemene (0.27%) Aromadendrene (0.09%) Methyl chavicol (86.57%) (E)-β-farnesene (0.24%) α-terpineol (0.25%) α-muurolene (0.25%) Germacrene B (0.007%) Cadina-1,4-diene (0.03%) Methyl eugenol (0.59%) Eugenol (0.01%) T-cadinol (0.24%) α-cadinol (0.004%) β-eudesmol (0.01%) Chavicol (0.01%)	Sheen *et al.*, 1991.
	Herb	α-pinene Sabinene β-pinene Myrcene Limonene 1,8-cineole Trans-ocimene Cis-thujan-4 ol Linalol Camphor Terpinen-4 ol α-terpineol Methyl chavicol Linalyl acetate Geraniol Bornyl acetate Eugenol α-copaene β-elemene α-cubebene β-caryophyllene Trans-bergamotene α-guaiene β-farnesene Humulene γ-muurolene Germacrene D α-bulnesene γ-cadinene Calamenene Cadinene isomer δ-cadinol	Baritaux *et al.*, 1992.

O. basilicum L.	Herb	α-pinene (0.03%) Camphene (0.09%) β-pinene (0.55%) Sabinene (0.25%) Myrcene (0.13%) α-terpinene (0.04%) Limonene (0.31%) 1, 8-cineole (5.61%) (Z)-β-ocimene (0.14%) (E)-β-ocimene (0.43%) p-cymene (0.24%) Terpinolene (0.10%) (Z)-3-hexenol (0.05%) Cis-linalool oxide (0.08%) Trans-sabinene hydrate (tr.) α-cubebene (0.12%) Trans-linalool oxide (0.08%) Octyl acetate (0.05%) α-copaene (0.14%) Camphor (0.72%) Benzaldehyde (0.07%) Linalool (41.72%) Terpinen-1 ol (tr.) Trans-α-bergamotene (tr.) Bornyl acetate (0.52%) β-elemene (1.61%) Terpinen-4 ol+ β-caryophyllene (1.35%) α-humulene (tr.) α-amorphene (tr.) Methyl chavicol (2.24%) α-terpineol (0.67%) Borneol (0.12%) Germacrene D (0.74%) β-bisabolene (tr.) Bicyclogermacrene (0.41%) Carvone (tr.) (E)-anethole (0.02%) Geraniol (tr.) Calamenene (0.02%) (Z)-methyl cinnamate (4.69%) Caryophyllene oxide (tr.) (E)-methyl cinnamate (31.94%) Cinnamyl acetate (1.22%)	Fleisher & Fleisher, 1992.
var. *glabratum* Chemotype-1	,,	Methyl chavicol (55-75%) Linalool (22-35%)	
Chemotype-2	,,	Eugenol (55-75%) Ocimene (10-15%)	Gupta, 1994.

O. basilicum L. var. *glabratum* Chemotype-2	Herb	Cresol (tr.-3%) Guaiacol (tr.-3%)	
Chemotype-3	,,	Camphor (60-76%) Camphene (11-18%) Limonene (5-10%) Cadinene (2-7%)	Gupta, 1994.
O. basilicum L.	,,	1,8-cineole (2.45-3.37%) Ocimene (tr.-1.75%) Linalool (0.04-2.69%) α-fenchyl alcohol (tr.-0.26%) Camphor (tr.-0.36%) Methyl chavicol (83.58-87.21%) Geranyl acetate (tr.-0.33%) Bornyl acetate (tr.-0.28%) Methyl eugenol (1.41-2.68%) Guaiene (tr.-0.25%) β-eudesemene (tr.-1.37%) β-bisabolene (tr.-0.19%) Eremophilene (tr.-0.23%) α-bulnesene (tr.-0.46%) γ-cadinene (tr.-0.13%) β-patchoulene (tr.-0. 96%)	Riaz *et al.*, 1994.
Chemotype-1	,,	α-pinene (0.24-3.00%) β-pinene (0.29-0.43%) Sabinene (0.35-1.10%) 1,8-cineole (0.98-9.30) γ-terpinene (0.73-2.93%) Terpinen-4 ol (1.49-4.40) β-caryophyllene (0.15-0.44%) Linalool (44.34-56.84%) Eugenol (1.60-20.77%) Isoeugenol (0.38-2.68%) Methyl chavicol (tr.-0.41%)	
Chemotype-2	,,	α-pinene (7.74%) β-pinene (2.74%)	Retamar *et al.*, 1995.

O. basilicum L. Chemotype-2	Herb	Sabinene (53.14%) α-phellandrene (0.89%) 1,8-cineole (4.50%) γ-terpinene (1.20%) Terpinen-4 ol (2.54%) β-caryophyllene (1.15%) Linalool (0.26%) Eugenol (5.98%) Isoeugenol (2.53%) Methyl chavicol (0.28%)	Retamar *et al.*, 1995.
O. basilicum L.	,,	Linalool Geraniol Eugenol Methyl chavicol γ-muurolene	Hethelyi *et al.*, 1995.
cv. 'Kinos'	,,	Linalool (54.4%) Eugenol (7.3%) Methyl chavicol (29.9%) Camphor (tr.)	
cv. 'Sgouros'	,,	Linalool (41.0%) Methyl chavicol (13.0%) Geraniol (9.0%) Camphor (tr.)	
cv. 'Mauromytikos'	,,	Methyl cinnamate (37.8%) Linalool (22.6%) 1,8-cineole (7.9%)	Petropoulos & Vlachou, 1995.
var. *glabratum*	,,	α-thujene (tr.) α-pinene (0.43%) Camphene (0.10%) Sabinene (0.14%) β-pinene (0.53%) Myrcene (0.18%) α-phellandrene (tr.) α-terpinene (tr.) p-cymene (tr.) Limonene (tr.) 1,8-cineole (6.87%) γ-terpinene (tr.) Cis-linalool oxide (0.39%) Trans-linalool oxide (0.60%) Linalool (43.73%) Camphor (0.58%) Terpinen-4 ol (tr.) α-terpineol (0.63%) Bornyl acetate (1.08%) (Z)-methyl cinnamate (4.63%)	Perez-Alonso *et al.*, 1995.

O. basilicum L. var. *glabratum*	Herb	(E)-methyl cinnamate (27.28%) β-elemene (0.30%) β-caryophyllene (0.17%) Trans-α-bergamotene (3.08%) α-humulene (0.46%) γ-muurolene (0.21%) Germacrene D (1.00%) (E,E)-α-farnesene (0.24%) γ-cadinene (1.51%) Cubenol (0.47%) T-cadinol (3.06%)	Perez-Alonso *et al.*, 1995.
O. basilicum L. Chemotype-1	,,	α-pinene (0.27-0.78%) α-thujene (tr.-0.18%) Camphene (tr.-0.32%) β-pinene (0.13-1.30%) Sabinene (0-0.56%) δ-3-carene (0-0.10%) α-phellandrene (0-0.16%) Myrcene (0.24-0.92%) α-terpinene (0.11-0.30%) Limonene (0.34-1.01%) 1,8-cineole (1.30-11.68%) (Z)-β-ocimene (tr.-0.16%) γ-terpinene (0.12-0.92%) (E)-β-ocimene (0.84-2.99%) p-cymene (tr.-2.41%) Terpinolene (0.11-0.53%) Fenchone (0.49-3.21%) 1-octen-3 ol (0-tr.) Linalool oxide ((0-tr.) α-fenchyl acetate (0.14-0.75%) Camphor (0.06-0.32%) Octyl acetate (tr.) Linalool (20.03-60.47%) Bornyl acetate (tr.) α-fenchol (0-tr.) β-caryophyllene+β-elemene (7.26-12.63%) Terpinen-4 ol (tr.) α-farnesene (1.00-5.48%) α-humulene (0.08-0.22%) Methyl chavicol (0.37-12.33%)	Chalchat *et al.*, 1996.

O. basilicum L. Chemotype-1	Herb	(E)-β-farnesene (tr.-0.72%) (Z)-β-farnesene (0.16%-1.67%) α-terpineol (0.56-2.44%) δ-guaiene (0.13-0.69%) Geranial (0-1.56%) α-amorphene (0.68-3.10%) δ-cadinene (0-tr.) β-sesquiphellandrene (tr.-1.00) Nerol (0.10-0.30%) Geraniol (0.12-1.39%) Methyl eugenol (0.10-40.44%) Eugenol (0.64-21.14%) T-cadinol (0.54-4.87%) β-eudesmol (0-0.13%) Cubenol (0-0.74%) Nerolidol (0-0.50%) Spathulenol (0-0.35%)	
Chemotype-2	,,	α-pinene (0.08-0.19%) α-thujene (tr.) Camphene (0.07-0.18%) β-pinene (0.10-0.21%) Sabinene (tr.-0.16%) α-phellandrene (tr.) Myrcene (0.23-1.58%) α-terpinene (tr.- 0.06%) Limonene (0.19-0.42%) 1,8-cineole (1.03-1.88%) (Z)-β-ocimene (0.09-0.23%) γ-terpinene (tr. -0.19%) (E)-β-ocimene (1.09-4.05%) p-cymene (tr.-0.11%) Terpinolene (0.8-0.25%) Linalool oxide (0-tr.) α-fenchyl acetate (tr.-0.39%) Linalool (61.73-73.12%) Bornyl acetate (0-0.25%) α-fenchol (0-1.35%) β-caryophyllene+β-elemene (2.84-5.09%) α-humulene (tr.-0.30%) Methyl chavicol (0.21-7.87%) Borneol (0.27-1.00%)	Chalchat *et al.*, 1996.

O. basilicum L. Chemotype-2	Herb	α-terpineol (0.40-1.10%) δ-guaiene (0.36-0.84%) α-terpinyl acetate (0.29-0.62%) α-amorphene (1.94-3.47%) Nerol (tr.-0.20%) Geraniol (0.24-0.89%) Methyl eugenol (0-0.67%) Eugenol (2.20-15.63%) T-cadinol (3.28-5.23%) Cubenol (0.20-1.89%) Nerolidol (0-0.79%) Spathulenol (0-0.44%)	Chalchat *et al.*, 1996.
var. *canum*	,,	Linalool (21.7-26.2%) Citronellal (tr.) Methyl chavicol (1.6-4.1%) Methyl eugenol (3.2-5.7%) Methyl cinnamate (28.8-34.5%) Geraniol (14.3-18.9%) Methyl isoeugenol (1.3-3.4%)	
var. *crispa*	,,	Linalool (67.5-73.4%) Citronellal (1.2-3.1%) Methyl chavicol (tr.) Methyl cinnamate (tr.) Geraniol (4.3-8.1%) Geranyl acetate (1.3-4.4%)	
var. *glabratum*	,,	Linalool (1.4-3.8%) Methyl chavicol (43.1-51.7%) Methyl eugenol (1.8-6.7%) Methyl cinnamate (15.7-26.9%) Geraniol (tr.) Methyl isoeugenol (5.6-7.4%)	
var. *glabratum* x var. *purpurascens*	,,	Linalool (21.8-27.4%) Citronellal (tr.) Methyl chavicol (11.8-19.7%) Methyl cinnamate (33.7-39.3%)	Thoppil, 1996.

O. basilicum L. var. *glabratum* x var. *purpurascens*	Herb	Geraniol (5.1-7.6%)	
var. *purpurascens*	,,	Linalool (36.2-41.6%) Citronellal (20.2-24.2%) Methyl eugenol (tr.) Methyl cinnamate (tr.) Geraniol (7.1-9.6%) Methyl isoeugenol (tr.) Geranyl acetate (tr.)	
var. *minimum*	,,	Linalool (7.6-8.8%) Methyl chavicol (tr.) Methyl eugenol (43.7-49.8%) Methyl cinnamate (5.4-8.8%) Geraniol (21.4-27.2%) Methyl isoeugenol (tr.) Geranyl acetate (2.8-5.9%)	
var. *pilosum*	,,	Linalool (tr.) Methyl chavicol (tr.) Methyl eugenol (1.3-4.4%) Methyl cinnamate (10.5-17.3%) Geraniol (6.1-8.6%) Methyl isoeugenol (15.2-26.1%) Geranyl acetate (31.9-43.6%)	
var. *thyrsiflorum*	,,	Linalool (tr.) Methyl chavicol (1.3-4.2%) Methyl eugenol (2.8-4.7%) Methyl cinnamate (74.6-81.8%)	Thoppil, 1996.
O. basilicum L.	,,	α-pinene (0-0.38%) Camphene (0-0.74%) Sabinene (0-0.38%) β-pinene (0-2.00%) Myrcene (0-0.94%) α-Terpinene (0-0.05%) p-cymene (0-tr.) Limonene (0-0.58%) 1,8-cineole (0.94-12.91%) (Z)-β-ocimene (0-tr.)	Marotti *et al.*, 1996.

O. basilicum L.	Herb	(E)-β-ocimene (0-1.02%) γ-terpinene (0-0.18%) Terpinolene (tr.-1.47%) Linalool (41.17-76.20%) α-fenchol (0-0.30%) Camphor (0.10-0.83%) Borneol (0.21-1.21%) Terpinen–4 ol (tr.-3.14%) α-terpineol (0.12-1.14%) Methyl chavicol (0-41.40%) α-fenchyl acetate (0-0.56%) Bornyl acetate (0.18-1.03%) δ-elemene (0-tr.) α-copaene (0-0.17%) β-elemene (0.10-0.66%) Cis-α-bergamotene (0-0.16%) α-caryophyllene (0.09-0.70%) Eugenol (0-3.89%) Trans-α-bergamotene (0-3.37%) α-guaiene (0-0.27%) α-cadinene (0-0.36%) α-humulene (0.26-0.82%) γ-muurolene (0.04-0.43%) Germacrene D (0.72-2.11%) Germacrene B (0.50-1.30%) α-farnesene (0.09-0.91%) γ-cadinene (0.38-1.37%) Calamenene (0.04-0.80%) T-cadinol (1.76-7.55%)	Marotti *et al.*, 1996.
	Leaf	α-pinene (0.04%) Camphene (0.04%) β-pinene (0.08%) 7-octen-4 ol (0.17%) Myrcene (0.05%) p-cymene (0.06%) Limonene (0.01%) 1,8-cineole (3.39%) δ-3-carene (0.02%) Fenchone (0.87%)	Thach *et al.*, 1996.

O. basilicum L.	Leaf	Linalool (2.26%) α-fenchol (1.34%) Camphor (1.79%) Borneol (0.84%) Methyl chavicol (58.03%) α-fenchyl acetate (0.19%) Anisaldehyde (3.56%) Bornyl acetate (0.36%) p-propylanisole (0.19%) Elemene (1.25%) Methyl eugenol (1.29%) Cadinene (0.96%)	Thach *et al.*, 1996.
	Herb	Linalool Methyl chavicol Geraniol Bornyl acetate β-elemene β-caryophyllene γ-cadinene Caryophyllene α-bisabolol	Lemberkovics *et al.*, 1996.
Chemotype 1	,,	Myrcene (0-0.9%) Limonene+1,8-cineole (3.8-6.8%) Linalool (69.3-85.6%) Methyl chavicol (0-0.5%) Eugenol (0-4.6%) β-elemene (1.2-2.3%) Bergamotene (3.0-12.7%) γ-cadinene (3.0-4.7%) T-cadinol (3.0-4.3%)	
Chemotype 2	,,	Limonene+1,8-cineole (2.4-4.1%) Camphor (0-1.5%) Methyl chavicol (89.8-91.5%) Eugenol (0-1.1%) β-caryophyllene (0-4.4%) Bergamotene (0-0.7%) γ-cadinene (0-0.9%) T-cadinol (0-1.3%)	
Chemotype 3	,,	Sabinene (0-1.2%) Myrcene (0-2.2%) Limonene+1,8-cineole (3.7-19.9%) Ocimene (0-3.5%) Linalool (15.6-31.8%)	Grayer *et al.*, 1996.

O. basilicum L. Chemotype 3	Herb	Camphor (0-2.1%) Methyl chavicol (43.7-56.1%) Geraniol (0-8.5%) Geranial (0-1.7%) β-elemene (0.6-1.5%) Bergamotene (0-4.9%) α-humulene (0-0.7%) β-bisabolene (0-1.5%) γ-cadinene (1.2-2.9%) T-cadinol (2.1-3.9%)	
Chemotype 4	,,	Myrcene (0-1.1%) Limonene+1,8-cineole (6.1-11.40%) Ocimene (0-1.0%) Fenchone (0-2.6%) Linalool (30.5-40.9%) Camphor (0-2.7%) Methyl chavicol (0-0.6%) Eugenol (38.9-48.0%) β-elemene (1.6-6.2%) Methyl eugenol (0-5.8%) Bergamotene (0-3.5%) γ-cadinene (1.0-3.9%) T-cadinol (2.2-3.3%)	
Chemotype 5	,,	Limonene+1,8-cineole (9.4%) Fenchone (2.2%) Methyl chavicol (45.3%) Methyl eugenol (43.2%)	Grayer *et al.*, 1996.
O. basilicum L.	,,	(E)-2-hexenal (Z)-3-hexenol α-thujene α-pinene Camphene Sabinene 1-octen-3 ol β-pinene Myrcene Octenol α-phellandrene δ-3-carene α-terpinene p-cymene Limonene β-phellandrene 1,8-cineole (Z)- β-ocimene (E)- β-ocimene γ-terpinene	Venskutonis *et al.*, 1996.

O. basilicum L.	Herb	Cis-sabinene hydrate Cis-linalool oxide Trans-linalool oxide Terpinolene α-thujone Linalool β-thujone α-fenchol Allo-ocimene Camphor Menthone Isomenthone Borneol Menthol Terpinen-4 ol α-terpineol Methyl chavicol Neral Geraniol Geranial Anethole Bornyl acetate Thymol Carvacrol Eugenol Geranyl acetate Methyl cinnamate Damascenone Methyl eugenol β-elemene α-ionone β-caryophyllene β-farnesene Trans-α-bergamotene α-humulene Allo-aromadendrene β-ionone Germacrene D Germacrene B (Z)-nerolidol (E)-nerolidol Caryophyllene oxide Farnesol isomer A Farnesol isomer B	Venskutonis *et al.*, 1996.
cv. 'Castelar'	,,	α-pinene (tr.-0.3%) β-pinene (0.2-0.5%) α-terpinene (tr.-0.1%) Limonene (0.1-0.3%) 1,8-cineole (2.9-4.2%) (E)- β-ocimene (0.1-1.0%) p-cymene (0-0.1%) Camphor (0.7-1.1%) Linalool (27.5-59.7%) Methyl chavicol (6.6-42.0%)	

O. basilicum L. cv. 'Castelar'	Herb	β-caryophyllene (0.1-0.5%) Eugenol (0-3.9%) Geranyl acetate (0-0.1%) Geraniol (0-0.2%) Bornyl acetate (0.3-0.5%) α-terpineol (0.4-0.7%)	
cv. 'Samalao'	,,	α-pinene (0.2%) β-pinene (0.7%) α-terpinene (0.1%) Limonene (0.2%) Camphor (0.1%) Linalool (50.6%) Methyl chavicol (7.4%) β-caryophyllene (0.1%) Eugenol (12.5%) Geraniol (0.1%) Bornyl acetate (0.2%) α-terpineol (1.2%)	
cv. 'Bella Vista'	,,	α-pinene (0.1%) β-pinene (0.3%) α-terpinene (0.1%) Limonene (0.2%) (E)- β-ocimene (0.2%) Camphor (1.6%) Linalool (36.1%) Methyl chavicol (34.7%) β-caryophyllene (0.2%) Eugenol (0.1%) Geranyl acetate (0.2%) Geraniol (0.1%) Bornyl acetate (0.4%) α-terpineol (0.9%)	
cv. 'La Consulta'	,,	α-pinene (tr.-0.1%) β-pinene (0.1-0.2%) α-terpinene (0-0.2%) Limonene (0.1-0.2%) (E)- β-ocimene (0-0.3%) Camphor (0.3-2.7%) Linalool (48.4-50.3%) Methyl chavicol (19.5-28.4%) β-caryophyllene (tr.-0.7%) Eugenol (tr.-0.1%) Geranyl acetate (0-0.1%) Geraniol (0.1-0.2%)	Mizrahi *et al.*, 1996.

O. basilicum L. cv. 'La Consulta'	Herb	Bornyl acetate (0.8-0.9%) α-terpineol (0.7-1.0%)	
cv. 'Merlo'	,,	α-pinene (0.2%) β-pinene (0.4%) Limonene (0.2%) (E)- β-ocimene (0.1%) p-cymene (0.2%) Camphor (0.3%) Linalool (45.4%) Methyl chavicol (25.0%) β-caryophyllene (0.2%) Eugenol (2.8%) Geraniol (0.2%) Bornyl acetate (0.3%)	Mizrahi *et al.*, 1996.
cv. 'Reunion'	Leaf	α-pinene (0.3%) Camphene (0.1%) β-pinene (0.3%) Myrcene (0.1%) α-terpinene (tr.) Limonene (0.3%) 1,8-cineole (2.6%) γ-terpinene (0.1%) p-cymene (tr.) fenchone (tr.) Camphor (0.6%) Linalool (1.5%) β-caryophyllene (0.5%) Methyl chavicol (87.2%) α-terpineol (tr.) Borneol (tr.) Geranial (tr.) Citronellol (tr.) Geraniol (tr.) Methyl cinnamate (tr.) Eugenol (tr.)	
cv. 'Large Lettuce'	,,	α-pinene (0.4%) Camphene (0.1%) β-pinene (0.7%) Myrcene (0.6%) α-terpinene (0.1%) Limonene (0.3%) 1,8-cineole (6.2%) γ-terpinene (0.1%) p-cymene (0.1%) fenchone (tr.) Camphor (0.4%) Linalool (65.4%) β-caryophyllene (0.5%) Methyl chavicol (13.5%) α-terpineol (0.6%) Borneol (0.1%)	Lachowicz *et al.*, 1996.

O. basilicum L. cv. 'Large Lettuce'	Leaf	Geranial (0.3%) Citronellol (0.1%) Geraniol (0.1%) Methyl cinnamate (tr.) Eugenol (tr.)	
cv. 'Anise'	,,	α-pinene (0.2%) Camphene (0.2%) β-pinene (0.2%) Myrcene (0.4%) α-terpinene (tr.) Limonene (0.3%) 1,8-cineole (1.6%) γ-terpinene (tr.) p-cymene (tr.) fenchone (0.1%) Camphor (1.2%) Linalool (43.7%) β-caryophyllene (0.2%) Methyl chavicol (27.3%) α-terpineol (0.4%) Borneol (0.4%) Geranial (0.6%) Citronellol (0.1%) Geraniol (tr.) Methyl cinnamate (11.2%) Eugenol (tr.)	Lachowicz *et al.*, 1996.
O. basilicum L.	Herb	Camphene β-pinene Sabinene Limonene 1,8-cineole (Z)- β-ocimene γ-terpinene (E)- β-ocimene p-cymene Camphor Linalool Muurolene β-caryophyllene β-elemene Trans-α-bergamotene Terpinen-4 ol Methyl chavicol Neral Borneol α-terpineol Germacrene D Bicyclogermacrene Geranial β-bisabolene Nerol Methyl cinnamate T-cadinol	Pasquier & Chalchat, 1997.

O. basilicum L.	Herb	Eugenol Thymol α-bisabolol	Pasquier & Chalchat, 1997.
cv. 'Midedji'	,,	α-pinene (0.1%) α-thujene (0.03%) Camphene (0.04%) β-pinene (0.06%) Sabinene (0.03%) Myrcene (0.20%) α-terpinene (0.10%) 1,8-cineole (1.40%) γ-terpinene (0.50%) (E)- β-ocimene (2.30%) p-cymene (0.30%) Terpinolene (0.20%) (Z)-3-hexenol (1.50%) Linalool oxide (0.50%) Linalool (45.30%) (Z,Z)-α-farnesene (6.70%) Methyl chavicol (1.00%) (E)- β-farnesene (0.60%) α-terpineol (1.80%) δ-guaiene (0.20%) Geranial (0.70%) α-amorphene (1.40%) Geranyl acetate (0.30%) Geraniol (0.80%) Methyl isoeugenol (1.70%) Nerolidol (0.07%) Spathulenol (0.09%) Eugenol (14.90%) T-cadinol (5.00%) Thymol (0.02%)	
cv. 'Womey'	,,	α-pinene (tr.) α-thujene (tr.) Camphene (tr.) β-pinene (tr.) Sabinene (tr.) Myrcene (0.16%) α-terpinene (0.08%) 1,8-cineole (1.50%) γ-terpinene (0.40%) (E)- β-ocimene (0.10%) p-cymene (1.20%) Terpinolene (tr.) Linalool oxide (0.10%) α-fenchyl acetate (0.50%) α-copaene (1.20%) Linalool (29.10%) (Z,Z)-α-farnesene (2.20%) Methyl chavicol (54.60%) (E)- β-farnesene (0.30%) (Z)- β-farnesene (0.40%)	Chalchat *et al.*, 1997.

O. basilicum L. cv. 'Womey'	Herb	α-terpineol (1.50%) Geranial (0.10%) α-amorphene (0.60%) α-bisabolene (0.50%) Geraniol (0.60%) Methyl isoeugenol (0.08%) Spathulenol (tr.) Eugenol (0.40%) T-cadinol (0.80%) Thymol (tr.)	
cv. 'Save'	,,	α-pinene (0.08%) α-thujene (tr.) β-pinene (0.09%) Sabinene (0.10%) Myrcene (tr.) α-terpinene (tr.) 1,8-cineole (2.20%) (E)- β-ocimene (tr.) p-cymene (0.16%) α-fenchyl acetate (0.07%) α-copaene (0.20%) (Z,Z)-α-farnesene (2.40%) Methyl chavicol (90.10%) (E)- β-farnesene (tr.) (Z)- β-farnesene (0.10%) α-terpineol (0.10%) δ-guaiene (0.10%) Geranial (2.10%) Methyl isoeugenol (0.10%)	Chalchat *et al.*, 1997.
O. basilicum L.	,,	α-pinene (0.16%) Sabinene (0.10%) β-pinene (0.25%) 1,8-cineole (0.43%) Linalool (1.55%) Camphor (tr.) α-terpineol (0.16%) Methyl chavicol (0.68%) Lavandulyl acetate (0.55%) (Z)-methyl cinnamate (tr.) δ-elemene (tr.) Eugenol (4.00%) α-copaene (0.99%) (E)-methyl cinnamate (2.50%) β-elemene (0.96%) Methyl eugenol (0.61%) β-caryophyllene (48.70%) trans-α-bergamotene (6.76%) α-humulene (6.00%) Bisabolene (tr.) γ-cadinene (1.15%)	Pallado *et al.*, 1997.

O. basilicum L.	Herb	(Z)-γ-bisabolene (1.10%) Spathulenol (1.50%) Caryophyllene oxide (1.25%) Cubenol (2.46%) Cadinol (18.14%) Hexadecene (tr.) Octadecene (tr.) Docosene (tr.) Nonacosane (tr.) Hentriacontane (tr.) Tritriacontane (tr.)	Pallado *et al.*, 1997.
	,,	α-pinene (0.16%) Sabinene (0.10%) β-pinene (0.25%) 1,8-cineole (0.43%) Linalool (1.55%) Camphor (tr.) α-terpineol (0.16%) Methyl chavicol (0.68%) Lavandulyl acetate (0.55%) (Z)-methyl cinnamate (tr.) β-elemene (tr.) Eugenol (4.00%) α-copaene (0.99%) (E)-methyl cinnamate (2.50%) Methyl eugenol (0.61%) β-elemene (0.96%) β-caryophyllene (48.70%) Trans-bergamotene (6.76%) α-caryophyllene (6.00%) Bisabolene (tr.) γ-cadinene (1.15%) (Z)-δ-bisabolene (1.10%) Spathulenol (1.50%) Caryophyllene oxide (1.25%) Cubenol (2.46%) Cadinol (18.14%)	D'Alpaos *et al.*, 1997.
cv. 'Reunion'	,,	α-pinene (0.3%) Camphene (tr.) β-pinene (0.3%) Sabinene (tr.) Myrcene (tr.) α-terpinene (tr.) Limonene (0.3%) 1,8-cineole (2.4%) (Z)- β-ocimene (0.2%) γ-terpinene (tr.) (E)- β-ocimene (2.2%) p-cymene (tr.)	Lachowicz *et al.*, 1997.

O. basilicum L. cv. 'Reunion'	Herb	Fenchone (tr.) Pentadecane (tr.) Camphor (1.0%) Linalool (1.5%) Linalyl acetate (tr.) α-fenchol (0.2%) Hexadecane (tr.) β-caryophyllene (0.4%) Methyl chavicol (82.6%) Heptadecane (tr.) α-terpineol (tr.) Borneol (tr.) Octadecane (tr.) Geraniol (tr.) Nonadecane (tr.) Eicosane (tr.) Methyl cinnamate (tr.) Heneicosane (tr.) Eugenol (tr.) Docosane (tr.) Tricosane (tr.)	
cv. 'Cinnamon'	,,	α-pinene (0.2%) Camphene (tr.) β-pinene (0.2%) Sabinene (0.1%) Myrcene (tr.) α-terpinene (tr.) Limonene (0.2%) 1,8-cineole (2.4%) (Z)- β-ocimene (tr.) γ-terpinene (0.1%) (E)- β-ocimene (0.4%) p-cymene (0.1%) Fenchone (tr.) Pentadecane (tr.) Camphor (0.5%) Linalool (27.3%) Linalyl acetate (0.1%) α-fenchol (tr.) Hexadecane (tr.) β-caryophyllene (tr.) Methyl chavicol (6.8%) Heptadecane (tr.) α-terpineol (0.5%) Borneol (0.3%) Octadecane (tr.) Geraniol (tr.) Nonadecane (tr.) Eicosane (tr.) Methyl cinnamate (28.1%) Heneicosane (tr.) Eugenol (3.9%) Docosane (tr.) Tricosane (tr.)	Lachowicz *et al.*, 1997.

O. basilicum L. cv. 'Anise'	Herb	α-pinene (0.2%) Camphene (0.2%) β-pinene (tr.) Sabinene (tr.) Myrcene (0.2%) α-terpinene (tr.) Limonene (0.3%) 1,8-cineole (1.3%) (Z)- β-ocimene (tr.) γ-terpinene (tr.) (E)- β-ocimene (0.4%) p-cymene (tr.) Fenchone (0.2%) Pentadecane (tr.) Camphor (2.5%) Linalool (27.8%) Linalyl acetate (tr.) α-fenchol (tr.) Hexadecane (tr.) β-caryophyllene (tr.) Methyl chavicol (40.9%) Heptadecane (tr.) α-terpineol (0.4%) Borneol (0.7%) Octadecane (0.2%) Geraniol (tr.) Nonadecane (tr.) Eicosane (tr.) Methyl cinnamate (3.4%) Heneicosane (tr.) Eugenol (tr.) Docosane (tr.) Tricosane (tr.)	
cv. 'Dark opal'	,,	α-pinene (0.8%) Camphene (0.1%) β-pinene (0.9%) Sabinene (0.4%) Myrcene (tr.) α-terpinene (tr.) Limonene (0.4%) 1,8-cineole (5.5%) (Z)-β-ocimene (tr.) γ-terpinene (tr.) (E)- β-ocimene (tr.) p-cymene (tr.) Fenchone (0.3%) Pentadecane (tr.) Camphor (tr.) Linalool (57.4%) Linalyl acetate (0.1%) α-fenchol (tr.) Hexadecane (tr.) β-caryophyllene (2.5%) Methyl chavicol (tr.) Heptadecane (tr.) α-terpineol (0.8%)	Lachowicz *et al.*, 1997.

O. basilicum L. cv. 'Dark opal'	Herb	Borneol (0.4%) Octadecane (tr.) Geraniol (0.7%) Nonadecane (tr.) Eicosane (tr.) Methyl cinnamate (0.7%) Heneicosane (tr.) Eugenol (tr.) Docosane (tr.) Tricosane (tr.)	
cv. 'Bush type'	,,	α-pinene (0.6%) Camphene (0.2%) β-pinene (0.5%) Sabinene (0.2%) Myrcene (0.4%) α-terpinene (tr.) Limonene (0.5%) 1,8-cineole (3.0%) (Z)-β-ocimene (tr.) γ-terpinene (0.2%) (E)-β-ocimene (tr.) p-cymene (0.5%) Fenchone (tr.) Pentadecane (tr.) Camphor (tr.) Linalool (52.0%) Linalyl acetate (tr.) α-fenchol (tr.) Hexadecane (tr.) β-caryophyllene (tr.) Methyl chavicol (tr.) Heptadecane (0.2%) α-terpineol (0.7%) Borneol (tr.) Octadecane (0.3%) Geraniol (0.9%) Nonadecane (tr.) Eicosane (tr.) Methyl cinnamate (tr.) Heneicosane (tr.) Eugenol (tr.) Docosane (tr.) Tricosane (tr.)	Lachowicz *et al.*, 1997.
cv. 'PK-1'	,,	α-pinene (0.31%) α-thujene (0.3%) Camphene (0.07%) β-pinene (0.61%) δ-3-carene (0.29%) Myrcene (0.54%) α-phellandrene (0.01%) α-terpinene (0.05%) Limonene (0.31%) 1,8-cineole (4.02%) (Z)-β-ocimene (0.05%)	Hasegawa *et al.*, 1997.

O. basilicum L. cv. 'PK3'	Herb	α-pinene (0.18%) Camphene (0.03%) β-pinene (0.04%) δ-3-carene (0.23%) Myrcene (0.48%) α-phellandrene (0.30%) Limonene (0.21%) 1,8-cineole (3.40%) (Z)-β-ocimene (0.03%) γ-terpinene (0.12%) (E)-β-ocimene (0.66%) p-cymene (0.04%) Terpinolene (0.01%) (Z)-3-hexenyl acetate (0.01%) (Z)-3-hexenol (0.05%) Octyl acetate (0.02%) α-copaene (0.06%) Camphor (0.26%) Linalool (21.09%) Octanol (0.09%) β-caryophyllene (4.33%) Methyl chavicol (56.20%) Neral (0.40%) α-terpineol (0.52%) α-borneol (0.03%) δ-guaiene (0.37%) Geraniol (1.35%) Methyl eugenol (0.23%) Eugenol (1.23%) Chavicol (0.11%)	
cv. 'PK 4'	,,	α-pinene (0.14%) α-thujene (0.04%) Camphene (0.06%) β-pinene (0.13%) δ-3-carene (0.07%) Myrcene (0.14%) α-phellandrene (0.01%) Limonene (0.21%) 1,8-cineole (1.05%) (Z)-β-ocimene (0.01%) γ-terpinene (0.01%) (E)-β-ocimene (1.99%) p-cymene (0.18%) Terpinolene (0.10%) (Z)-3-hexenol (0.01%) Octyl acetate (0.13%) α-copaene (0.04%) Camphor (0.29%) Linalool (0.14%) Octanol (0.03%) β-caryophyllene (4.05%) Methyl chavicol (82.79%) Neral (0.23%) α-terpineol (0.42%) Methyl eugenol (0.69%)	Hasegawa *et al.*, 1997.

O. basilicum L. cv. 'PK 5'	Herb	Myrcene (0.01%) Limonene (0.04%) 1,8-cineole (0.01%) (E)-β-ocimene (0.08%) Benzaldehyde (0.02%) Octyl acetate (0.02%) α-copaene (0.06%) Camphor (0.03%) Linalool (3.28%) Octanol (0.01%) β-caryophyllene (4.12%) Methyl chavicol (0.04%) α-terpineol (0.03%) δ-guaiene (0.70%) Geraniol (0.22%) Methyl cinnamate (73.65%) Methyl eugenol (0.02%) Eugenol (0.06%) Chavicol (0.01%)	
cv. 'Lettuce'	,,	α-pinene (0.19%) Camphene (0.05%) β-pinene (0.39%) δ-3-carene (0.22%) Myrcene (0.39%) α-phellandrene (0.02%) Limonene (0.22%) 1,8-cineole (4.37%) (Z)-β-ocimene (0.05%) γ-terpinene (0.09%) (E)-β-ocimene (1.24%) p-cymene (0.02%) Terpinolene (0.15%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.03%) Octyl acetate (0.14%) α-copaene (0.11%) Camphor (0.48%) Linalool (33.76%) Octanol (0.06%) β-caryophyllene (4.04%) Methyl chavicol (35.91%) Neral (0.04%) α-terpineol (0.64%) α-borneol (0.15%) Geranyl acetate (1.19%) Geraniol (0.06%) Eugenol (4.36%) Chavicol (0.35%)	
cv. 'Fino verde'	,,	α-pinene (0.08%) Camphene (0.03%) β-pinene (0.11%) δ-3-carene (0.07%)	Hasegawa *et al.*, 1997.

O. basilicum L. cv. 'Fino verde'	Herb	Myrcene (0.31%) α-phellandrene (0.02%) α-terpinene (0.01%) Limonene (0.17%) 1,8-cineole (1.24%) (Z)-β-ocimene (0.06%) γ-terpinene (0.11%) (E)-β-ocimene (1.28%) p-cymene (0.06%) Terpinolene (0.15%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.01%) Octyl acetate (0.24%) α-copaene (0.13%) Camphor (0.44%) Linalool (49.48%) Octanol (0.14%) β-caryophyllene (6.74%) Methyl chavicol (0.5%) Neral (0.01%) α-terpineol (0.54%) α-borneol (0.16%) Geranyl acetate (1.93%) Geraniol (0.44%) Eugenol (16.19%) Chavicol (0.01%)	
cv. 'Sweet'	,,	α-pinene (0.12%) Camphene (0.02%) β-pinene (0.28%) δ-3-carene (0.17%) Myrcene (0.38%) α-phellandrene (0.02%) Limonene (0.17%) 1,8-cineole (3.37%) (Z)-β-ocimene (0.03%) γ-terpinene (0.07%) (E)-β-ocimene (0.70%) p-cymene (0.02%) Terpinolene (0.11%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.03%) Octyl acetate (0.07%) α-copaene (0.11%) Camphor (0.28%) Linalool (31.05%) Octanol (0.06%) β-caryophyllene (4.59%) Methyl chavicol (39.09%) Neral (0.05%) α-terpineol (0.56%) α-borneol (0.06%) Geranyl acetate (1.60%) Geraniol (0.12%) Eugenol (3.55%) Chavicol (0.23%)	Hasegawa *et al.*, 1997.

O. basilicum L. cv. 'Genovese'	Herb	α-pinene (0.21%) Camphene (0.06%) β-pinene (0.50%) δ-3-carene (0.29%) Myrcene (0.60%) α-phellandrene (0.01%) α-terpinene (0.01%) Limonene (0.24%) 1,8-cineole (4.72%) (Z)-β-ocimene (0.07%) γ-terpinene (0.02%) (E)-β-ocimene (1.43%) p-cymene (0.03%) Terpinolene (0.16%) (Z)-3-hexenyl acetate (0.03%) (Z)-3-hexenol (0.05%) Octyl acetate (0.37%) α-copaene (0.19%) Camphor (0.48%) Linalool (41.56%) Octanol (0.11%) β-caryophyllene (9.06%) Methyl chavicol (0.65%) α-terpineol (1.10%) δ-guaiene (1.78%) Geranyl acetate (2.28%) Geraniol (0.17%) Eugenol (14.81%) Chavicol (0.08%)	Hasegawa *et al.*, 1997.
O. basilicum L.	,,	α-pinene (0.5%) Camphene (0.3%) Sabinene (0.1%) β-pinene (0.6%) Myrcene ((0.2%) Decane (tr.) p-cymene (tr.) 1,8-cineole (2.8%) Limonene (0.5%) Cis-β-ocimene (tr.) Trans-β-ocimene (0.9%) Fenchone (0.2%) Terpinolene (tr.) Linalool (1.1%) α-fenchol (tr.) Camphor (0.7%) Menthone (0.3%) Isomenthone (tr.) Borneol (0.1%) Menthol (0.4%) Estragole (86.1%) Trans-anethole 0.1%) Bornyl acetate (0.2%) Menthyl acetate (tr.) Methyl eugenol (0.5%) β-elemene (0.3%)	Baratta *et al.*, 1998.

O. basilicum L.	Herb	β-caryophyllene (0.1%) Trans-β-bergamotene (1.9%) α-humulene (0.1%) Trans-β-farnesene (0.1%) Cis-β-farnesene (0.2%) γ-cadinene (0.3%) Spathulenol (0.2%) T-cadinol (0.3%)	Baratta *et al.*, 1998.
	Leaf	α-pinene (0.6%) Camphene (0.4%) β-pinene (1.3%) p-cymene (0.5%) 1,8-cineole (5.0%) Limonene (1.0%) γ-terpinene (0.3%) Linalool (39.3%) Camphor (0.9%) Borneol (0.6%) Terpinen-4 ol (1.8%) Methyl chavicol (1.9%) Octyl acetate (2.3%) Bornyl acetate (0.9%) δ-elemene (3.4%) α-copaene (0.6%) β-bourbonene (0.5%) β-cubebene (2.0%) β-elemene (0.8%) β-caryophyllene (0.8%) Trans-α-bergamotene (4.0%) α-humulene (1.6%) Bicyclogermacrene (0.7%) Germacrene A (1.0%) δ-guaiene (0.4%) γ-cadinene (7.7%) Humulene epoxide II (1.5%) 1-epi-cubenol (2.3%) α-muurotol (11.0%) β-eudesmol (0.5%)	Silva *et al.*, 1998.
	,,	Neral (40.0-42.9%) Geranial (42.8-47.6%) Linalool (3.8-4.9%)	Tansi & Nacar, 2000
var.' *k. maras*'	,,	1,8-cineole (4.10-14.06%) Linalool (44.35-60.00%) Methyl cinnamate (0.18-37.44%) Methyl eugenol (1.98-28.26%)	Nacar & Tansi, 2000

O. basilicum L. var. *'hatay'*	Herb	1,8-cineole (3.49-38.08%) Linalool (4.28-36.5%) Methyl cinnamate (7.03-54.47%) Methyl eugenol (0-25.64%)	
var. *'German'*	,,	1,8-cineole (3.07-9.23%) Linalool (29.14-80.50%) Methyl cinnamate (4.07-45.25%) Methyl eugenol (0.4-5.2%)	Nacar & Tansi, 2000

MATERIALS AND METHODS

1. *In vitro* multiplication

Plants of *O. basilicum* L. var. *purpurascens* Benth. were collected from Kannur district of Kerala, South India and authenticated at Calicut University Herbarium (CALI 51323). The collected plants were grown in the net house of Botany Department. Nodal segments (1-1.5 cm) from four-month old potted plants were used as explants to initiate the cultures. Small cut twigs were brought to the laboratory in water. Expanded leaves were removed and stems were washed in Labolene detergent for 10 min. and later washed thoroughly under running water after a quick rinse in 70% alcohol. Surface sterilization was carried out with 0.1% $HgCl_2$ for 5-7 min. These stems were then rinsed with double distilled sterile water and implanted on to the nutrient medium.

Murashige and Skoog (1962) basal medium (Table. 1) with 3% sucrose, 100 mg/l myoinositol and 1% agar was used. The induction media consist of basal MS medium supplemented with different concentrations and combinations of auxins and cytokinins, and cytokinin alone. The p^H of the medium was adjusted to 5.7-5.8 before dispensing into culture tubes. Media were sterilized at 122^0 C for 15 min. Each experiment was set up with 10-12 replicates and repeated twice. The cultures were grown at 25 ± 3^0 C with humidity of 55-60% under fluorescent day light tubes emitting 2000 lux for 16/8 hrs. light/dark period and were subcultured every 4-6 weeks. Leaf explants were collected from *in vitro* grown plants. The callus generated from leaf in agarified medium was used as explant for liquid culture.

Establishment of plants in soil

Four to six weeks old regenerated plantlets were subcultured to half MS medium for rooting. The rooted plants were removed from the tube and were potted in a sterilized mixture of soil and sand (1:1). They were initially irrigated with half strength MS solution for one week. Established plants were transplanted to the field in earthen pots and watered regularly. The morphologically variant somaclones were isolated and used for further analysis.

Table. 1. Murashige and Skoog (1962) basal medium.

	Stock Chemicals	mg/l	Stock Concentration	Stock
I	NH_4NO_3	1650.00	50x	82.50 g/l
	KNO_3	1900.00		95.00 g/l
	KH_2PO_4	170.00		8.50 g/l
	$MgSO_4.7H_2O$	370.00		18.50 g/l
II	$CaCl_2.2H_2O$	440.00	50x	22.00 g/l
III	Na_2EDTA	37.30	100x	3.70 g/l
	$FeSO_4.7H_2O$	27.80		2.80 g/l
IV	$MnSO_4.4H_2O$	22.30	100x	2.23 g/l
	$ZnSO_4.7H_2O$	8.60		860 mg/l
	H_3BO_3	6.20		620 mg/l
	KI	0.83		83 mg/l
	$Na_2MoO_4.2H_2O$	0.25		25 mg/l
	$CoCl_2.6H_2O$	0.025		2.5 mg/l
	$CuSO_4.5H_2O$	0.025		2.5 mg/l
V	VITAMINS			
	Glycine	2.00	100x	200 mg/l
	Nicotinic acid	0.50		50 mg/l
	Pyridoxine – HCl	0.50		50 mg/l
	Thiamine – HCl	0.10		10 mg/l

2. Cytological Analysis

Somatic chromosome spreads were prepared with the help of improved techniques (Sharma & Sharma, 1990). Young root tips were collected from vegetative cuttings grown in the pots at the period showing peak mitotic activity (8.30-9.00 am). The root tips were thoroughly washed in distilled water and treated in pre-treatment chemicals. Saturated solution of para-dichlorobenzene with a trace of aesculin was used as pre-treatment chemical. Small quantity of saponin was also added to remove the oil particles from the cells. The pre-treatment solution is initially chilled to 0-5^0 C for 4-5 min. and root tips were treated at 12-15^0 C for 2½-3 hrs. Then the treated root tips were washed in water and fixed in 1:3 acetic acid:ethanol mixture (Carnoy's fluid) over night.

Pre-treated root tips were washed in distilled water followed by treatment in 1N HCl for 15 min. at room temperature. The root tips were again thoroughly washed to remove last trace of acid. After acid treatment materials were stained in 2% aceto-orcein stain for 3-4 hrs. at room temperature. Stained root tips were washed in 45% acetic acid to remove excess stain and then squashed in 45% acetic acid. The photomicrographs were taken using LEICA GALEN III Microscope attached with Pentax photosystem.

In somaclonal variants the leaf meristem was used instead of root meristem for mitotic preparation with the same pre-treatment procedure.

Karyomorphological studies

Chromosome Image Analysis System (CHIAS)

Karyograms were generated with the aid of computer-based programs such as Adobe Photoshop, Auto CAD and a data based analysing system (MicroSoft Excel). Photographs were scanned and stored as digital images. These digital images were converted into greyscale images using Photoshop program. Identification numbers were allotted to each chromosome and then loaded to Auto CAD for karyomorphometric analysis. After determining the centromeric position, arm lengths of each chromosome were measured and centromeric indices were calculated. On the basis of arm ratio and centromeric indices, homologous chromosomes were identified and were classified (Table. 2) according to Abraham and Prasad (1983). The images were reloaded to Photoshop and karyotypes were generated.

Karyotype formula was calculated depending upon the length of chromosome, position of the centromere and presence or absence of the secondary constriction.

Disparity Index (DI) of the chromosomes were calculated after Mohanty *et al.* (1991), by using the formula:

$$DI = \frac{\text{Longest chromosome - Shortest chromosome}}{\text{Longest chromosome + Shortest chromosome}} \times 100$$

The Variation Coefficient (VC) among the chromosome complements was determined after Verma (1980) as follows:

$$VC = \frac{\text{Standard deviation}}{\text{Mean length of chromosomes}} \times 100$$

Table. 2. Details of chromosome nomenclature in relation to centromere location based on arm ratios and centromeric index (Abraham & Prasad, 1983).

Nomenclature	Notation	R_1 s/l	R_2 l/s	I_1 100s/c	I_2 100l/c
Median	M	1.00	1.00	50.00	50.00
Nearly median	Nm	0.99 to 0.61	1.01 to 1.63	49.99 to 38.01	50.01 to 61.99
Nearly submedian	Nsm (-)	0.60 to 0.34	1.64 to 2.99	38.00 to 25.01	62.00 to 74.99
Submedian	SM	0.33	3.00	25.00	75.00
Nearly submedian	Nsm (+)	0.32 to 0.23	3.01 to 4.26	24.99 to 18.20	75.01 to 81.80
Nearly subterminal	Nst (-)	0.22 to 0.15	4.27 to 6.99	18.19 to 12.51	81.81 to 87.49
Subterminal	ST	0.14	7.00	12.50	87.50
Nearly subterminal	Nst (+)	0.13 to 0.07	7.01 to 14.38	12.49 to 5.01	87.51 to 94.99
Nearly terminal	Nt	0.06 to 0.01	14.39 to 19.99	5.00 to 0.01	95.00 to 99.99
Terminal	T	0	α	0	100

The Total Forma percentage (TF%) or mean centromeric index value was calculated after Huziwara (1962) by the formula:

$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

3. Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD method reveals sequence polymorphisms between template DNAs based on the selective amplification of DNA sequence. The template DNA can be prepared with any DNA purification protocol appropriate for the biological sample under study.

DNA Extraction and RNase treatment

Total DNA was extracted from leaf tissues of *O. basilicum* L. var. *purpurascens* Benth. and its somaclonal variant following CTAB method of Doyle and Doyle (1987) with minor modifications (Ausubel *et al.,* 1995) as described below:

1. Grind the young leaves (5 g) in liquid nitrogen with mortar and pestle and mix with preheated (65^0 C) 25 ml of CTAB buffer (100 mM Tris-HCl (p^H 8), 1.4 M NaCl, 20 mM EDTA (p^H 8), 2% CTAB, 0.2% mercaptoethanol, 10 μl RNase).

2. Incubate the mixture at 60^0 C for 30 to 60 min. in a water bath. Then add to it an equal volume of chloroform: isoamyl alcohol (24:1), mix thoroughly and centrifuge at 10,000 rpm for 10 min. at 4^0 C in a refrigerated centrifuge (Hitachi, Himac CR21, Japan).

3. Transfer the supernatant (aqueous phase) from top of the tube to a new centrifuge tube and then add 2/3 volume of ice-cold isopropyl alcohol and mix by gentle inversions.

4. After incubating the mixture at –20^0 C for 20 min. to enhance the precipitation of DNA, centrifuge at 10,000 rpm for 15 min. at 10^0 C.

5. Discard the supernatant and invert the tube on a paper towel for few minutes. Then rehydrate the pellets in 15 ml of TE buffer (10 mM Tris-HCl, 1mM EDTA, p^H 8).

6. Add 10 μl of RNase and incubate at 37^{0}C for 30 minutes.

7. Centrifuge at 10,000 rpm for 10 min. at 10^0 C after thorough but gentle mixing with equal volume of phenol (p^H 7).

8. Add equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the supernatant, mix gently and centrifuge at same conditions as in step 7.

9. Take the aqueous phase and add equal volume of chloroform: isoamyl alcohol (24:1), shake and centrifuge at 10,000 rpm for 10 min.

10. To the supernatant add one-tenth volume of 3 M sodium acetate (p^H 5.2) and 2.5 volumes of absolute ethanol and incubate at -20^0 C for one hour or at -70^{0}C for 30 min.

11. Centrifuge and wash the pellets in 70% ethanol. Air dry the pellets and dissolve in TE buffer and estimate the yield.

DNA was quantified and estimated spectrometrically (Shimadzu, UV 160A, Japan). 15 μl of isolated DNA is diluted in 3 ml of water and its optical density at wavelength 260 nm was noted. From the data obtained the quantity of DNA is calculated using the formula:

Quantity of DNA (in micrograms) = A_{260} x quantity of DNA which corresponds to optical density one

Where A_{260} is the optical density of the given sample at 260 nm UV.

Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)

The Arbitrarily Primed PCR (AP-PCR) is a modification of the PCR that generates informative genomic finger printing. AP-PCR combines PCR and primers of arbitrary sequence to amplify genomic DNA and produce a fingerprint. For the present reaction 15 different 10-mer oligonucleotide primers of OPX series, Operon Technologies Inc., Almeda, USA were used and sequence of each primer is given in the table 7.

The PCR was performed in 25 μl reaction mixture containing 20-30 ng of genomic DNA, 100 μM of each of the four dNTPs, 0.2 μM of each primer, 0.5 units of DNA polymerase, 2.5 μl of 10x reaction buffer (10 mM 3- Tris (hydroxymethyl) methyl amino propane sulphonic acid p^H 8, 1.5 mM $MgCl_2$, 50 mM KCl) and water up to 25 μl. The reaction mixtures were overlaid with 15 μl of mineral oil. Amplification was performed in an OmniGene thermal cycler (Hybaid, UK) under

programmed cycling conditions as follows: 1 cycle of 4 min. at 94° C; 45 cycles of 1 min at 94° C, 1 min. at 36° C and 2 min. at 72° C; 1 cycle at 72° C for 10 min. 10 µl of each reaction product was subjected to electrophoresis (99 V for 2hrs.) in a 1.5% agarose gel in 1x TBE (89 mM Tris borate, 10 mM EDTA, p^H 8) and the DNA bands were stained by 6 µl of ethidium bromide. The gels were then photographed on an UV transilluminator. EcoR I + Hind III double digest of the λ phage DNA (Genei, Bangalore) was used as the molecular weight standard.

4. Essential oil Analysis

A. Gas Chromatography – Mass Spectrometry (GC-MS)

The fresh aerial plant materials of *O. basilicum* L. var. *purpurascens* Benth. and somaclonal variant with flowers were collected. Flaked and powdered plant materials were hydrodistilled separately in a Clevenger apparatus for 4 hrs. at 100° C. The quantity of the essential oil was measured and isolated oil was dried over anhydrous sodium sulphate and stored in a small amber coloured bottle at 4-6° C.

GC-MS was carried out on a Shimadzu QP-2000 instrument at 70eV and 250° C. GC column: ULBON HR-1, fused silica capillary 0.25 mm x 50m with film thickness 0.25 µ; Carrier gas: Helium; Flow rate: 2 ml/min.; Temperature programme: Initial temperature was 100°C for 1 min. and then heated at the rate of 5° C per min. to 250° C. Mass spectral identification was based on published spectra (de Brauw *et al.*, 1979-1988).

B. Scanning Electron Micrography (SEM)

Glandular trichomes are regarded as the solo sites of terpene biosynthesis in the leaves of essential oil plants (Colson *et al.*, 1991). The leaves of *O. basilicum* L. var. *purpurascens* Benth.somatic as well as somaclonal variant were examined under scanning electron microscope for identification of the types and distribution of foliar glands.

Air-dried leaf samples were mounted on specimen stubs using Scotch 3M double adhesive tapes. Samples were coated with gold in a Hitachi vacuum evaporator model HUS 5 GB to a thickness of 200 A°. Coated samples were viewed in a Hitachi Scanning Electron Microscope (Model S. 450, Japan) operated at 15 KV and photographed. Each specimen was photographed at three different magnifications of x60, x90 and x300.

RESULTS

1. *In vitro* multiplication

The MS medium was used with varied hormonal combinations, for the initiation of multiple shoot cultures. Among the different explants tested (leaves and nodes) only the nodal explant responded positively.

For the induction of multiple shoot regeneration, explants were inoculated in the medium with the combinations of cytokinins and auxins, and cytokinin alone. The nodal segments were cultured on MS medium supplemented with different concentrations of BAP and KIN with IAA and NAA (Tables 3 & 4). The multiple shoot initiation was noticed in the medium with 1 mg/l KIN and 0.2 mg/l NAA (Fig. 1.1). About 20% of the cultures produced 6-8 shoots from each node. The frequency of shoots and percentage of initiation was higher in the medium containing 2 mg/l BAP and 0.3 mg/l IAA. 70% of the total cultures produced 8-12 shoots per culture (Fig. 1.2). Most of the combinations of KIN with NAA, culture establishment were in the form of elongation of shoots arising from the axillary buds in the nodal segments. Whereas the combinations with BAP produced only a little callus and occasional shoot elongation. The nodal cultures in the medium containing KIN and NAA produced morphologically variant plantlets (Figs. 1.11 & 1.12) with three leaves at each node (Table 3). These mutants were used for further analysis.

Leaf explants responded differently in various combinations of hormones. It produced very little callus in the medium containing BAP and IAA and responded by swelling (Fig. 1.3). In the medium with BAP (0.5 mg/l) and NAA (2 mg/l) it produced white granular callus (Fig. 1.5) within 10–12 days. After three weeks, cultures started to produce roots but no shoots (Fig. 1.6). 1 mg/l KIN and 3 mg/l NAA initiated yellow coloured friable callus from the leaves (Fig. 1.7). But higher concentrations of KIN (2 mg/l KIN + 2 mg/l NAA) resulted in compact and firm green callus from leaves (Fig. 1.9). The leaf callus were subcultured to liquid media, one containing 1 mg/l BAP + 0.5 mg/l 2, 4-D and the other with 1 mg/l BAP + 0.2 mg/l 2, 4-D for callus proliferation. Among the two media the second one produced large amount of callus on a shaker after two months. These calli were subcultured for organogenesis on the medium containing 1 mg/l BAP (Fig. 1.8). In some cases the root initiation (4-6 numbers) was noticed from the callus (Fig. 1.10) but shoot regeneration was absent.

The clumps of multiple shoots were separated and subcultured for rooting on ½ MS medium (Fig. 1.4). Rooted plantlets were transferred to the sterilized soil:sand mixture (Fig. 1.13). About 95% field survival was obtained in the pots. The gross morphological variations were noticed. Mutants with three leaves at each node produced normal axillary branches (Fig. 1.14). The leaf size of the donor plant varies from 79x43 mm to 54x21 mm (Fig.1.17A) and that of the somaclonal variant is 110x60 mm to 60x31 mm (Fig. 1.17B). Meristems at each node of the main stem were active and produced three axillary branches and suppressed the growth of the main meristem. On removing the axillary branches the meristem continued its growth until it loses its activity (Fig. 1.16). Delayed flowering is noticed in axillary branches (after 6 months) compared to the normal (3-4 months). The main stem did not produce flowers and exhibited stunted growth after one month (Fig. 1.15). Both the somaclonal variants and natural plants were seedless.

Table. 3. The effect of KIN and NAA on shoot multiplication and induction of somaclones on *in vitro* nodal explants.

KIN mg/l	NAA mg/l	% frequency of response		Nature of response	% of soma-clones
		Shoot	Callus		
2	4	10	60	2 axillary branches	0
2	2	10	40	Single shoot	0
2	0.5	20	20	Single shoot	5
2	0.2	35	20	2-3 shoots, axillary branching, callus at cut ends.	0
1	3	20	60	Rooting of explants	10
1	2	20	30	Rooting	0
1	0.4	--	50	Rooting	0
1	0.3	30	10	2-3 axillary branches, callus at cut ends.	5
1	0.2	60	--	Multiple shoot with pronounced axillary branches	5
1	0.1	35	--	2 shoots	5
0.5		80	--	Axillary shoot elongation.	0

Table. 4. Effect of phytohormones on shoot multiplication from *in vitro* nodal explants.

BAP mg/l	NAA mg/l	IAA mg/l	2,4-D mg/l	% frequency of response		Nature of response
				Shoot	Callus	
2		0.1		70	--	2 shoots
2		0.3		80	--	Multiple shoots; callus at cut ends.
2		0.5		30	--	2 shoots.
2		1		--	--	Little callus at cut ends.
1			0.5	--	100	Yellow friable callus
1			0.2	--	100	,,
1	0.5			30	50	2 shoots; rooting of explant.
0.5	2			40	50	,,
1				80	--	Elongation of axillary branches
0.5				80	--	,,

Figs. 1.1 & 1.2. Multiple shoot induction from nodal explants of *O. basilicum* L. var. *purpurascens* Benth.

Fig. 1.1. MS medium with 1 mg/l KIN+ 0.2 mg/l NAA.

Fig. 1.2. MS medium with 2 mg/l BAP+0.3 mg/l IAA.

Fig. 1.4. Rooting of the isolated plantlet in ½ MS medium.

Figs. 1.3 & 1.5 to 1.10. Callus formation from the leaf explants of *O. basilicum* L. var. *purpurascens* Benth.

Fig. 1.3. Swelling of the explant in the MS medium with 2 mg/l BAP+0.5 mg/l IAA

Fig. 1.5. White granular callus from MS medium containing 0.5 mg/l BAP+2 mg/l NAA.

Fig. 1.6. Root initiation from the callus.

Fig. 1.7. Yellow friable callus from MS medium containing 1 mg/l KIN+3 mg/l NAA.

Fig. 1.8. Callus proliferation in MS medium containing 1mg/l BAP.

Fig. 1.9. Firm green callus from MS medium containing 2 mg/l KIN+2 mg/l NAA.

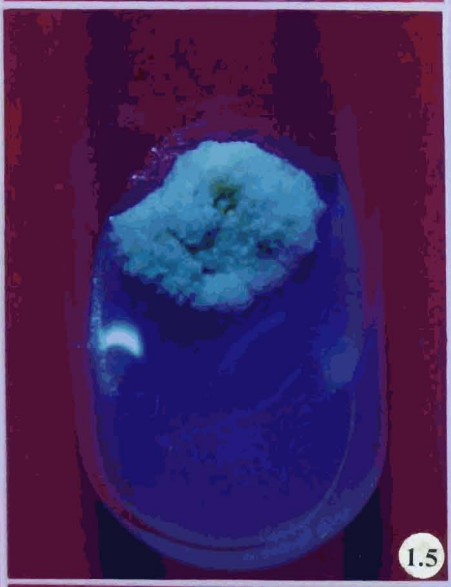
Fig. 1.10. Root initiation from the callus.


1.1


1.2


1.3


1.4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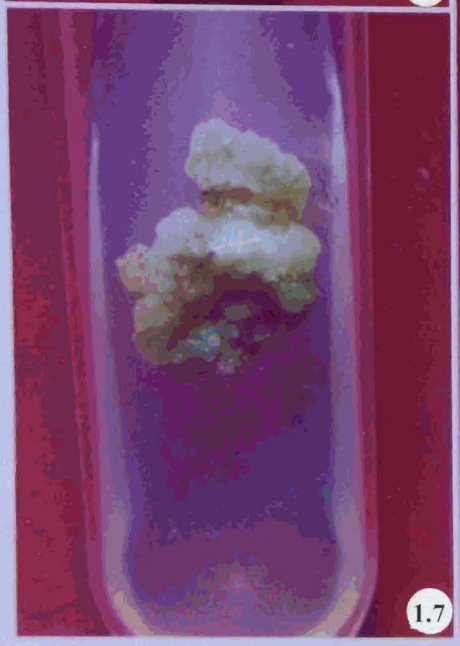
1.6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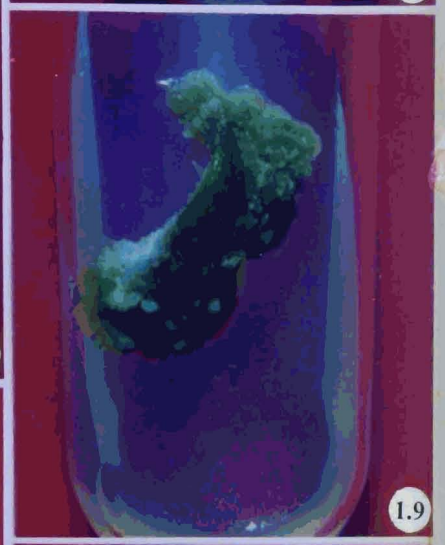
1.8


1.9


1.10

Figs. 1.11 to 1.16. Growth stages of somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth.

Fig. 1.11. Somaclonal variant with three leaves at each node (1 mg/l KIN+0.2 mg/l NAA)

Fig. 1.12. Root induction in ½MS medium.

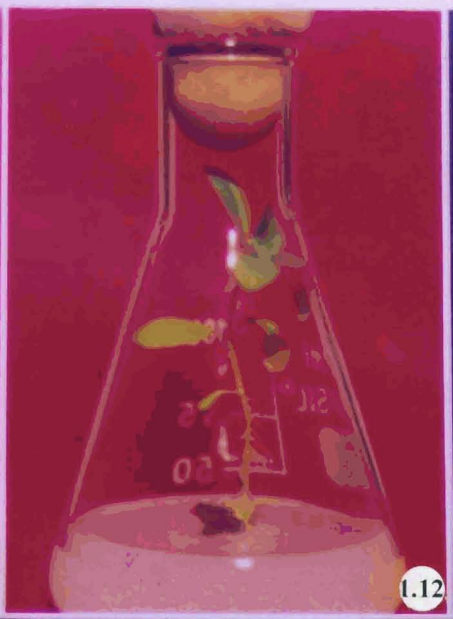
Fig. 1.13. Plantlet of the variant 2 weeks after transplantation.

Fig. 1.14. The variant one month after transplantation producing normal two leaved axillary branches.

Fig. 1.15. & 1.16. The main stem of the variant in which the apical meristem ceased its activity.

Fig. 1.17. Leaf size variation in *O. basilicum* L. var. *purpurascens* Benth. (A) and its somaclonal variant (B).


1.11


1.12


1.13


1.14


1.15


1.16

1.17

2. Cytological Analysis.

The present study is aimed to examine the karyomorphological changes in the somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth. and to know the cytological basis of variation. The ploidy levels of both, the donor plant and the variant were invariably tetraploid (2n=4x=48). Neither chimeral nor aneuploid variations were found. Chromosome sets of normal diploid and somaclonal variant are shown in figs. 2.1 & 2.2. Chromosome morphology of regenerated plant showed slight variation. Some of the chromosomes did not show the exact duplication of the standard chromosomes and exhibited structural changes, there by change in basic karyotype also. The somaclonal variant showed increase in total chromosome length and average chromosome length (Tables. 5 & 6). Changes in DI, VC and TF% were also noticed. The karyotype formulae deduced from both the donor ($A_4B_{40}C_4$) as well as the somaclonal variant ($A_4B_{34}C_{10}$) showed increase in the nearly submedian chromosomes at the cost of nearly median chromosomes in the latter.

The general description of the common chromosome types found in the investigated plant and its somaclonal variant are given below.

Type A: Chromosomes with two constrictions ranging from 2.6775 μm to 2.1245 μm in size and with nearly median (Nm) primary constriction.

Type B: Chromosomes ranging from 1.9238 μm to 1.2220 μm in size with nearly median (Nm) primary constriction

Type C: Chromosomes ranging from 1.7206 μm to 1.4298 μm in size with nearly submedian (Nsm-) primary constriction.

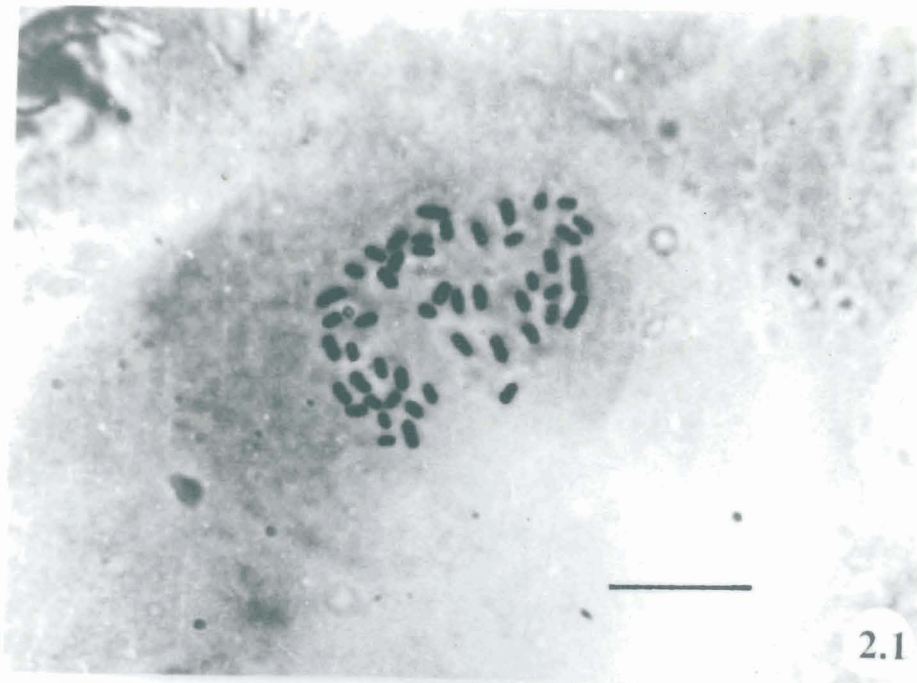
Diagrammatic representation of the different chromosome types observed in the present investigation is shown in Fig. 2.3. Detailed karyotypic description, computer image of karyotypes (Figs. $2.1A_1$-$2.1A_3$ & $2.2A_1$-$2.2A_3$), karyograms (Figs. 2.1B & 2.2B) and idiograms (Figs. 2.1C & 2.2C) are given as follows.

Figs. 2.1 & 2.2. Mitotic metaphases

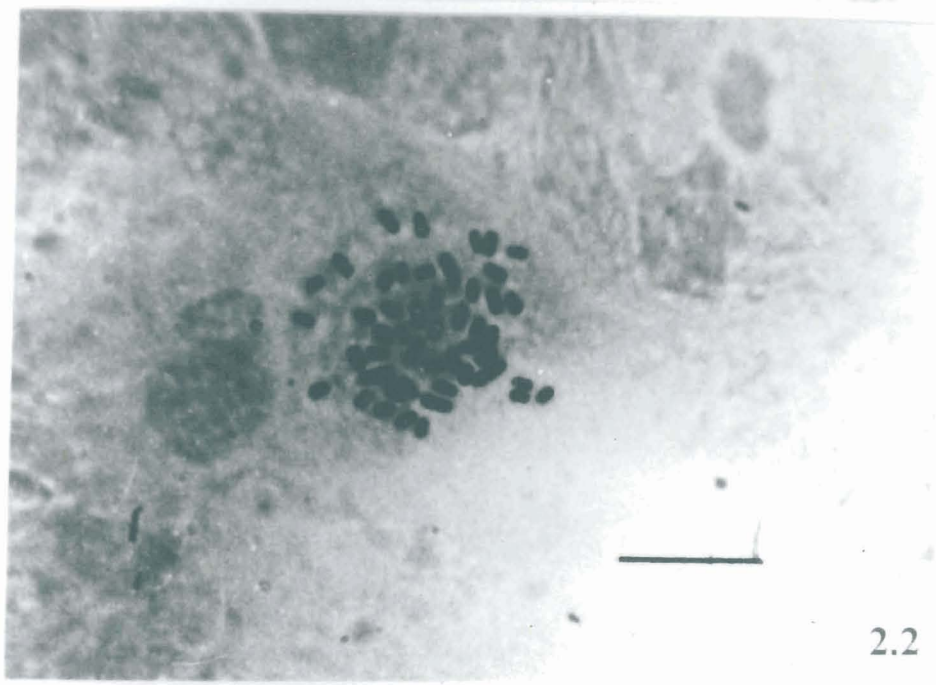
Fig. 2.1. *Ocimum basilicum* L. var. *purpurascens* Benth. (2n=48)

Fig. 2.2. *O. basilicum* L. var. *purpurascens* Benth. (Somaclonal variant) (2n=48)

Bar=10μm



2.1



2.2

Ocimum basilicum L. var *purpurascens* Benth.

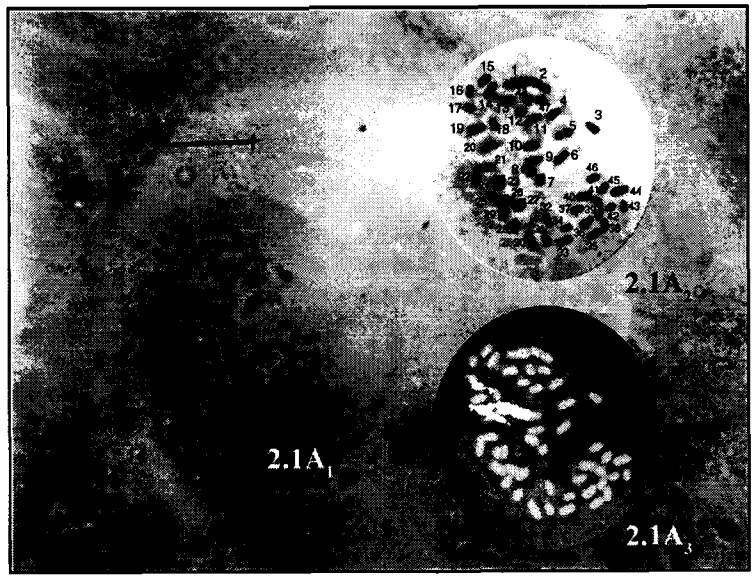
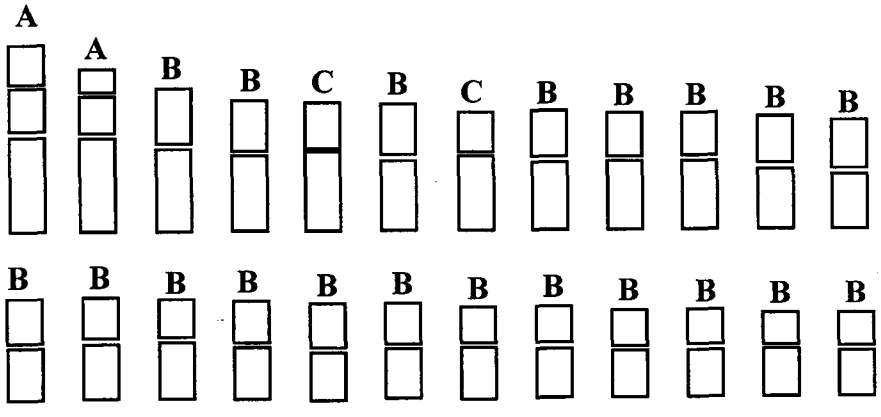


Fig. 2.1A$_1$: Computer scanned original image (Bar=10μm)
Fig. 2.1A$_2$: Resolved image
Fig. 2.1A$_3$: Inverted image

Karyogram. 2.1B



Idiogram. 2.1C

Ocimum basilicum L. var. *purpurascens* Benth. ($2n=2x=48=A_4B_{40}C_4$)

Normal somatic chromosome number : 48
Chromosome pairs with secondary constriction : 2
Range of chromosome length : 2.4242 µm – 1.1340 µm
Average chromosome length : 1.4908 µm
Total chromosome length : 71.5602 µm
Disparity Index (DI) : 36.26
Variation Coefficient (VC) : 21.0961
TF value (%) : 42.41

Table. 5. Detailed Karyomorphometrical Data

Chromo-some Type	No. of pairs	Total length (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of primary constriction
A*	1	2.4242	1.1468	1.2774	0.8970	1.1139	47.31	52.69	Nm
A*	1	2.1245	0.8599	1.2645	0.6800	1.4705	40.48	59.52	Nm
B	1	1.8904	0.7693	1.1211	0.6862	1.4573	40.70	59.30	Nm
B	1	1.7334	0.7037	1.0297	0.6834	1.4633	40.60	59.40	Nm
C	1	1.7206	0.6387	1.0819	0.5904	1.6939	37.12	62.88	Nsm(-)
B	1	1.6556	0.7037	0.9519	0.7393	1.3527	42.50	57.50	Nm
C	1	1.5771	0.5606	1.0165	0.5515	1.8117	35.55	64.45	Nsm(-)
B	1	1.5643	0.6387	0.9256	0.6900	1.4492	40.83	59.17	Nm
B	1	1.5514	0.6130	0.9384	0.6532	1.5308	39.51	60.49	Nm
B	1	1.5386	0.5995	0.9391	0.6384	1.5665	38.96	61.04	Nm
B	1	1.4729	0.6516	0.8213	0.7934	1.2604	44.24	55.76	Nm
B	1	1.4337	0.6779	0.7558	0.8969	1.1149	47.28	52.72	Nm
B	1	1.3687	0.6387	0.7300	0.8749	1.1429	46.66	53.34	Nm
B	1	1.3552	0.5866	0.7686	0.7632	1.3103	43.29	56.71	Nm
B	1	1.3424	0.5474	0.7950	0.6886	1.4523	40.78	59.22	Nm
B	1	1.3295	0.5995	0.7300	0.8212	1.2177	45.09	54.91	Nm
B	1	1.3031	0.6258	0.6773	0.9240	1.0823	48.02	51.98	Nm
B	1	1.2903	0.5866	0.7037	0.8336	1.1996	45.46	54.54	Nm
B	1	1.2517	0.5217	0.7300	0.7147	1.3993	41.68	58.32	Nm
B	1	1.2124	0.5217	0.6907	0.7553	1.3239	43.03	56.97	Nm
B	1	1.1861	0.5217	0.6644	0.7852	1.2735	43.98	56.02	Nm
B	1	1.1732	0.5088	0.6644	0.7658	1.3058	43.37	56.63	Nm
B	1	1.4168	0.4696	0.6772	0.6934	1.4421	40.95	59.05	Nm
B	1	1.1340	0.4824	0.6516	0.7403	1.3507	42.54	57.46	Nm

* Chromosome pairs with secondary constriction

s: Short arm; **l**: Long arm; R_1 & R_2: Arm ratios; **I_1 & I_2**: Centromeric indices.

Ocimum basilicum L. var. *purpurascens* Benth.
(Somaclonal variant)

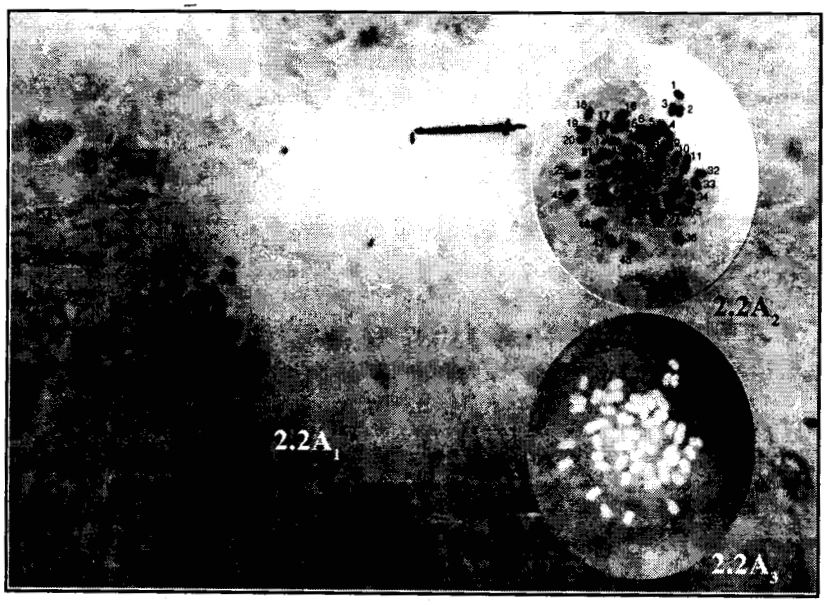
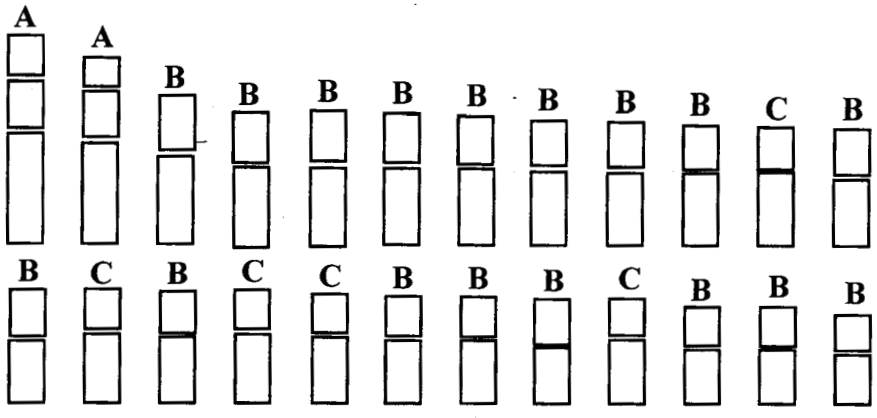


Fig. 2.2A$_1$: Computer scanned original image (Bar=10μm)
Fig. 2.2A$_2$: Resolved image
Fig. 2.2A$_3$: Inverted image

Karyogram. 2.2B



Idiogram. 2.2C

Ocimum basilicum L. var. *purpurascens* Benth. ($2n=2x=48=A_4B_{34}C_{10}$) (Somaclonal variant)

Normal somatic chromosome number	: 48
Chromosome pairs with secondary constriction	: 2
Range of chromosome length	: 2.6775 μm – 1.2220 μm
Average chromosome length	: 1.6332 μm
Total chromosome length	: 78.3922 μm
Disparity Index (DI)	: 37.33
Variation Coefficient (VC)	: 19.4342
TF value (%)	: 39.71

Table. 6. Detailed Karyomorphometrical Data

Chromo-some Type	No. of pairs	Total length (μm)	s (μm)	l (μm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of primary constriction
A*	1	2.6775	1.1957	1.4818	0.8069	1.2393	44.66	55.34	Nm
A*	1	2.3920	1.0398	1.3522	0.7690	1.3004	43.47	56.53	Nm
B	1	1.9238	0.7409	1.1829	0.6263	1.5966	38.51	61.49	Nm
B	1	1.7807	0.6889	1.0918	0.6310	1.5848	38.69	61.31	Nm
B	1	1.7551	0.6889	1.0662	0.6461	1.5477	39.25	60.75	Nm
B	1	1.7159	0.6761	1.0398	0.6502	1.5379	39.40	60.60	Nm
B	1	1.7031	0.6633	1.0398	0.6379	1.5676	38.95	61.05	Nm
B	1	1.6248	0.6242	1.0006	0.6238	1.6030	38.42	61.58	Nm
B	1	1.6120	0.6242	0.9878	0.6319	1.5825	38.72	61.28	Nm
B	1	1.5992	0.6113	0.9879	0.6188	1.6161	38.23	61.77	Nm
C	1	1.5991	0.5978	1.0013	0.5970	1.6750	37.38	62.62	Nsm(-)
B	1	1.5479	0.6370	0.9109	0.6993	1.4300	41.15	58.85	Nm
B	1	1.5344	0.6498	0.8846	0.7346	1.3613	42.35	57.65	Nm
C	1	1.5344	0.5594	0.9750	0.5737	1.7429	36.46	63.54	Nsm(-)
B	1	1.5209	0.5850	0.9359	0.6251	1.5998	38.46	61.54	Nm
C	1	1.5209	0.5466	0.9743	0.5610	1.7825	35.94	64.06	Nsm(-)
C	1	1.4825	0.5459	0.9366	0.5829	1.7157	36.82	63.18	Nsm(-)
B	1	1.4561	0.5594	0.8967	0.6238	1.6030	38.42	61.58	Nm
B	1	1.4561	0.5594	0.8967	0.6238	1.6030	38.42	61.58	Nm
B	1	1.4433	0.6370	0.8063	0.7900	1.2658	44.13	55.87	Nm
C	1	1.4298	0.5202	0.9096	0.5719	1.7486	36.38	63.62	Nsm(-)
B	1	1.3387	0.5594	0.7793	0.7178	1.3931	41.79	58.21	Nm
B	1	1.3259	0.5466	0.7793	0.7014	1.4257	41.22	58.78	Nm
B	1	1.2220	0.5074	0.7146	0.7100	1.4084	41.52	58.48	Nm

* Chromosome pairs with secondary constriction

s: Short arm; l: Long arm; R_1 & R_2: Arm ratios; I_1 & I_2: Centromeric indices.

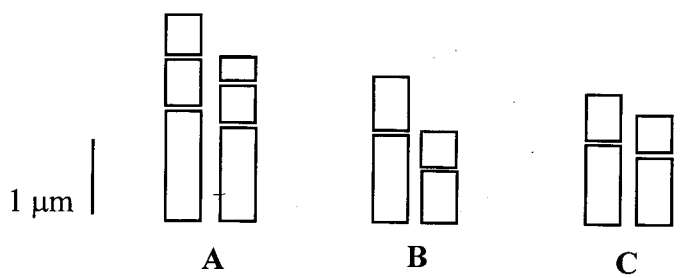


Fig. 2.3. Diagramatic representation of the chromosome types observed in *O. basilicum* L. var. *purpurascens* Benth. and its somaclonal variant.

3. Random Amplified Polymorphic DNA (RAPD) Analysis

To amplify specific DNA segment using PCR, 20-30 ng of template DNA is required. The total DNA extracted is quantified spectrometrically and the DNA required for the reaction mixture preparation is calculated.

Optical density at wavelength 260 nm of the DNA extracted from the donor plant is 0.125 and that of somaclonal variant is 0.109.

So the quantity of DNA in the sample is

0.125x50 μg=6.25 μg and

0.109x50 μg=5.45 μg respectively.

(Where 50 μg is the quantity of DNA that corresponds to optical density one at wavelength 260 nm.)

15 μl of each sample is used for spectrometrical analysis. So, 1 μl contains 6.25/15=0.42 μg/l or 420 ng/μl and 5.45/15=0.36 μg/μl or 360 ng/μl respectively. (1 μg=1000 ng)

Only 20-30 ng of template DNA is required for PCR reaction mixture. So each sample is diluted to 30 times and 2 μl each was used for reaction. 2 μl of DNA of donor plant contains 28 ng and that of somaclonal variant has 24 ng template DNA.

To obtain more information on genetic diversity between somaclonal variant and donor plant RAPD analysis was carried out using 15 primers of arbitrary sequence (Figs. 3.1-3.3). Approximately 100 bands were amplified for each sample. Of the 15 primers used 14 successfully amplified the extracted DNA with consistently reproducible banding. One of the 15, OPX 02 had to be excluded from the final comparison because of the inconsistent amplification (Fig. 3.1). The number of bands resolved per primer ranged from a minimum of 3 to a maximum of 11. The size of amplification product also differed and ranged from 564 bp to 4277 bp.

The RAPD fingerprints of the somaclonal variant differed from that of the donor plant with three primers used. Although both genotypes gave unique products

with every primer, some primers OPX 04 (Fig. 3.1), OPX 15 & OPX 16 (Fig. 3.3) revealed additional bands for somaclonal variant. Altogether these primers generated 13 new RAPD bands in the somaclonal variant that were absent in the donor plant fingerprints, while all the bands that were present in the donor plant fingerprints were scored in the somaclonal variant. In many cases intensity differences between corresponding bands were pronounced.

The primers and the characterization of the consistent bands are listed in table 7. Primer OPX 04 scored three additional bands and primer OPX 15 has 7 additional bands for the somaclonal variant.

However, the primer OPX 16 generated an additional fragment of 4277bp and two other minor bands only from the DNA extracted from the somaclonal variant. This is the single band, which have size greater than 3530bp among the total bands scored.

Table. 7. Nucleotide sequences of the primers selected and characterization of the recorded RAPD markers.

Primer	Sequences (5'-3'):	No. of markers		Size range bp (base pairs)
		A#	B#	
OPX 01	CTGGGCACGA	4	4	564 to 1584
OPX 02	TTCCGCCACC	--	--	--
OPX 03	TGGCGCAGTG	6	6	831 to 3530
OPX 04	CCGCTACCGA	5	8	564 to 3530
OPX 05	CCTTTCCCTC	5	5	564 to 2027
OPX 06	ACGCCAGAGG	11	11	564 to 3530
OPX 07	GAGCGAGGCT	5	5	564 to 1584
OPX 08	CAGGGGTGGA	3	3	831 to 983
OPX 09	GGTCTGGTTG	6	6	831 to 983
OPX 11	GGAGCCTCAG	6	6	564 to 1584
OPX 12	TCGCCAGCCA	3	3	564 to 1584
OPX 13	ACGGGAGCAA	4	4	831 to 1904
OPX 14	ACAGGTGCTG	4	4	831 to 1904
OPX 15	CAGACAAGCC	3	10	564 to 3530
OPX 16	CTCTGTTCGG	3	6	564 to 4277

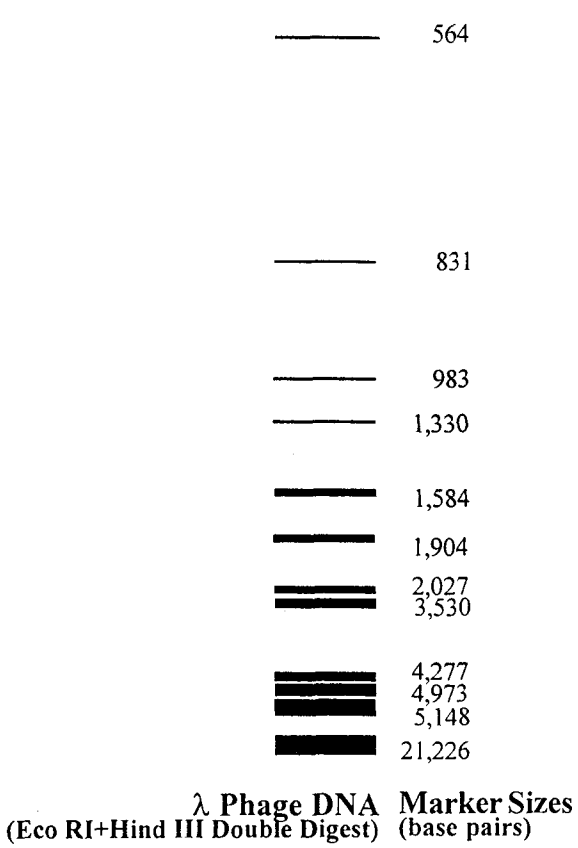
A#. *O. basilicum* L. var. *purpurascens* Benth.; B#. Somaclonal variant.

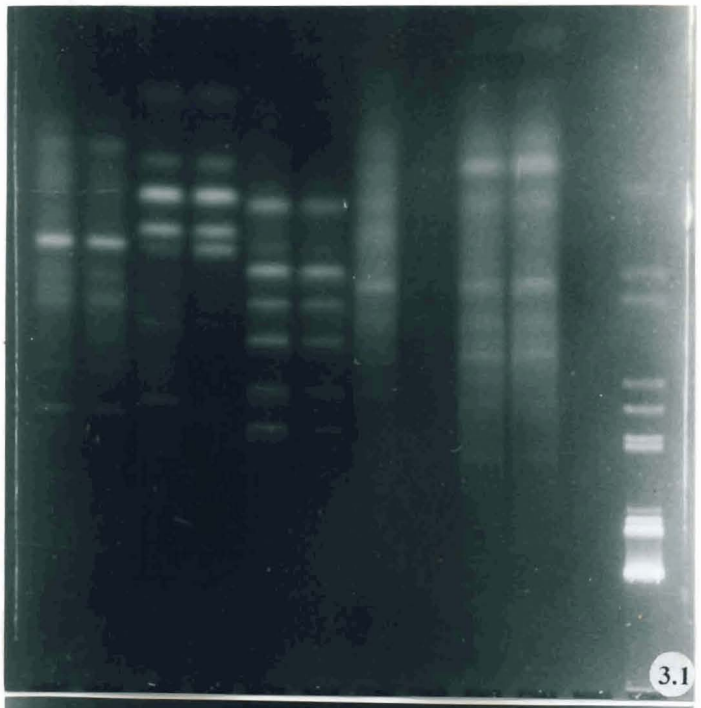
Figs. 3.1 to 3.3. Agarose gel electrophoresis of amplified sequence from a RAPD reaction using primers OPX 01 to OPX 09 and OPX 11 to OPX 16 of *O. basilicum* L. var. *purpurascens* Benth. and its somaclonal variant in alternate lanes. Last lane in each figure is the molecular weight marker (Eco R1+Hind III double digest λ phage DNA)

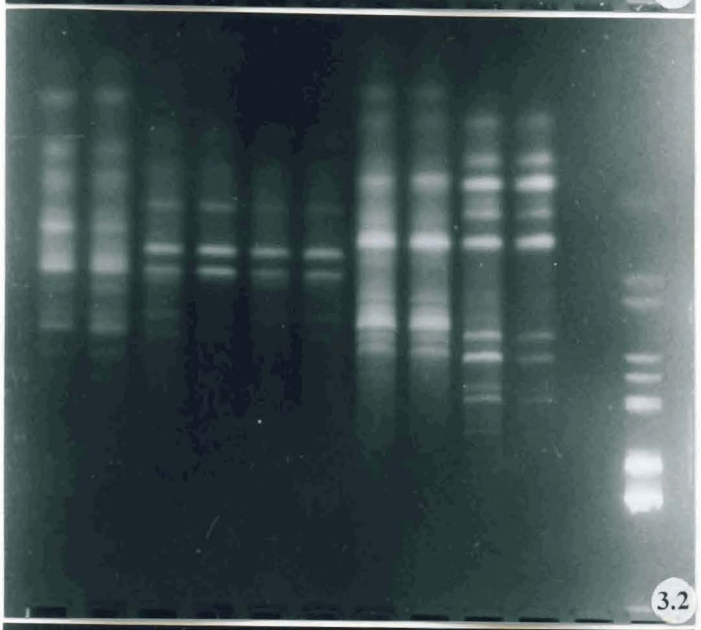
Fig. 3.1. Primers OPX 01 to OPX 05 from weight marker end.

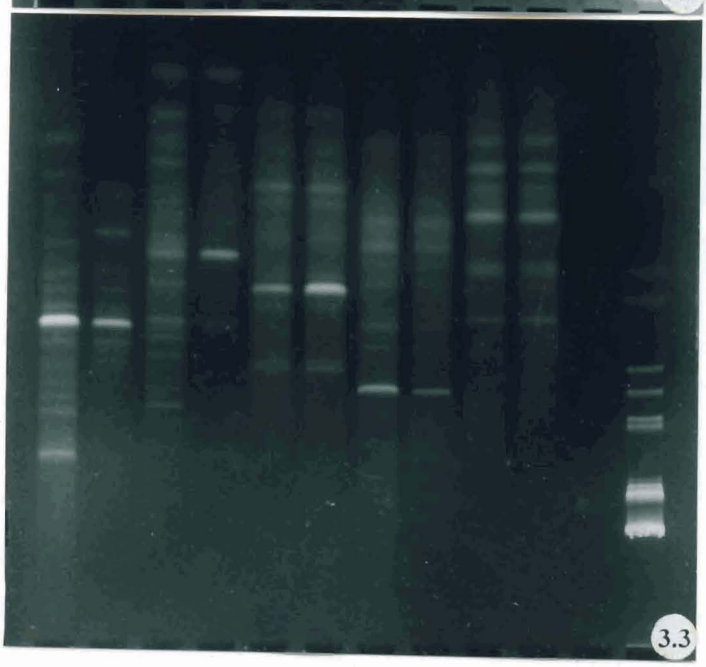
Fig. 3.2. Primers OPX 06 to OPX 09 & OPX 11 from weight marker end.

Fig. 3.3. Primers OPX 12 to OPX 16 from weight marker end.




3.1


3.2


3.3

4. Essential oil Analysis

A. Gas Chromatography-Mass Spectrometry (GC-MS)

Plants obtained from *in vitro* regeneration should be examined to verify its genetic variability and biochemical changes, since it is important for the field performance of regenerants. The present studies on somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth. were undertaken to screen desirable qualities like essential oil composition and yield.

The essential oils of *O. basilicum* L. var *purpurascens* Benth. and its somaclonal variant were analyzed quantitatively and qualitatively. The oil yield of somaclonal variant (0.9%) was higher than that of the donor plant (0.6%). The essential oils were analyzed by GC-MS and 22 compounds were identified which included monoterpenes, sesquiterpenes and phenols. The GC-MS pattern of somaclonal variant essential oil was distinctly different when compared with the pattern of the donor plant oil (Figs. $4A_1$ to $4A_{45}$).

The quantitative and qualitative differences observed in the essential oil directly influenced the colour of the oil distilled. The oil from the *in vivo* grown plant have light yellow colour whereas essential oil of *in vitro* grown plant showed slight dark yellow.

The results of GC-MS analysis of *O. basilicum* L. var. *purpurascens* Benth. and *O. basilicum* L. var. *purpurascens* Benth. (Somaclonal variant) are listed in the table 8.

There is clear difference between the compositions of two oils analyzed. The analysis of the oil samples revealed a range of variation in their constituents. The major components identified from the essential oils of *in vivo* and *in vitro* plants were the same (methyl chavicol, methyl cinnamate and camphor), showing variation in percentage composition of methyl chavicol (37.9% & 38.6%) and camphor (17.5% & 21.8%). The percentage of methyl cinnamate (27.4%) is the same in both oils.

2-butanol (0.5%), camphene (0.6%), limonene (3.3%), γ-terpinene (tr.) and β-bisabolene (tr.) were present in the essential oil of *in vivo* grown plant, whereas

these components were absent in the somaclonal variant. The somaclonal variant is characterized by the presence of cis-sabinene hydrate (0.6%) and alloaromadendrene (tr.), which were absent in the donor plant. In addition to these, two unidentified components were also present in the essential oil of somaclonal variant.

Table. 8. List of essential oil components identified in the present investigation

Components			Composition in %	
			A#	B#
1	2-butanol	Alcohol	0.5	--
2	Camphene	Monoterpene	0.6	--
3	Myrcene	,,	1.7	tr.
4	Cis-sabinene hydrate	,,	--	0.6
5	Limonene	,,	3.3	--
6	Cis-ocimene	,,	1.7	tr.
7	γ-terpinene	,,	tr.	--
8	Methyl chavicol	Phenol	37.9	38.6
9	Camphor	Monoterpene	17.5	21.8
10	Terpinen-4 ol	,,	1.2	1.4
11	Anethole	Phenol	2.8	1.6
12	UI*		--	tr.
13	β-bisabolene	Sesquiterpene	tr.	--
14	Methyl cinnamate	Aromatic Compound	27.4	27.4
15	UI*		1.2	1.2
16	β-caryophyllene	Sesquiterpene	1.4	1.7
17	Alloaromadendrene	,,	--	tr.
18	α-humulene	,,	tr.	tr.
19	Cubebene	,,	tr.	tr.
20	Valencene	,,	tr.	tr.
21	γ-caryophyllene	,,	tr.	tr.
22	α-himachalene	,,	tr.	tr.
23	β-Gurjunene	,,	1.0	1.3
24	Aromadendrene	,,	tr.	0.5
25	UI*		tr.	tr.
26	UI*		--	tr.

A#-*O. basilicum* L. var. *purpurascens* Benth.

B#-Somaclonal variant, UI* unidentified components.

Fig. 4A$_1$. Gas Chromatogram of herb oil of *O. basilicum* L. var. *purpurascens* Benth.

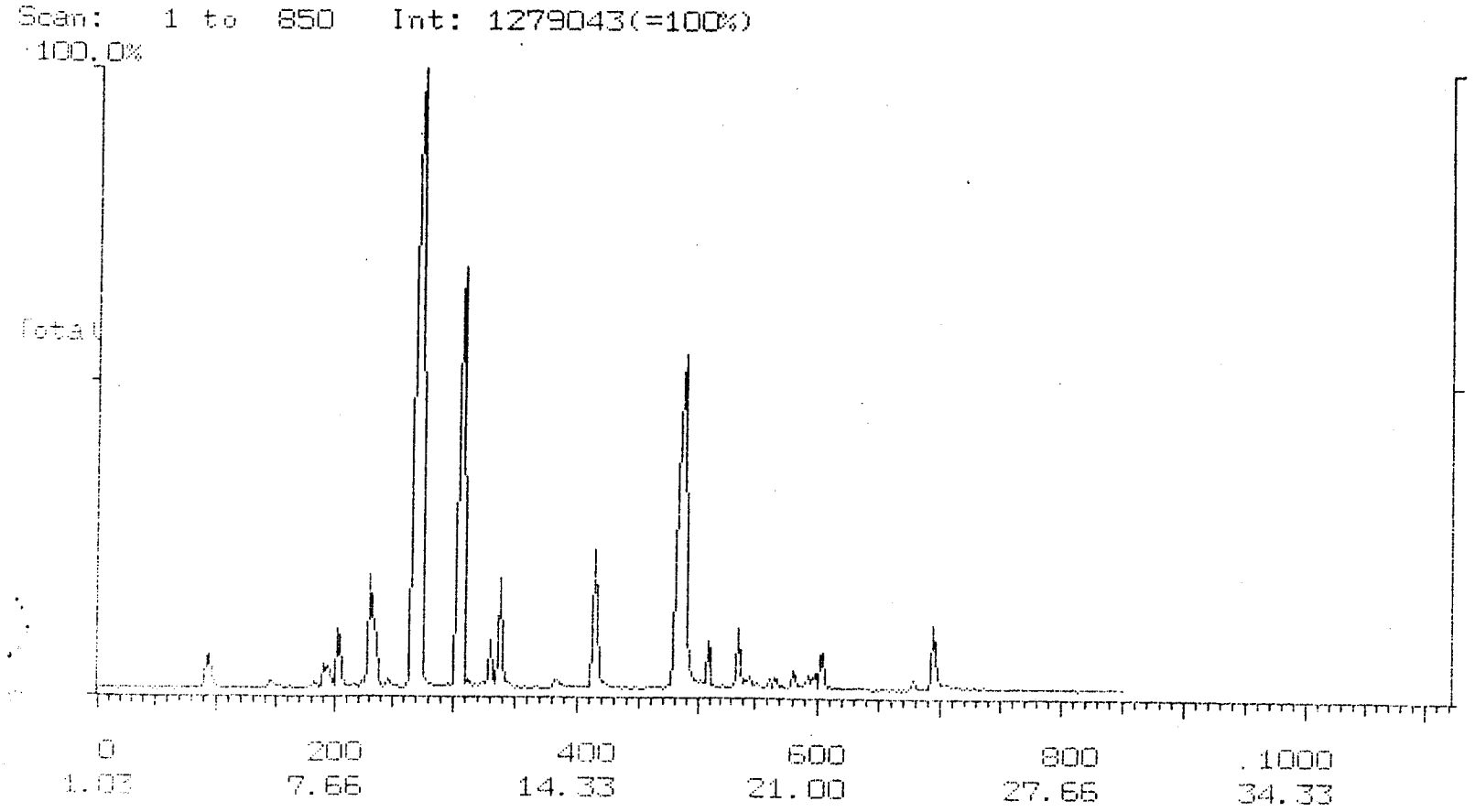


Fig. 4A$_2$. Mass Spectrum of 2-butanol

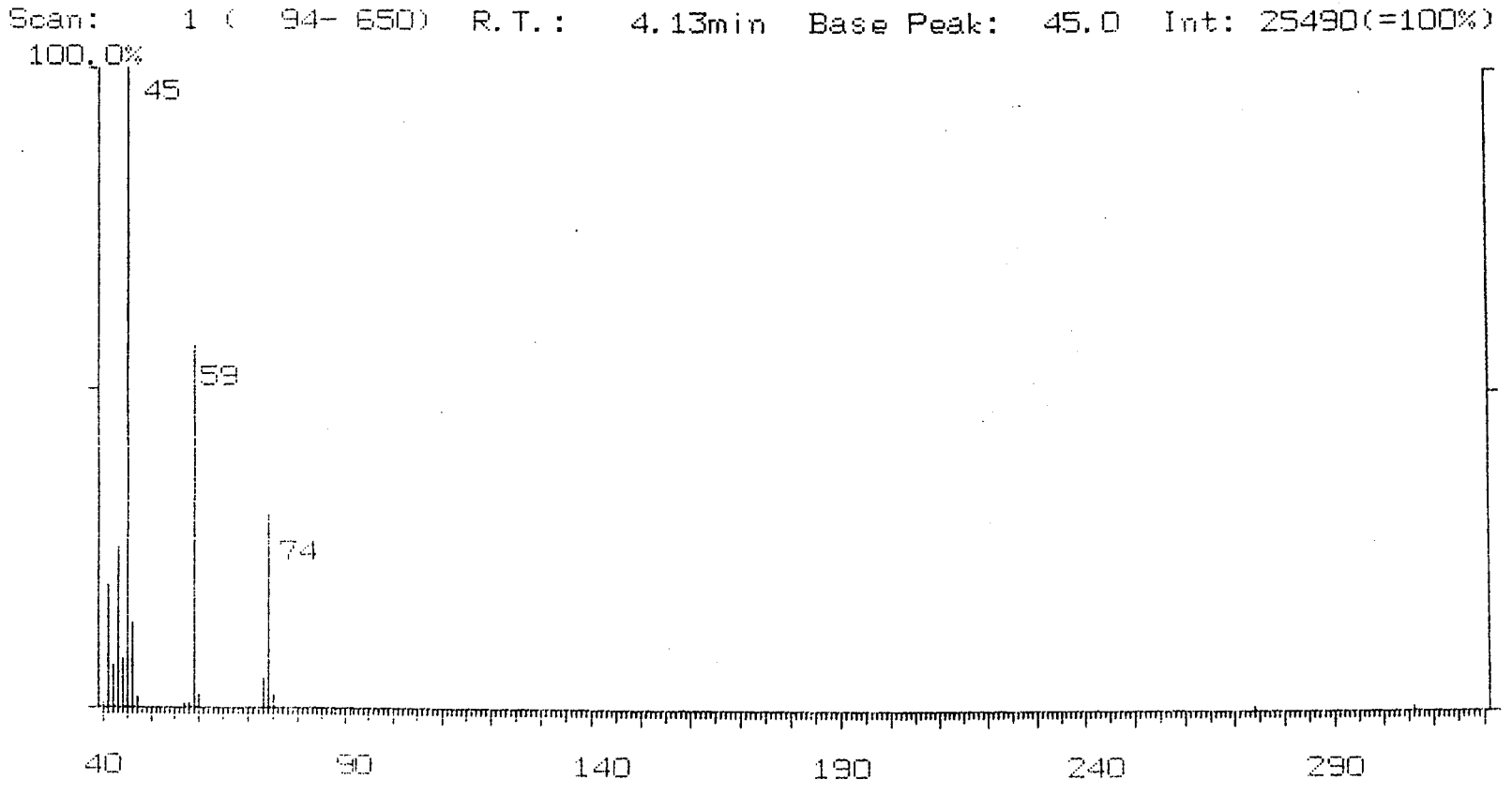


Fig. 4A$_3$. Mass Spectrum of Camphene

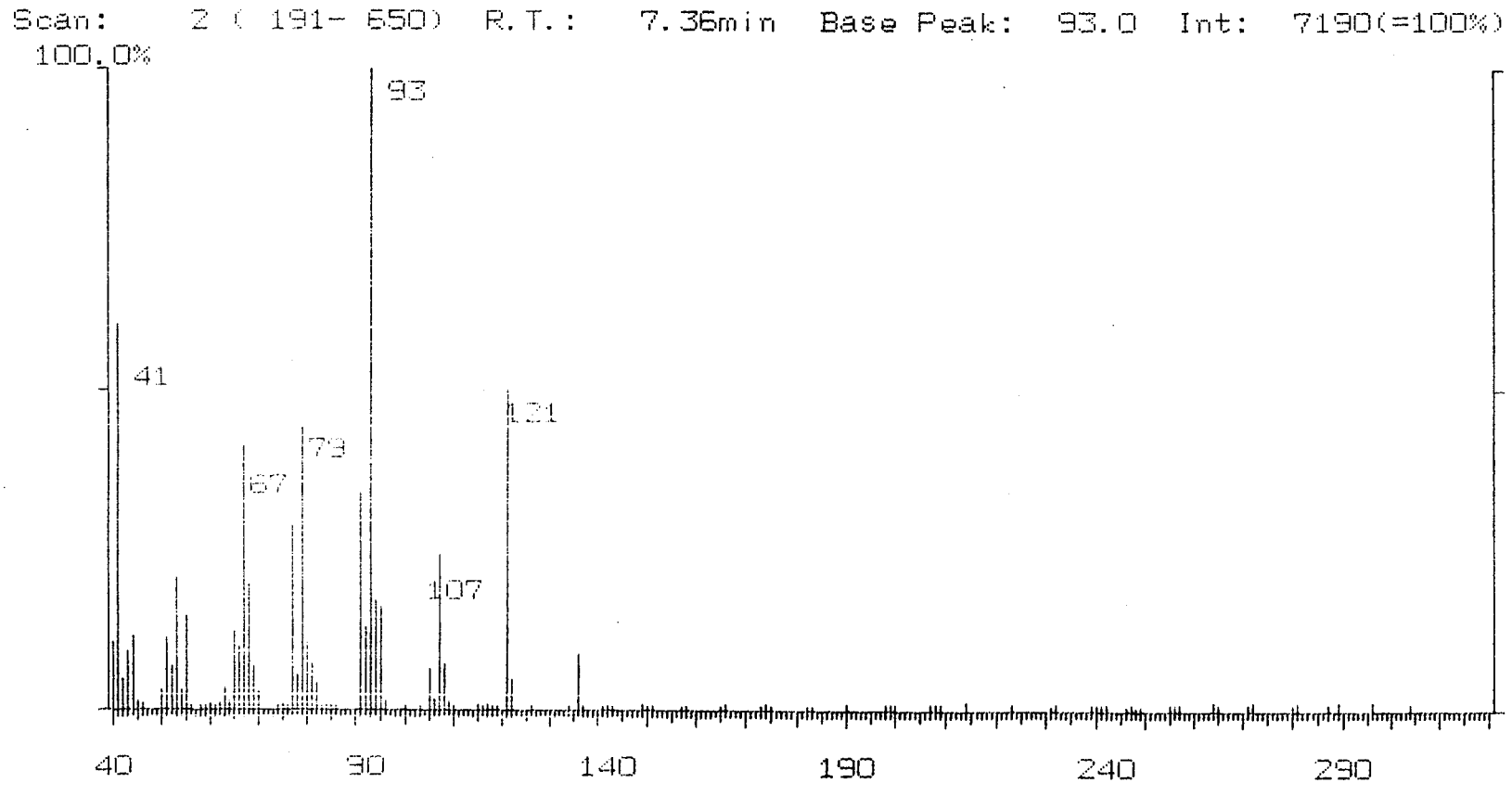


Fig. 4A$_4$. Mass Spectrum of Myrcene

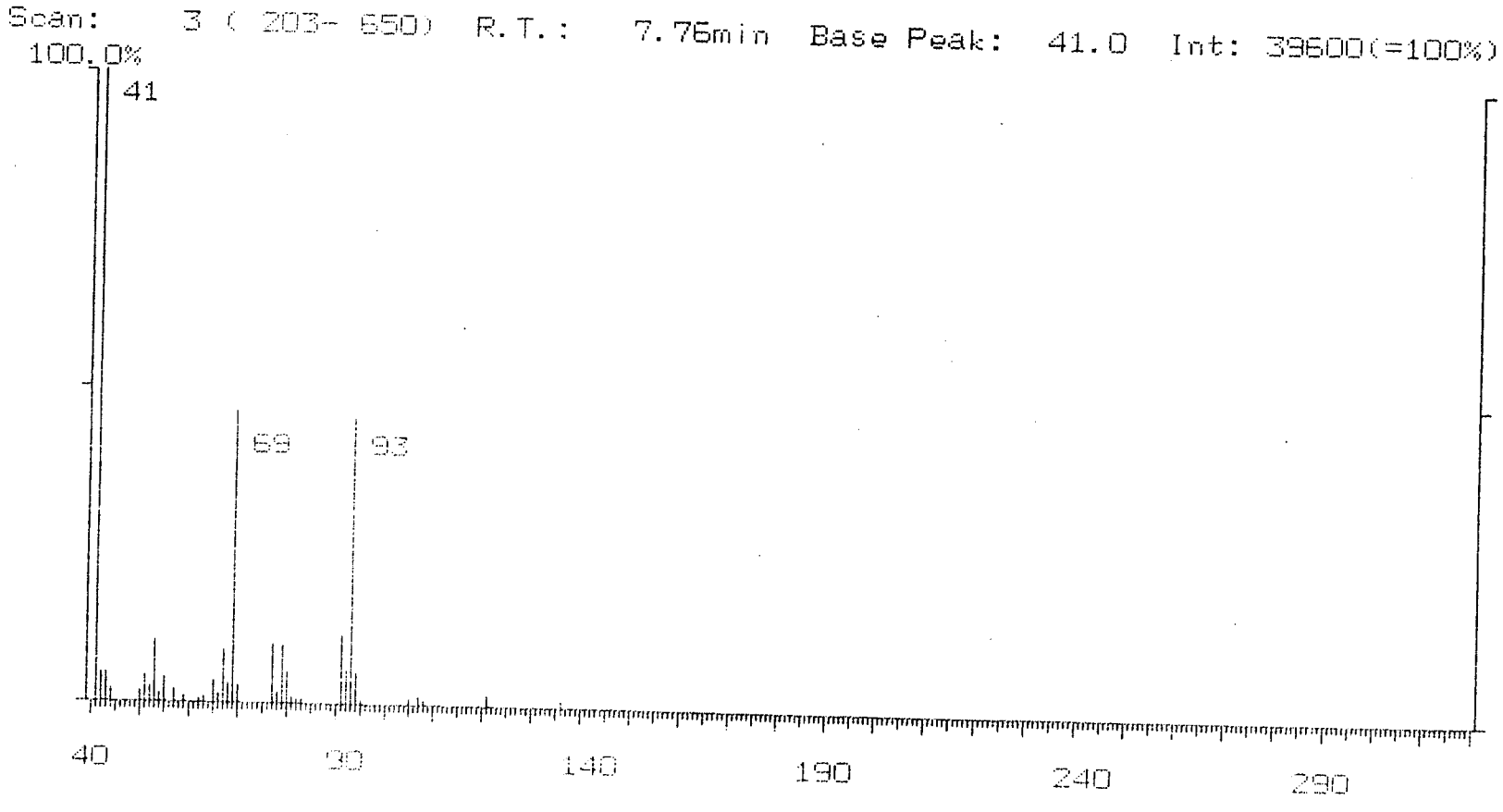


Fig. 4A$_5$. Mass Spectrum of Limonene

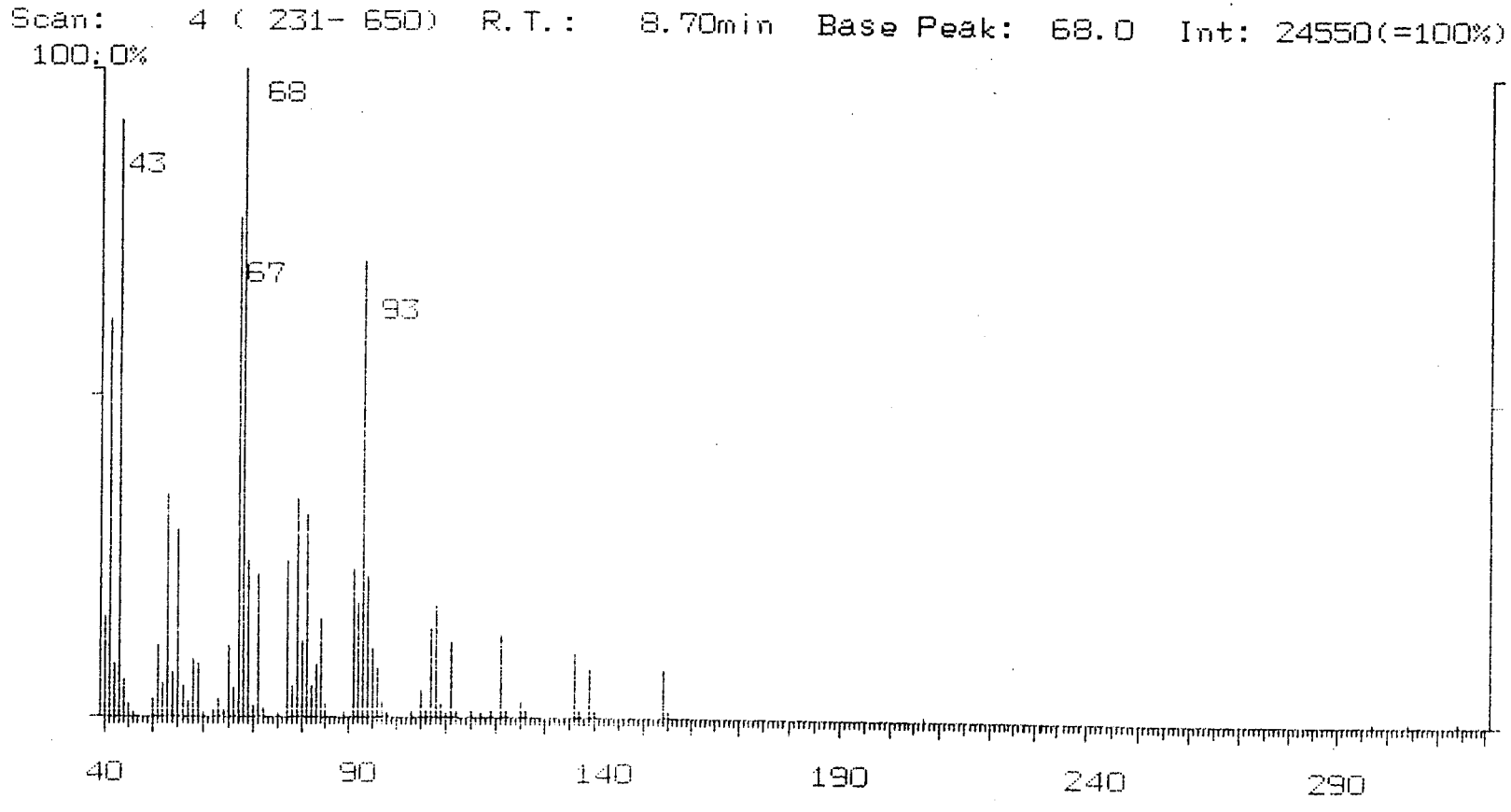


Fig. 4A$_6$. Mass Spectrum of Cis-ocimene

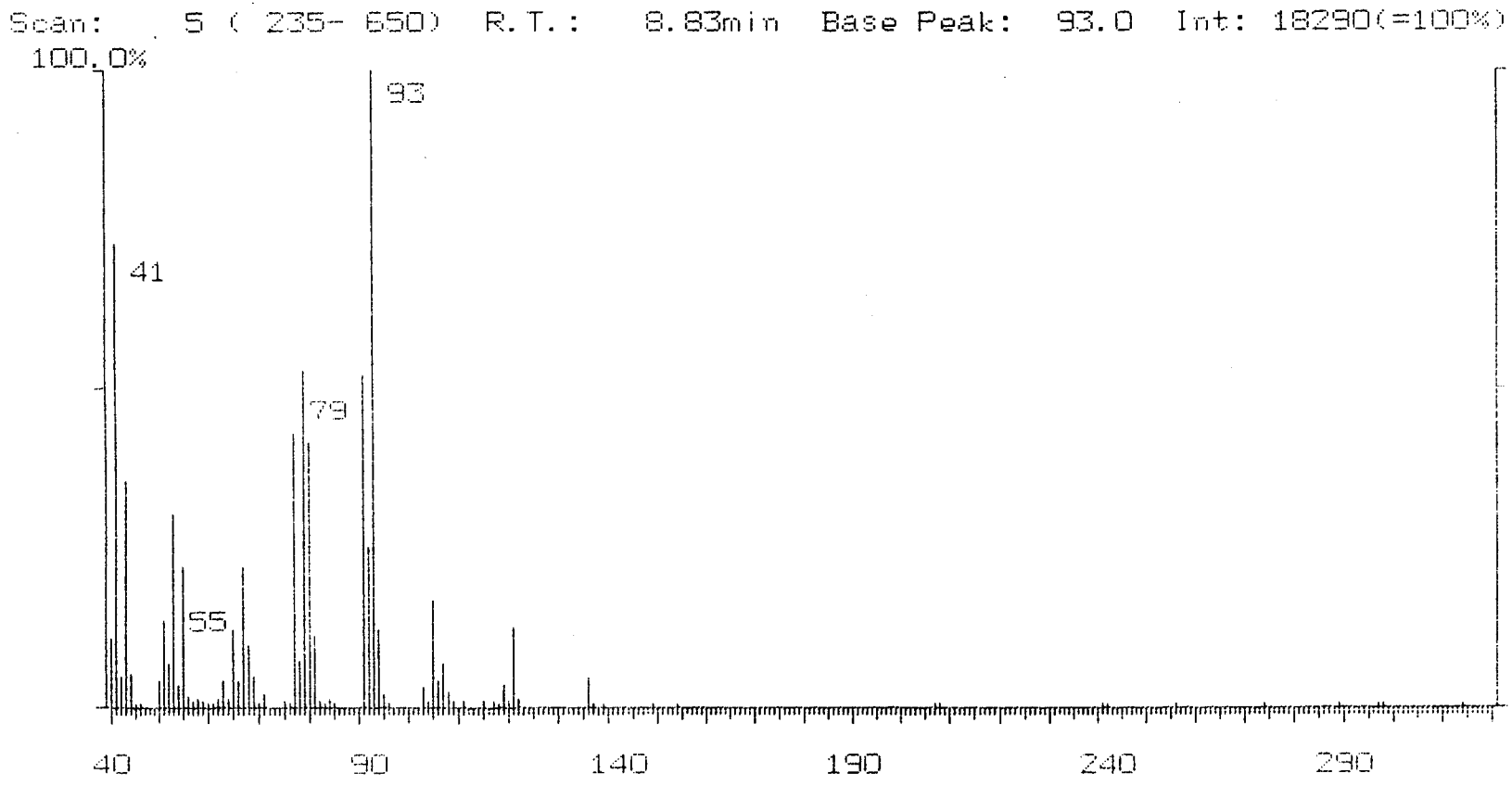


Fig. 4A$_7$. Mass Spectrum of γ-terpinene

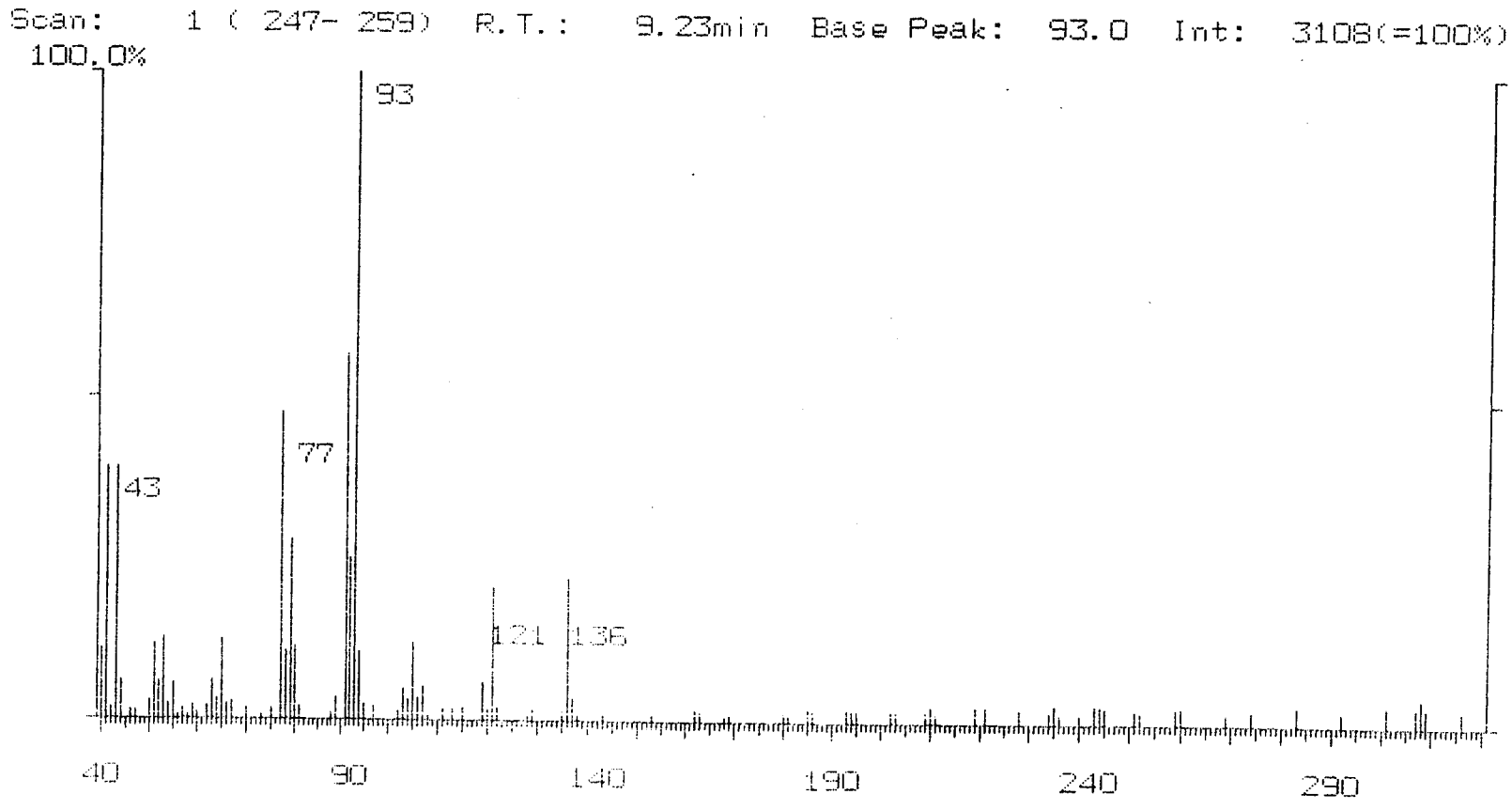


Fig. 4A$_8$. Mass Spectrum of Methyl chavicol

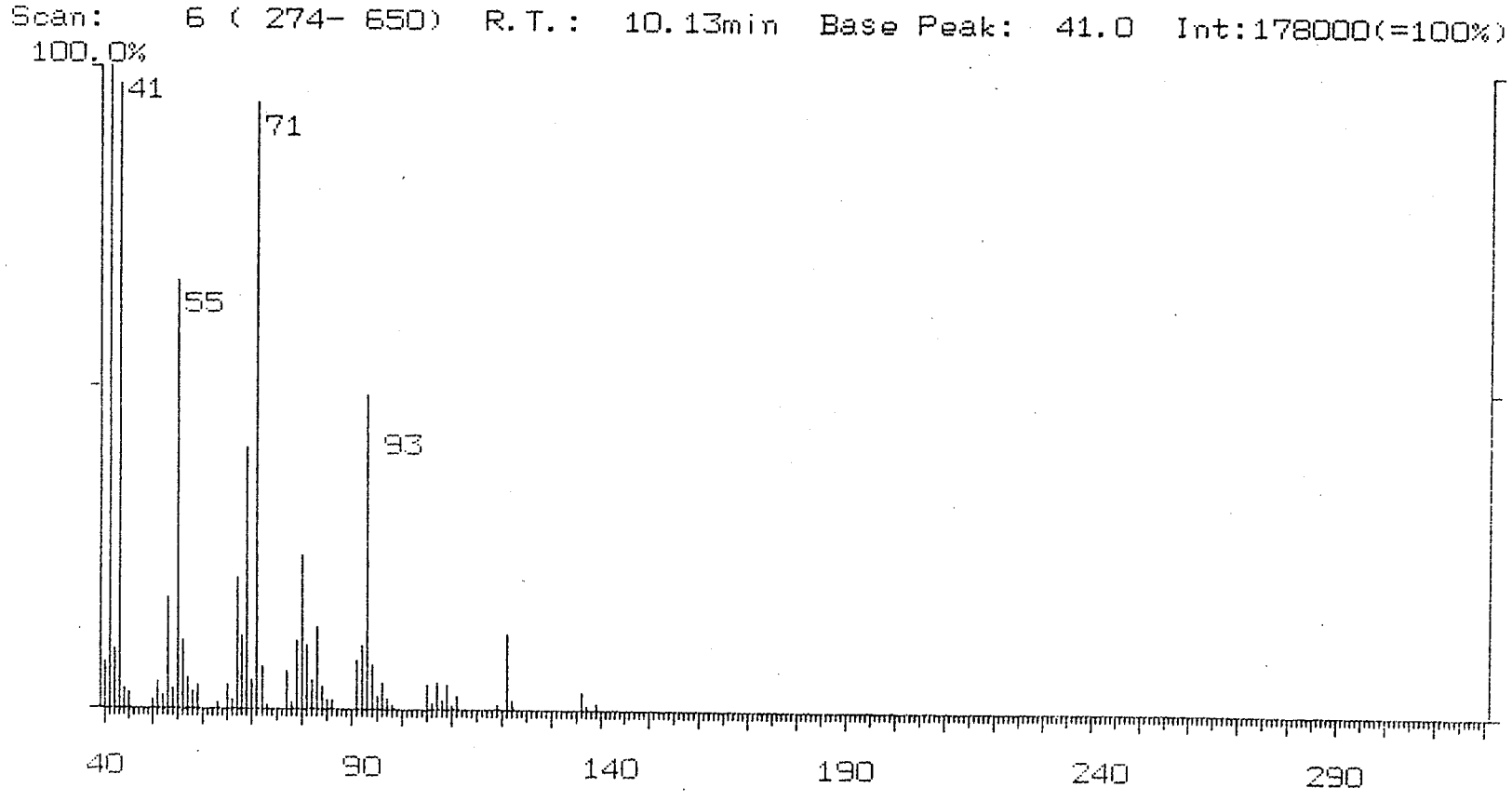


Fig. 4A$_9$. Mass Spectrum of Camphor

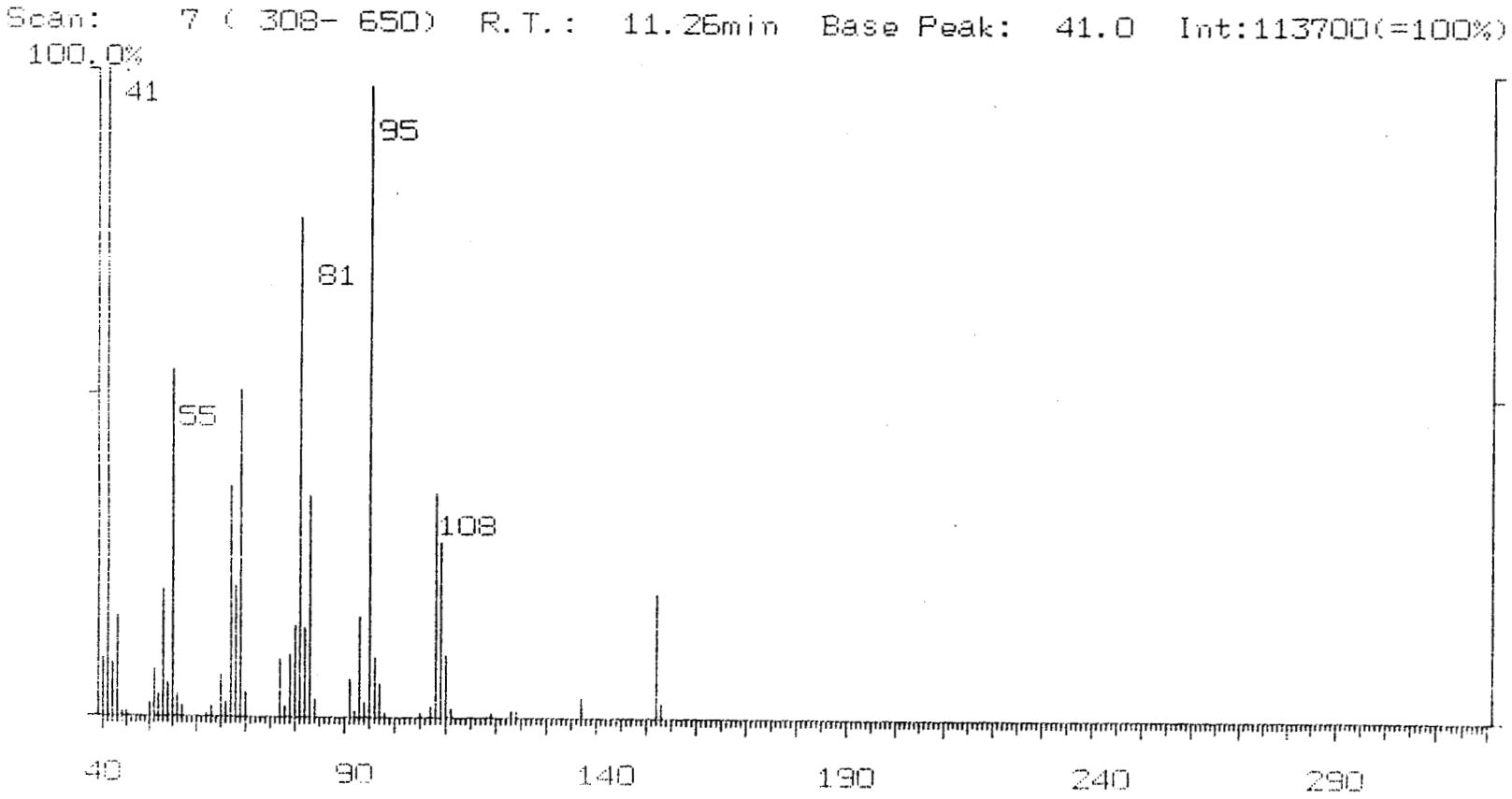


Fig. 4A$_{10}$. Mass Spectrum of Terpinen-4 ol

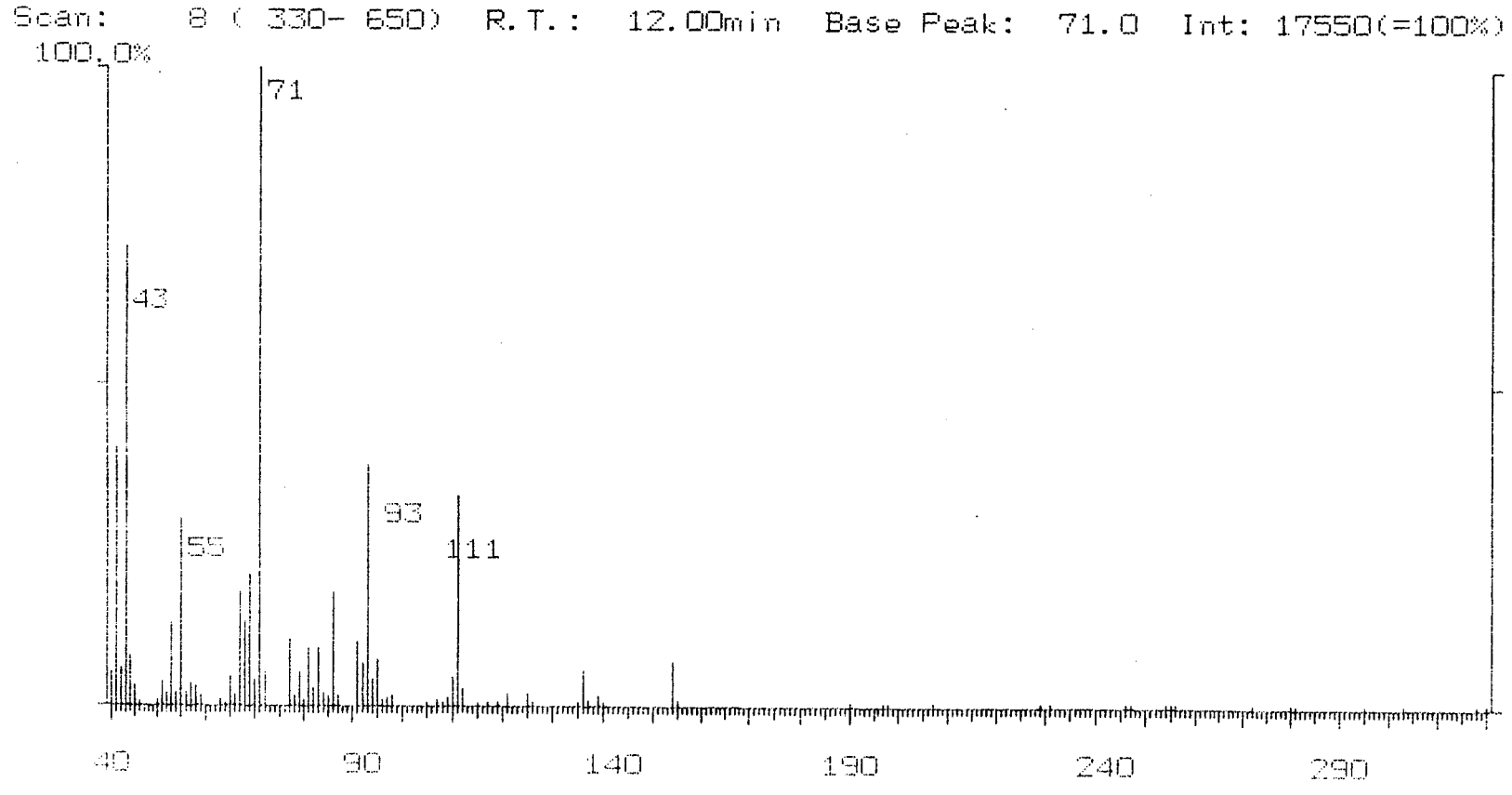


Fig. 4A$_{11}$. Mass Spectrum of Anethole

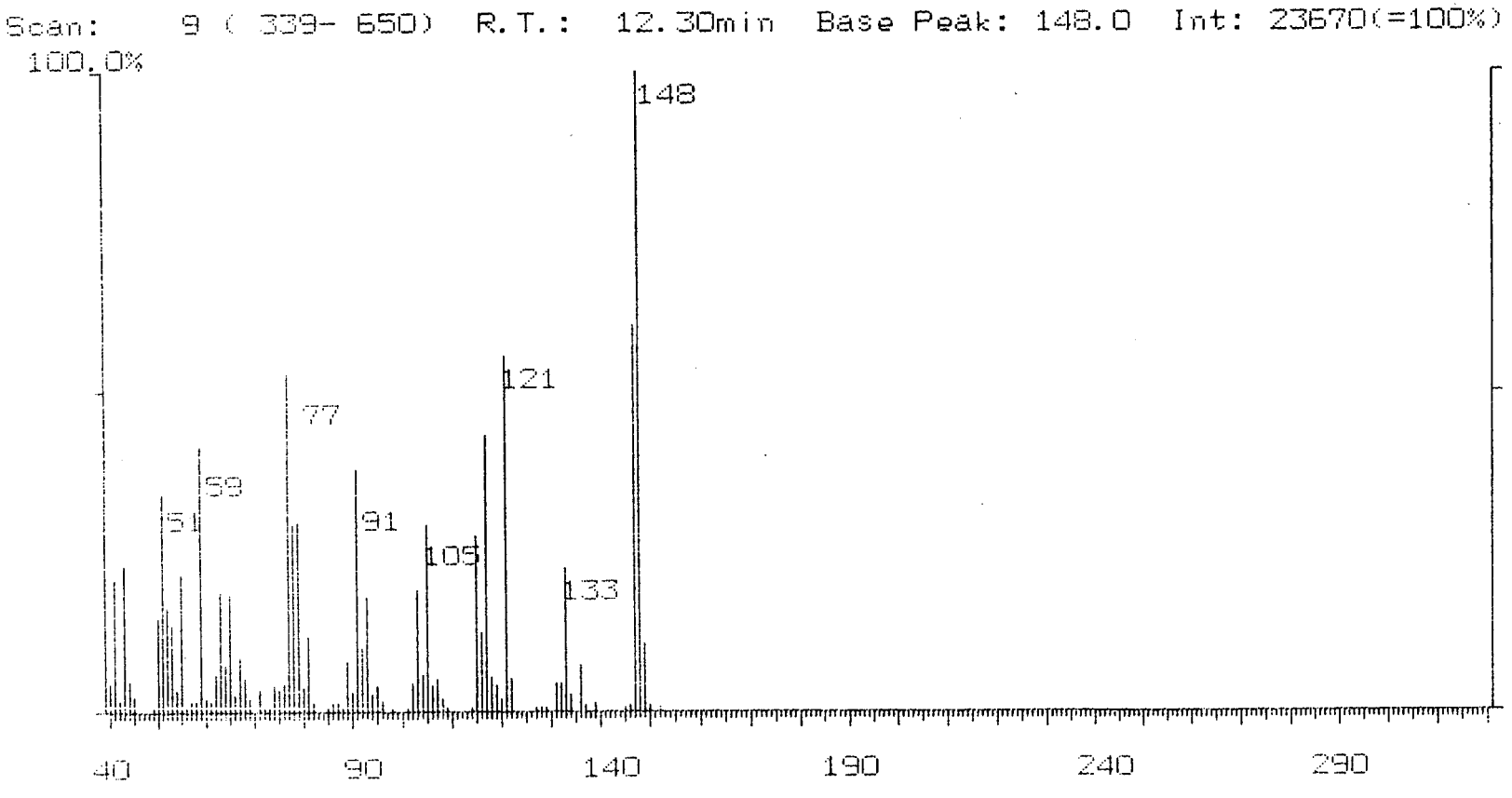


Fig. 4A$_{12}$. Mass Spectrum of β-bisabolene

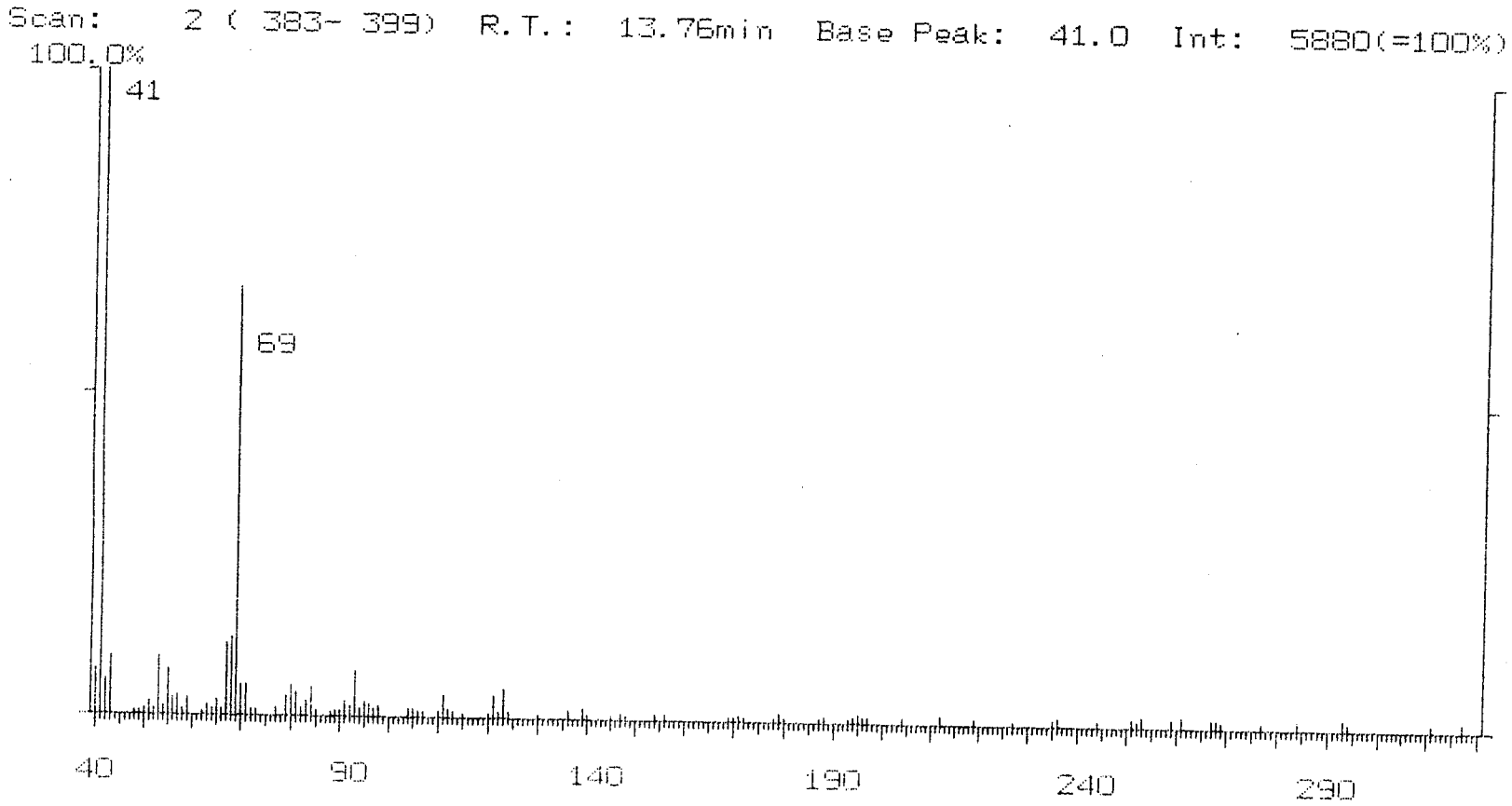


Fig. 4A$_{13}$. Mass Spectrum of Methyl cinnamate

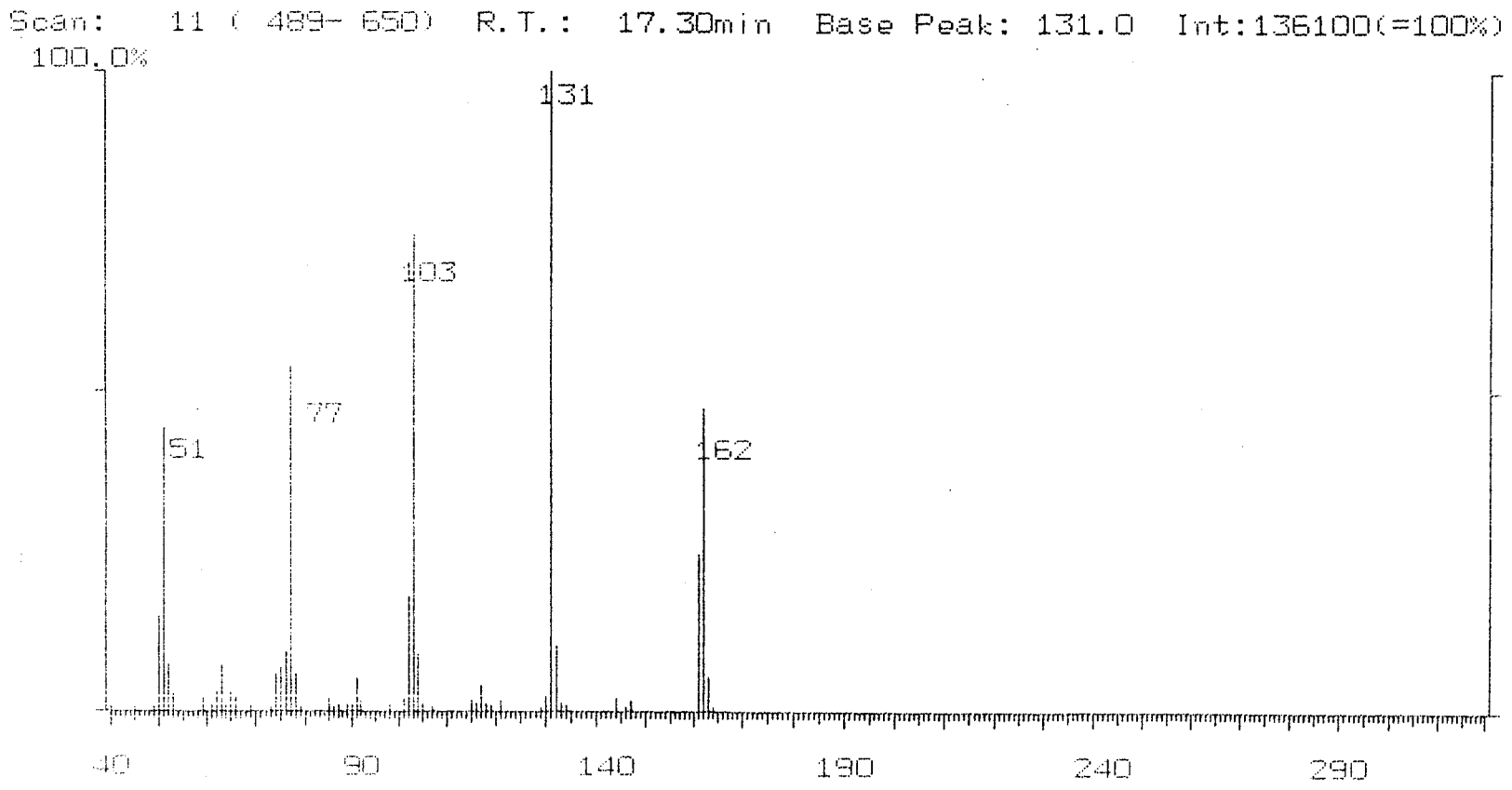


Fig. 4A$_{14}$. Mass Spectrum of unidentified compound

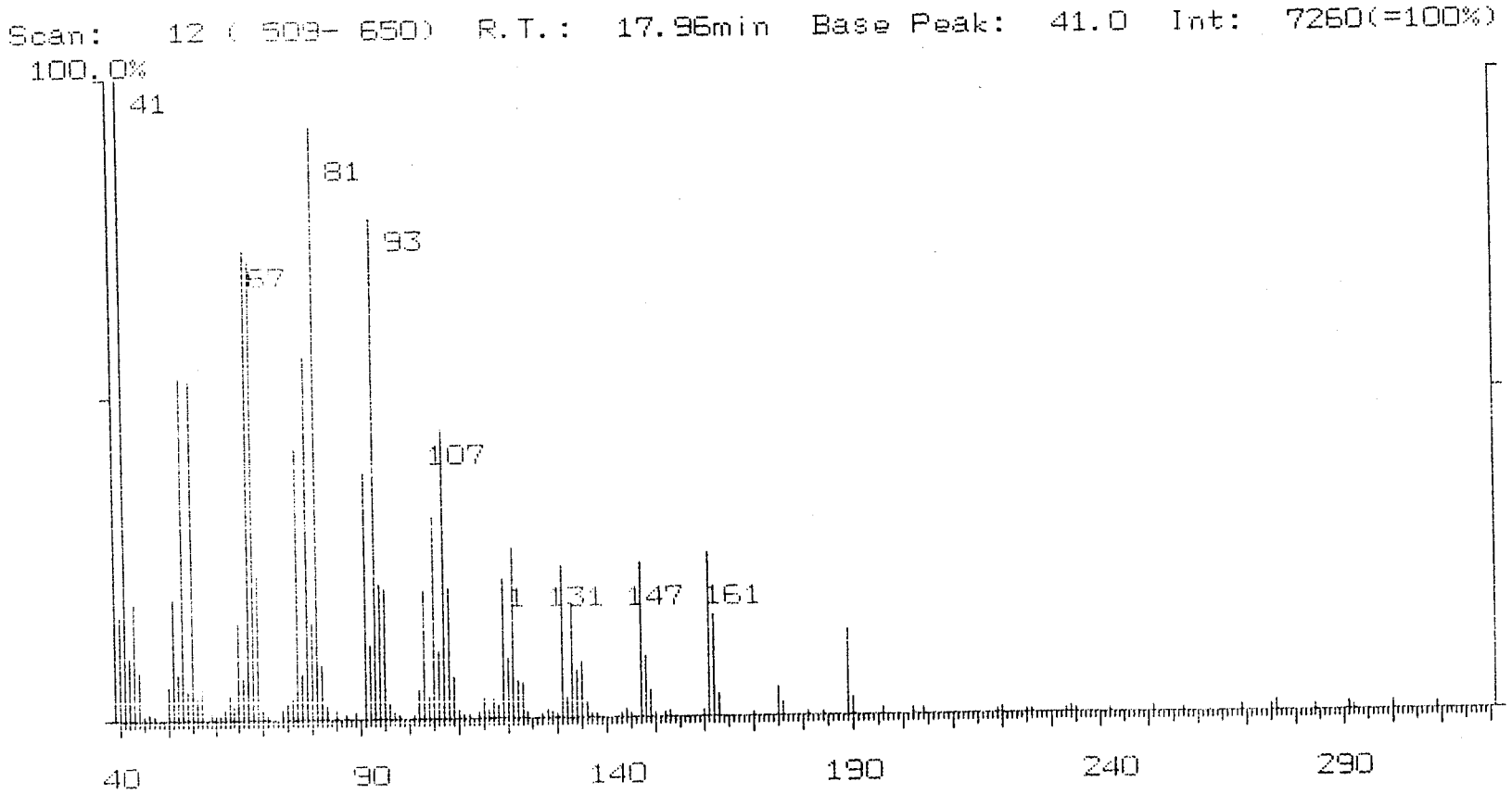


Fig. 4A$_{15}$. Mass Spectrum of β-caryophyllene

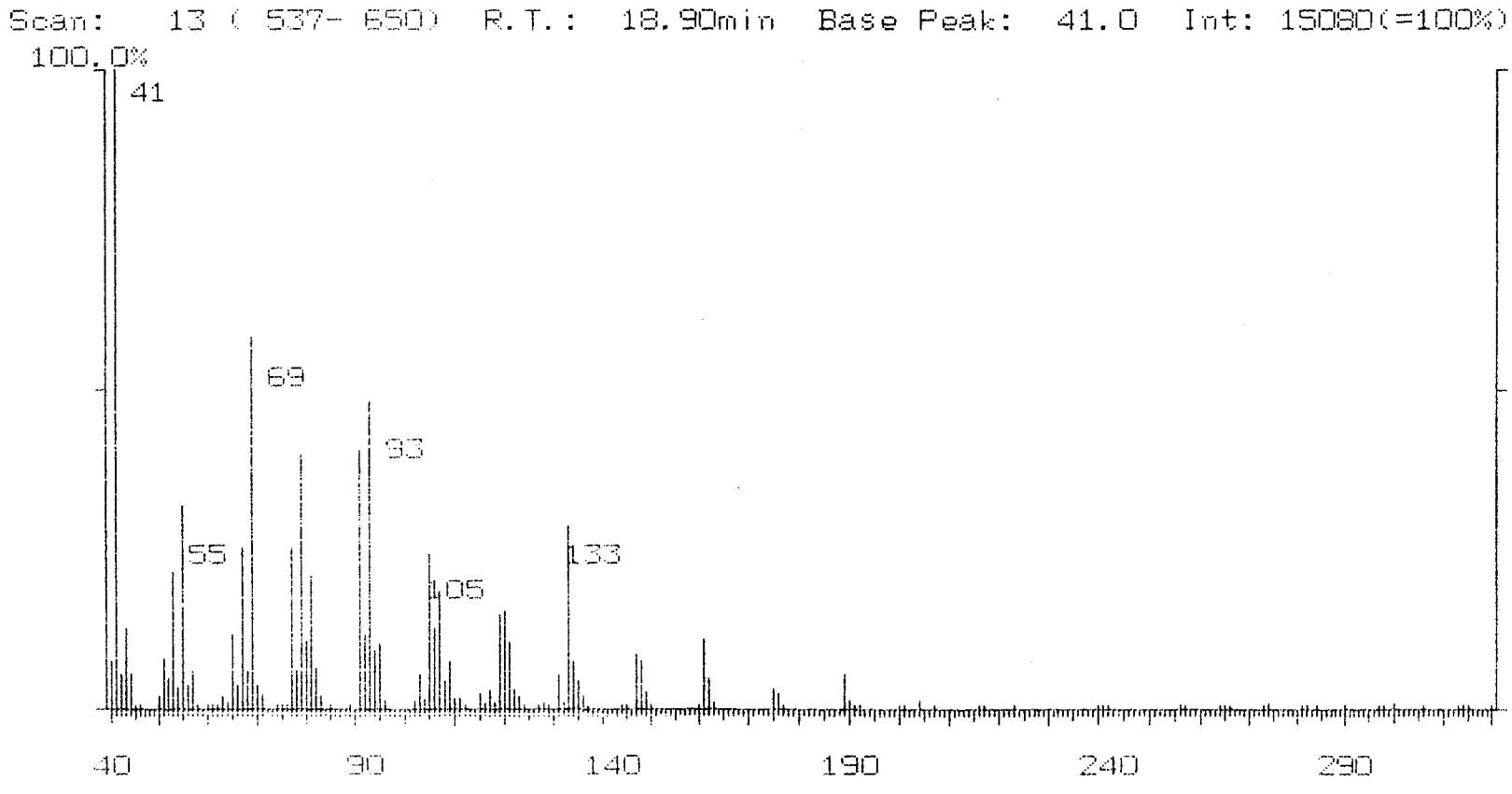


Fig. 4A$_{16}$. Mass Spectrum of α-humulene

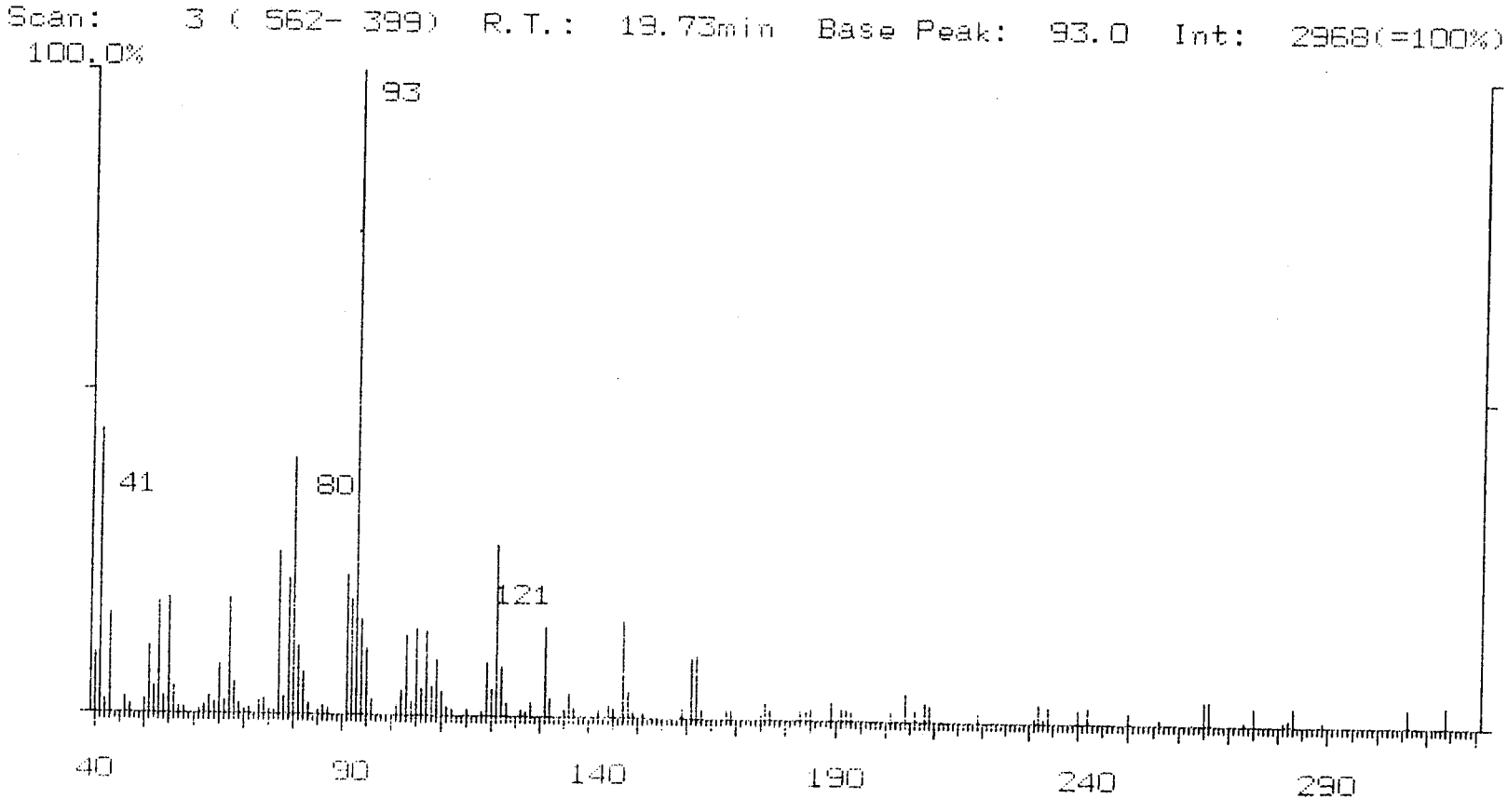


Fig. 4A$_{17}$. Mass Spectrum of α-cubebene

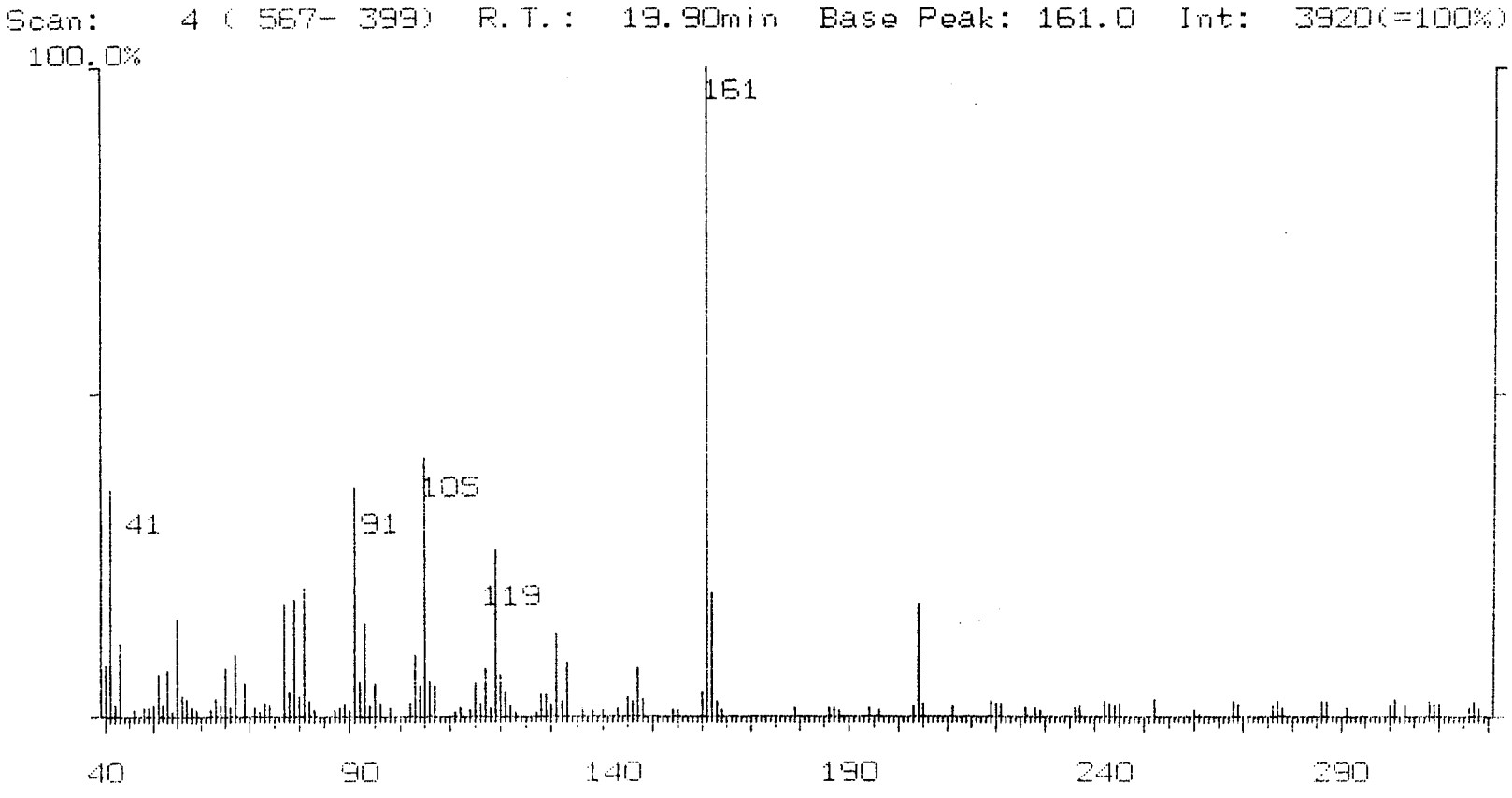


Fig. 4A$_{18}$. Mass Spectrum of Valencene

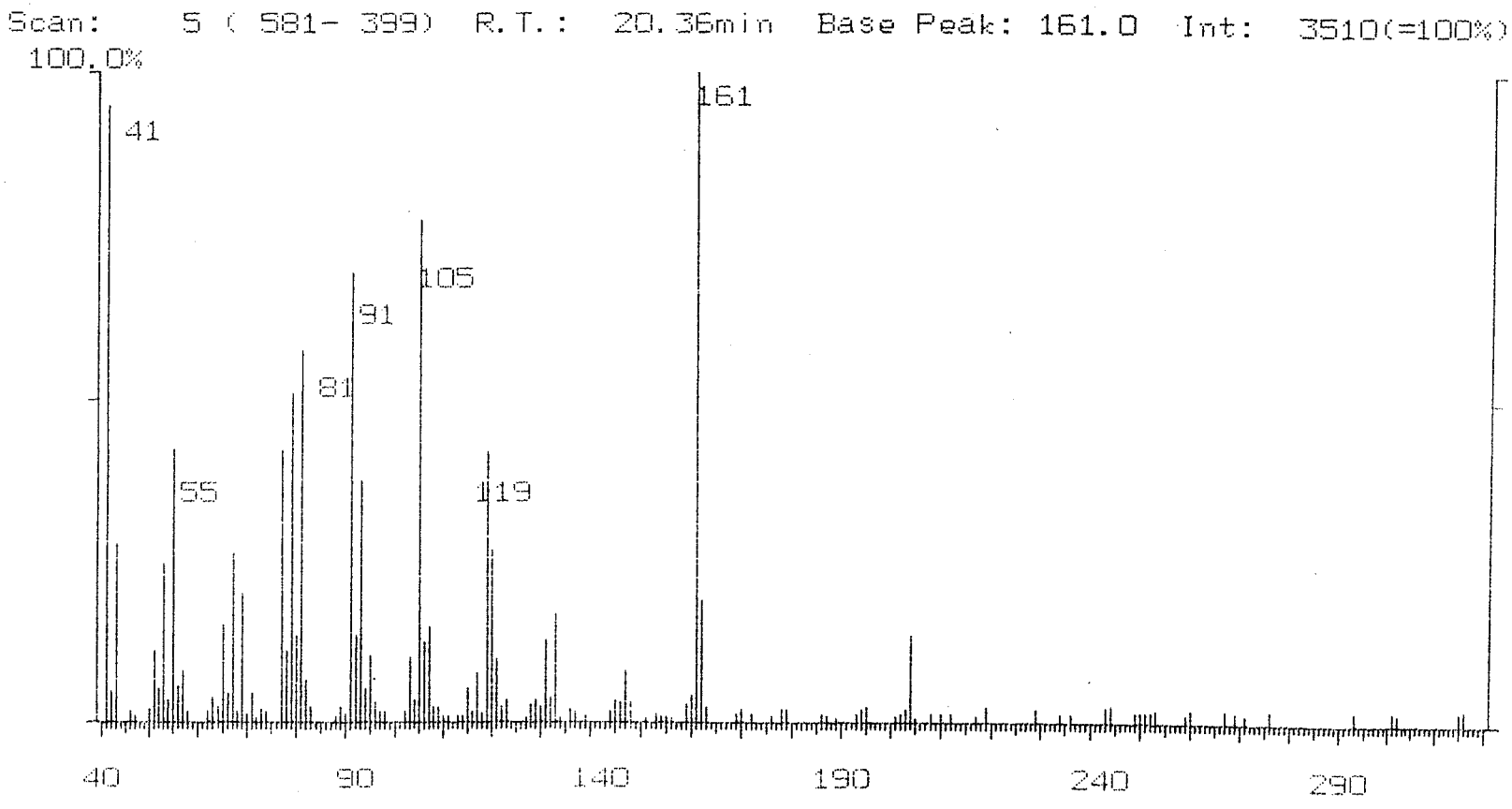


Fig. 4A$_{19}$. Mass Spectrum of γ-caryophyllene

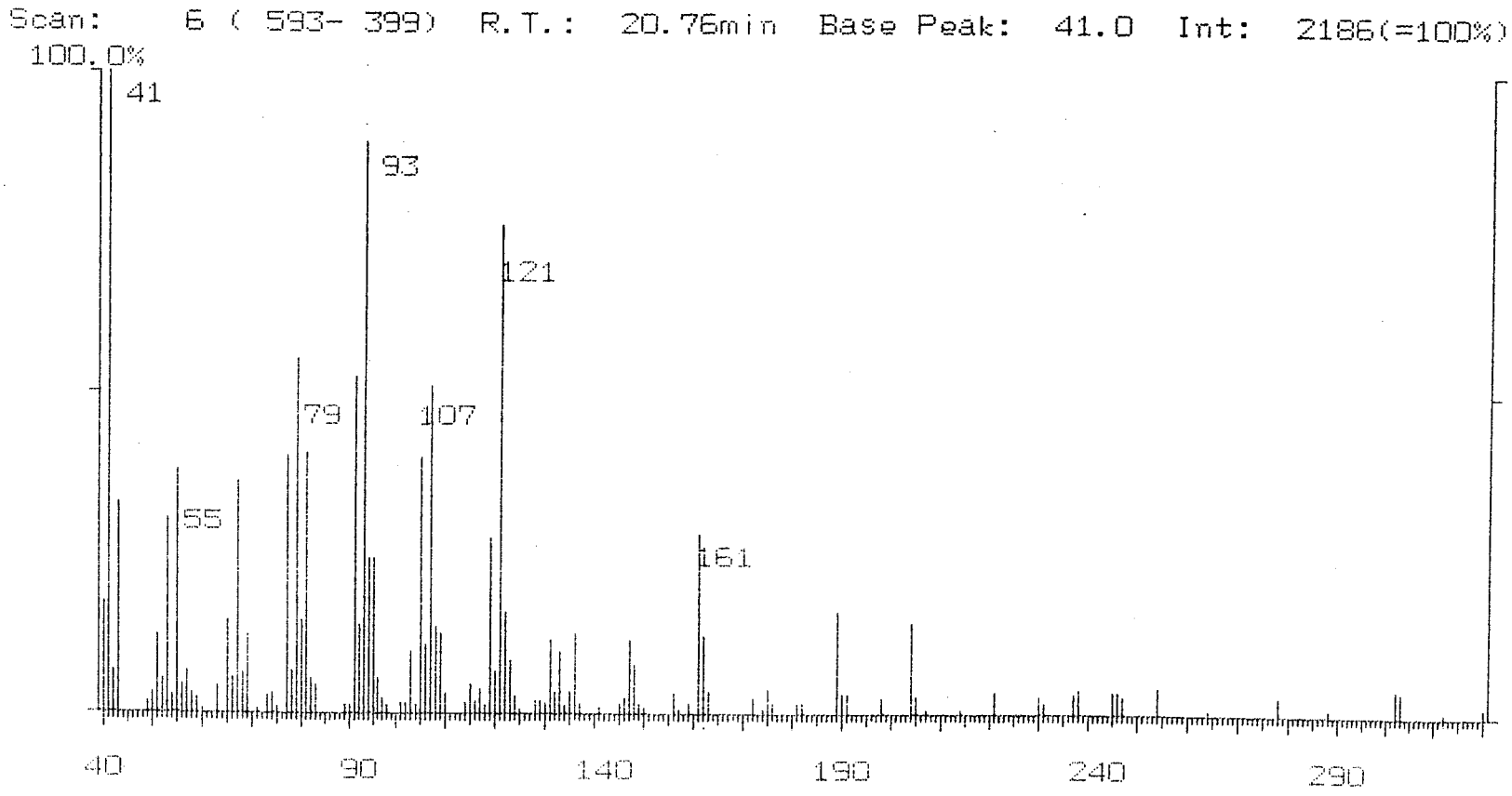


Fig. 4A$_{20}$. Mass Spectrum of α-himachalene

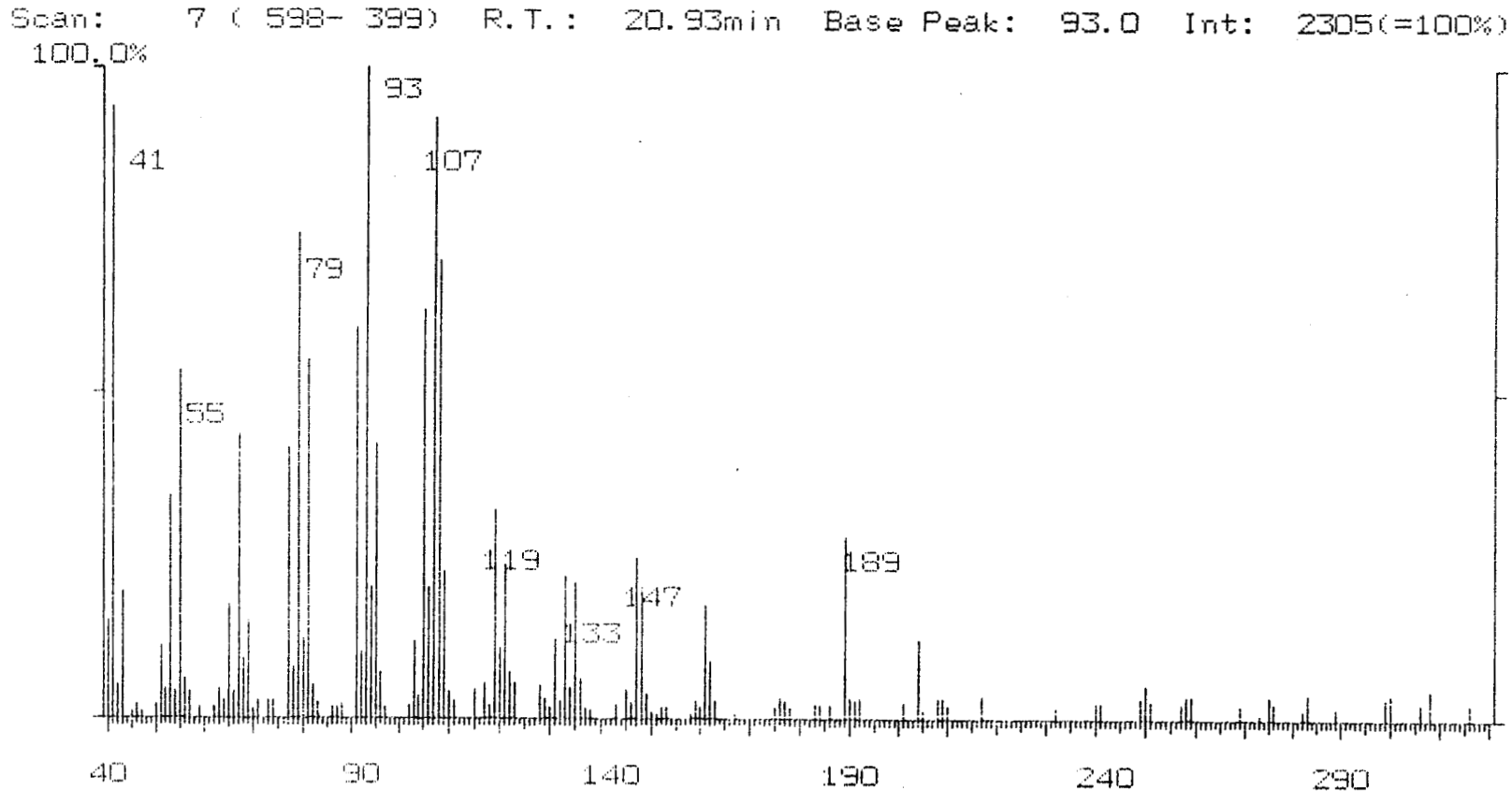


Fig. 4A$_{21}$. Mass Spectrum of β-gurjunene

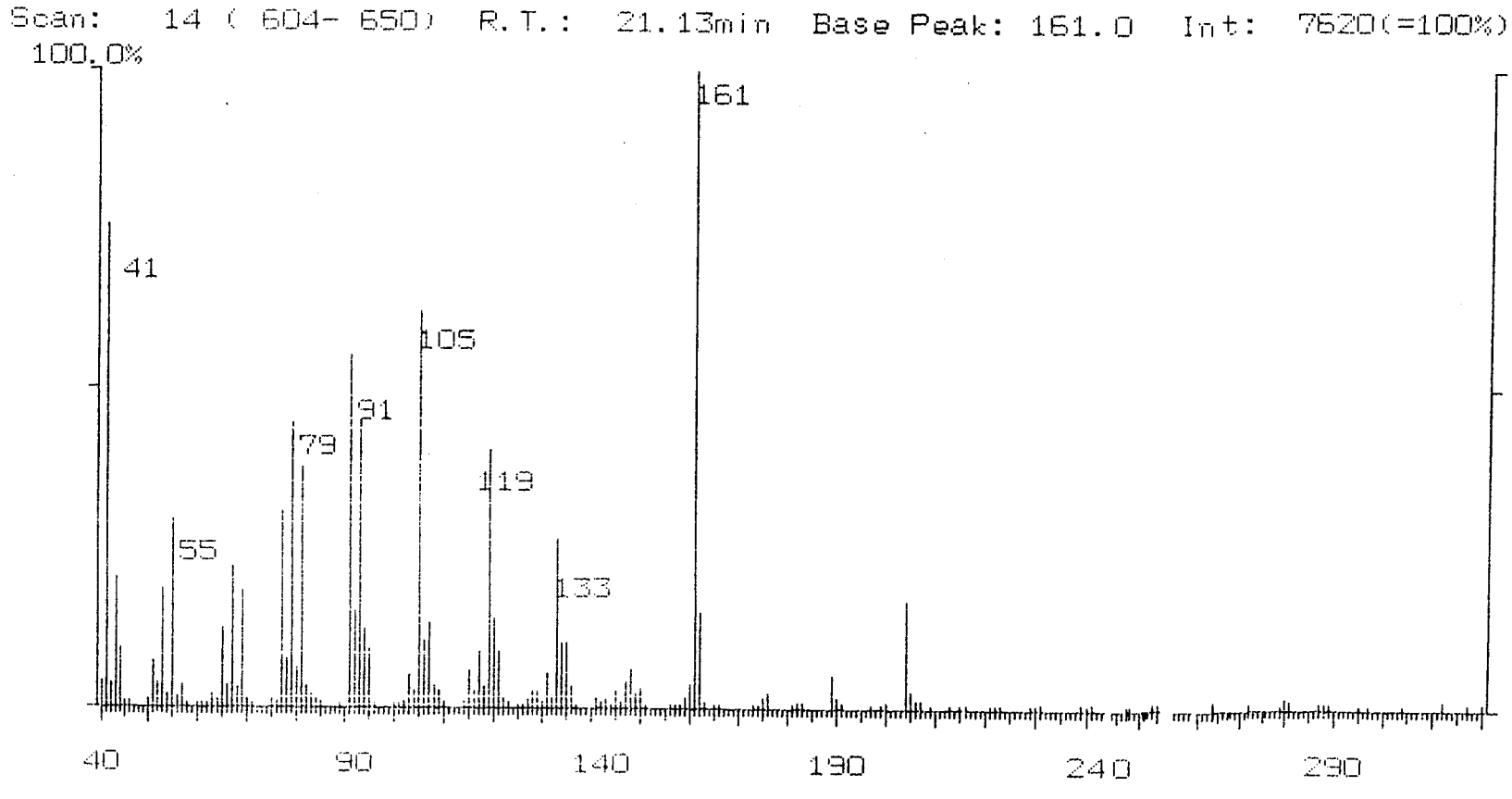


Fig. 4A$_{22}$. Mass Spectrum of Aromadendrene

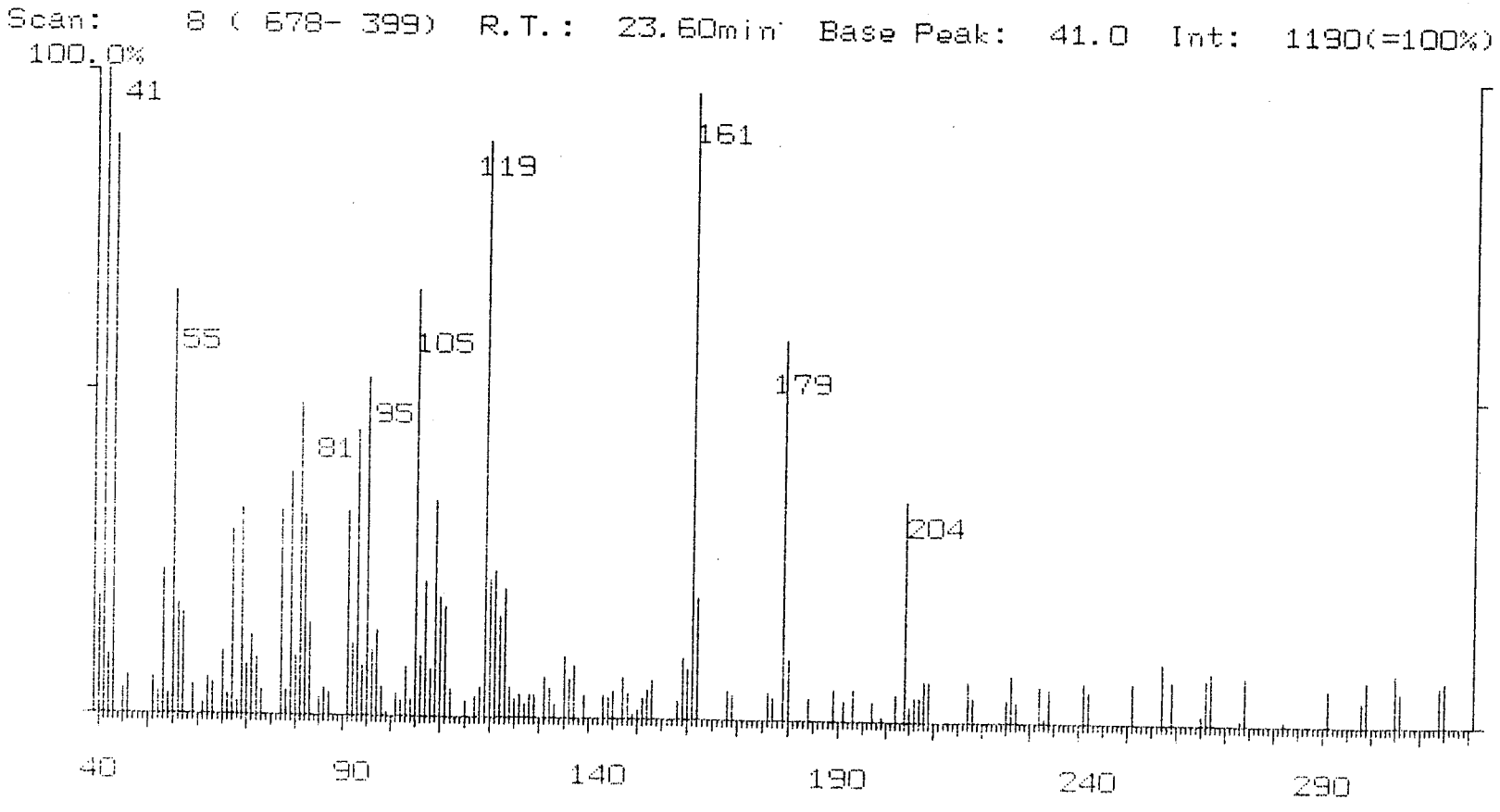


Fig. 4A$_{23}$. Mass Spectrum of unidentified compound

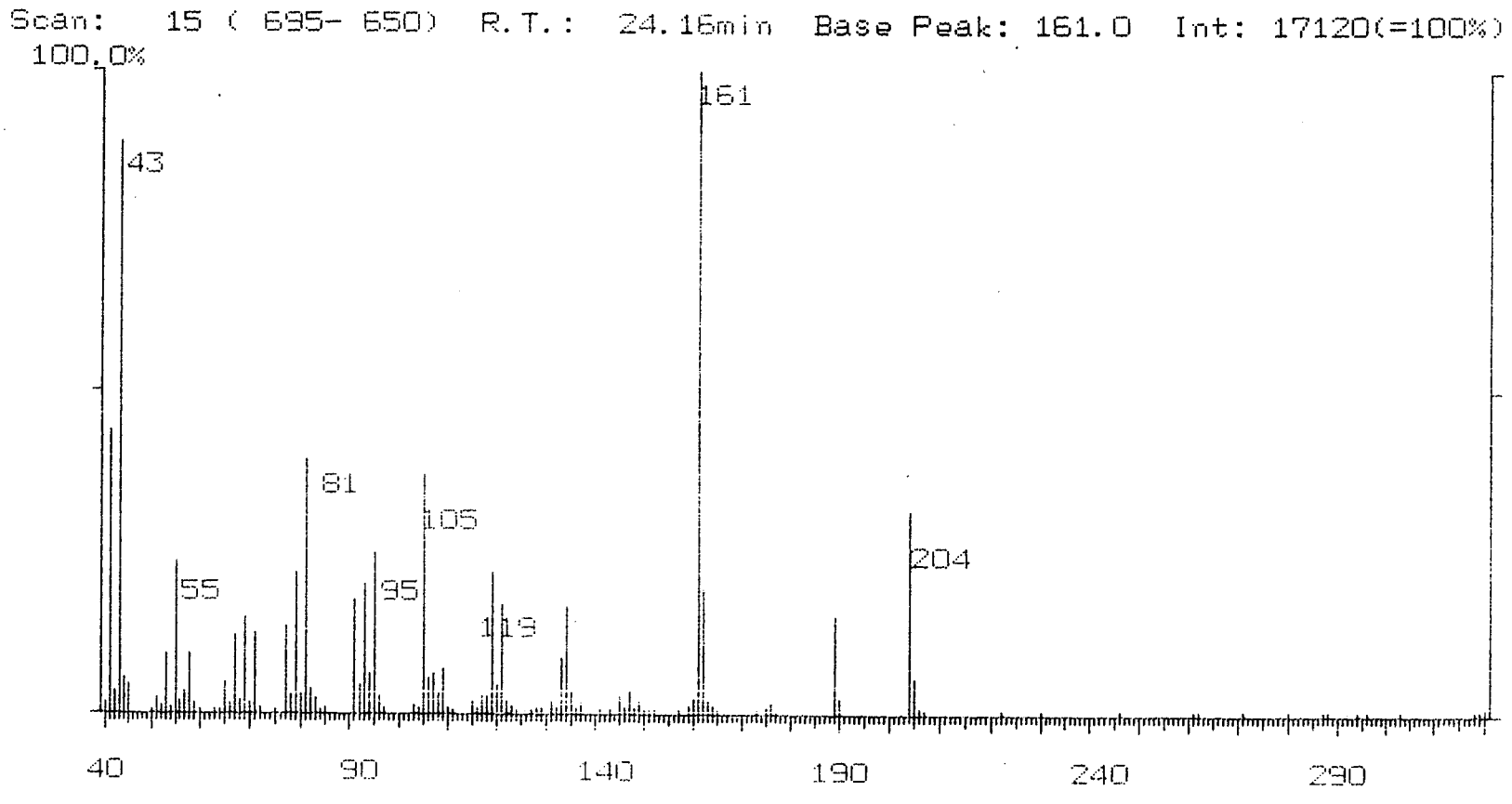


Fig. 4A$_{24}$. Gas Chromatogram of herb oil of *O. basilicum* L. var. *purpurascens* Benth. (Somaclonal variant)

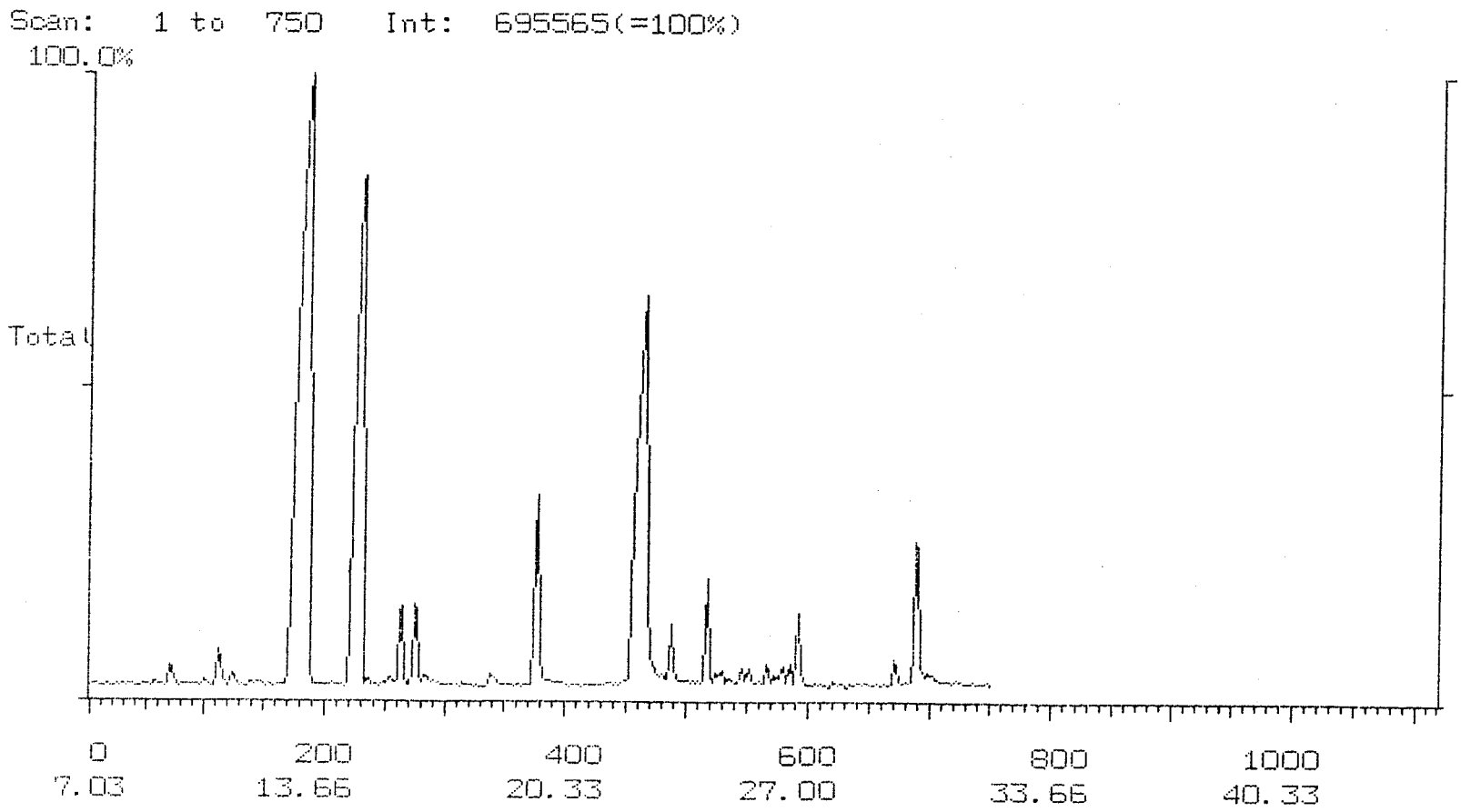


Fig. 4A$_{25}$. Mass Spectrum of Myrcene

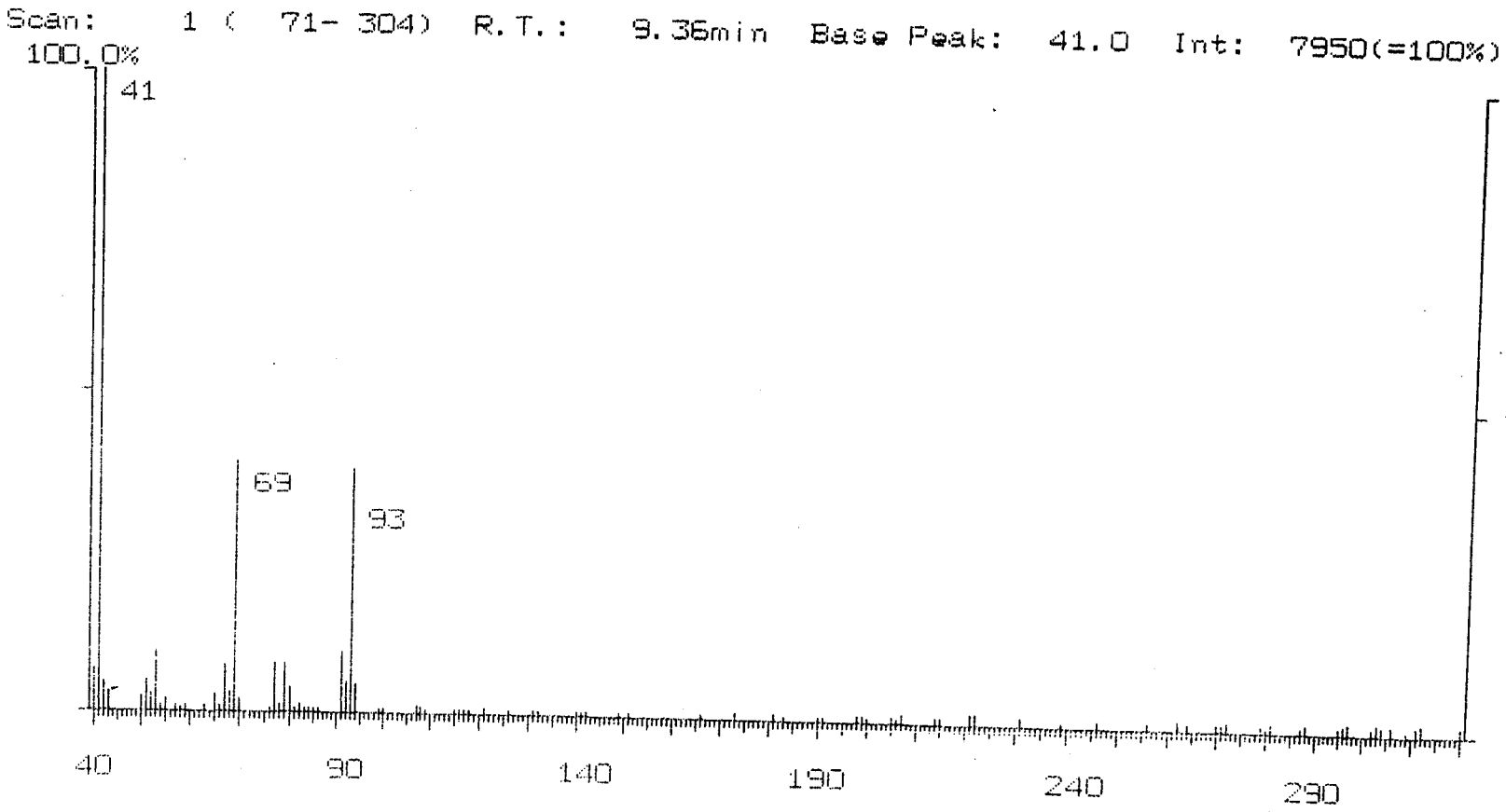


Fig. 4A$_{26}$. Mass Spectrum of Cis-sabinene hydrate

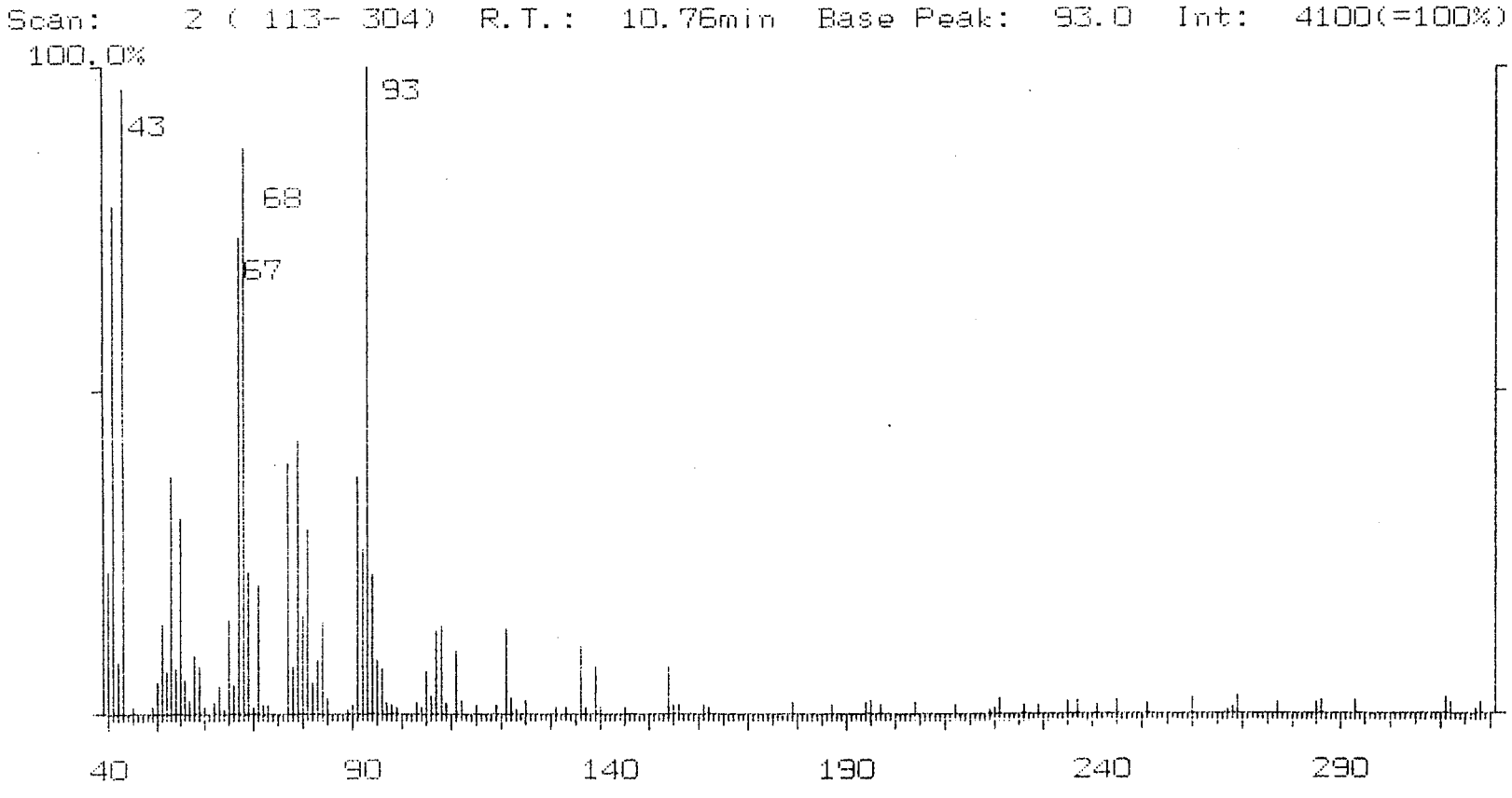


Fig. 4A$_{27}$. Mass Spectrum of Cis-ocimene

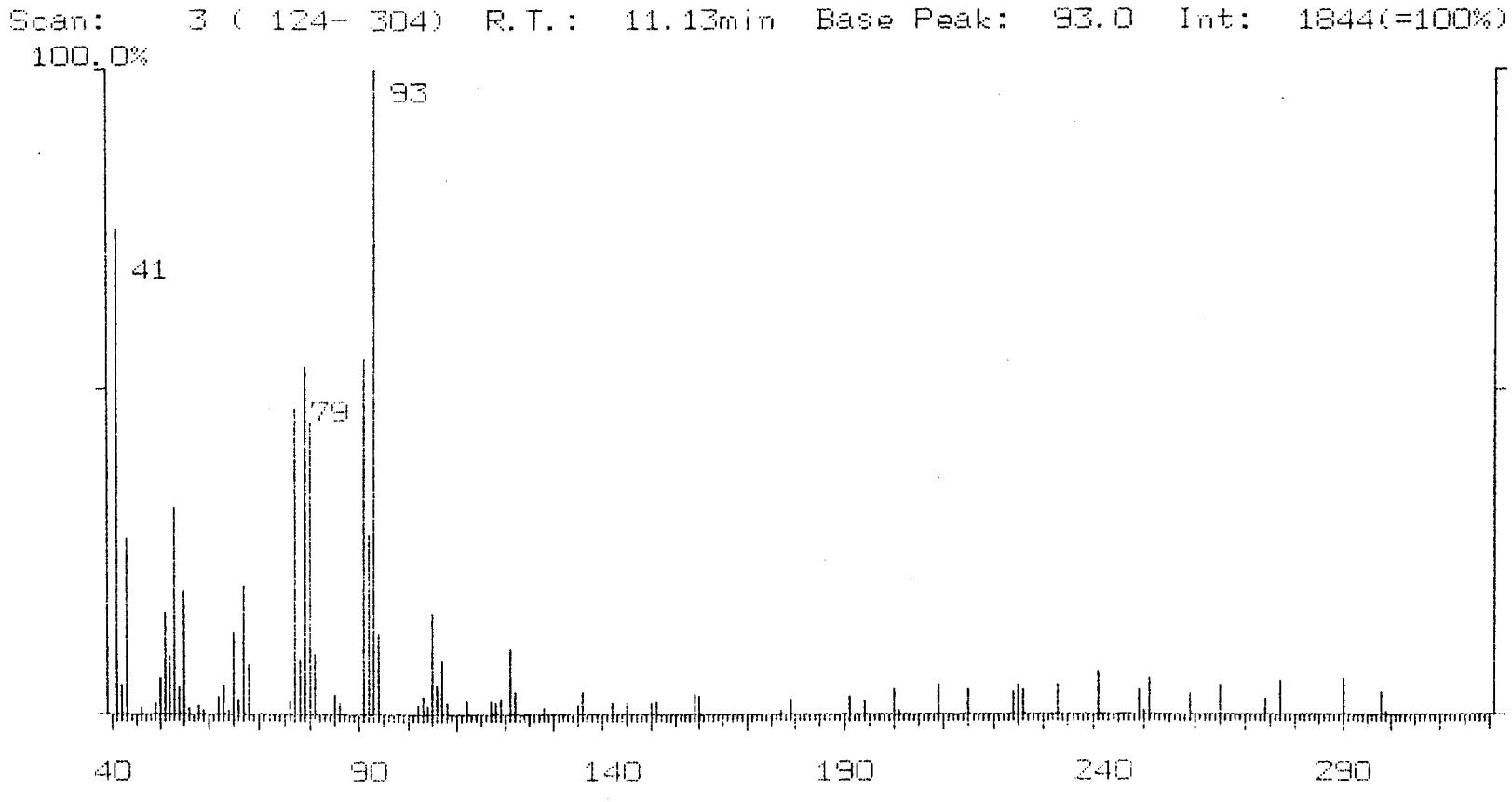


Fig. 4A$_{28}$. Mass Spectrum of Methyl chavicol

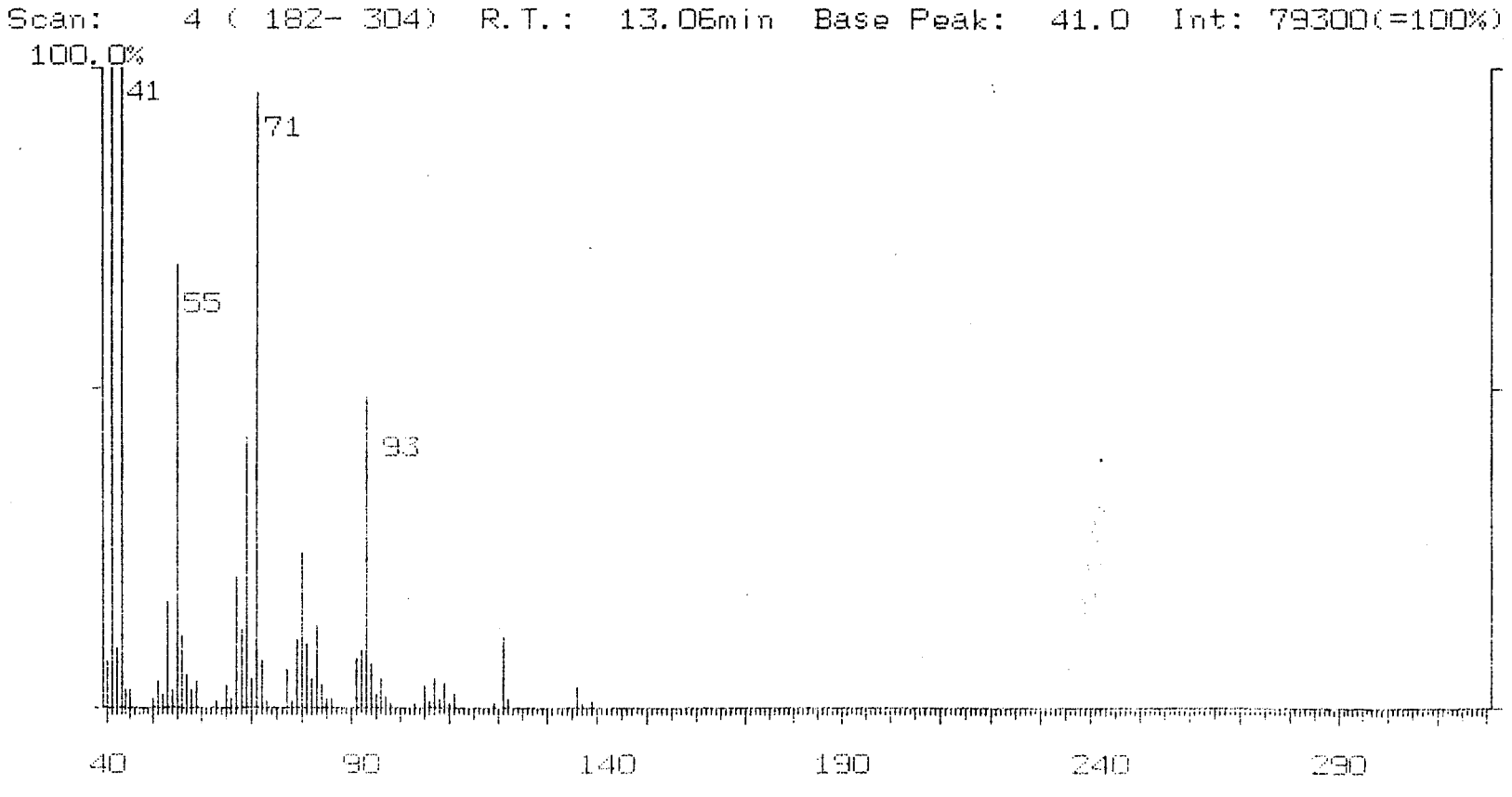


Fig. 4A$_{29}$. Mass Spectrum of Camphor

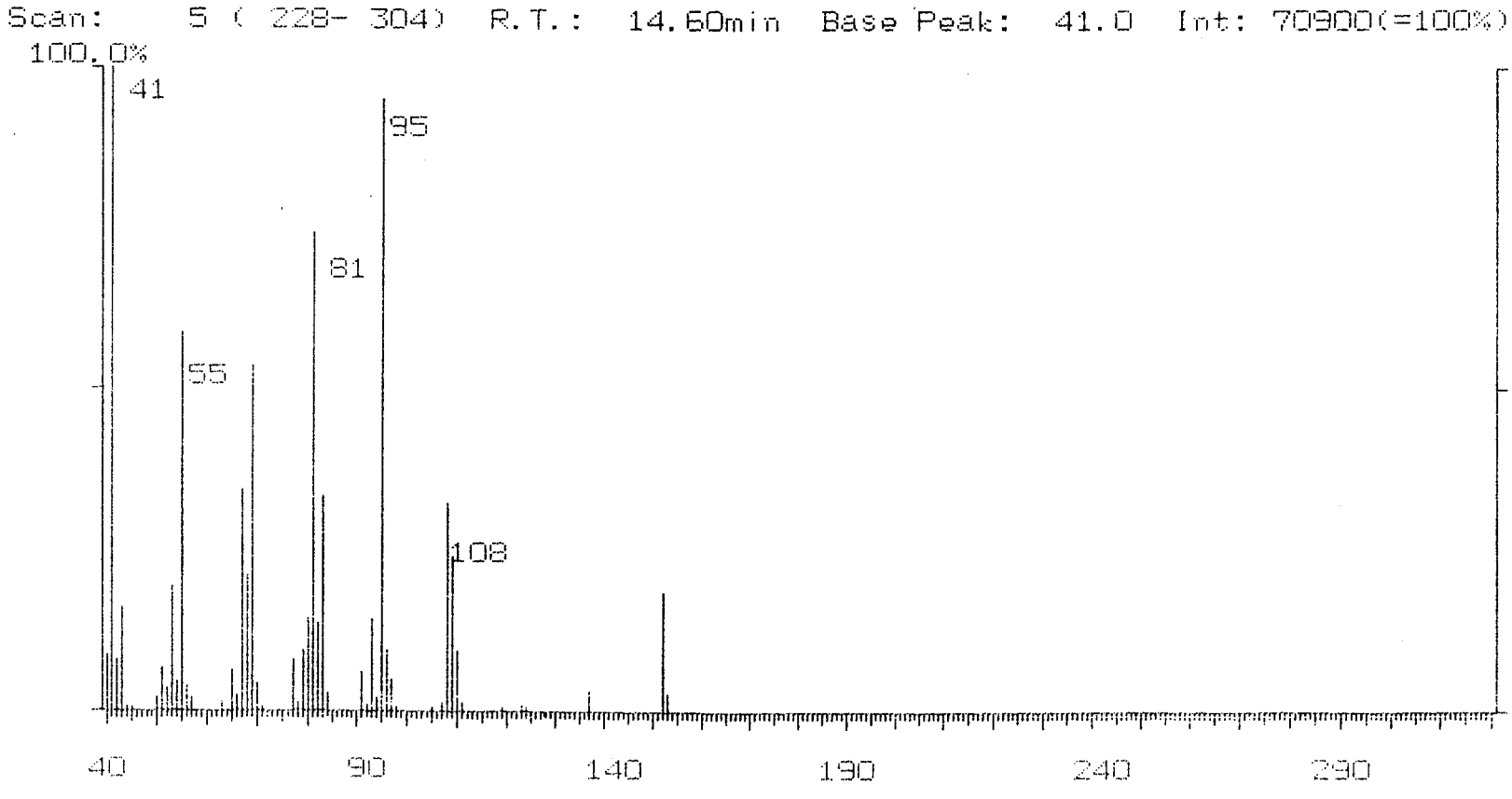


Fig. 4A$_{30}$. Mass Spectrum of Terpinen-4 ol

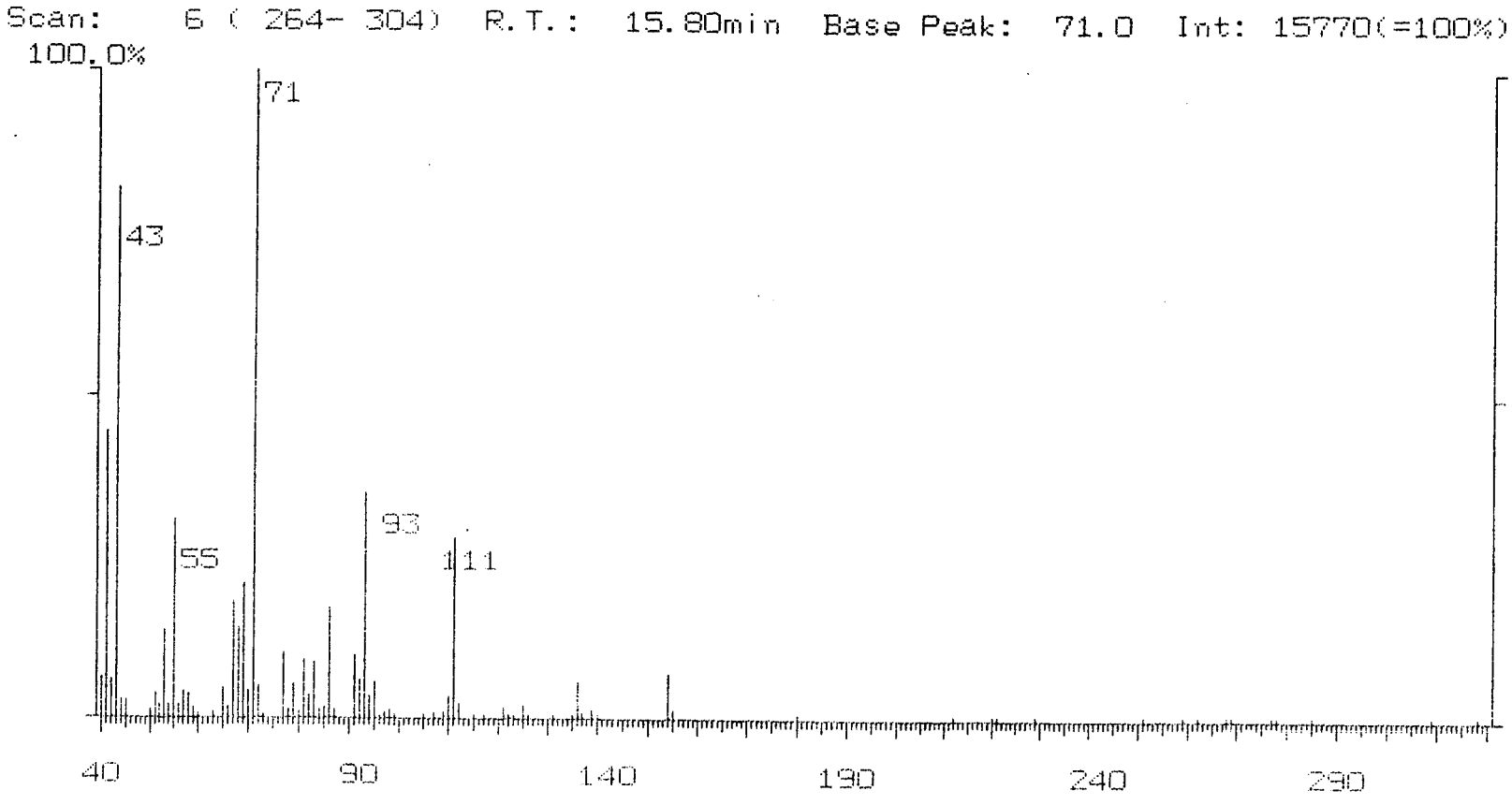


Fig. 4A$_{31}$. Mass Spectrum of Anethole

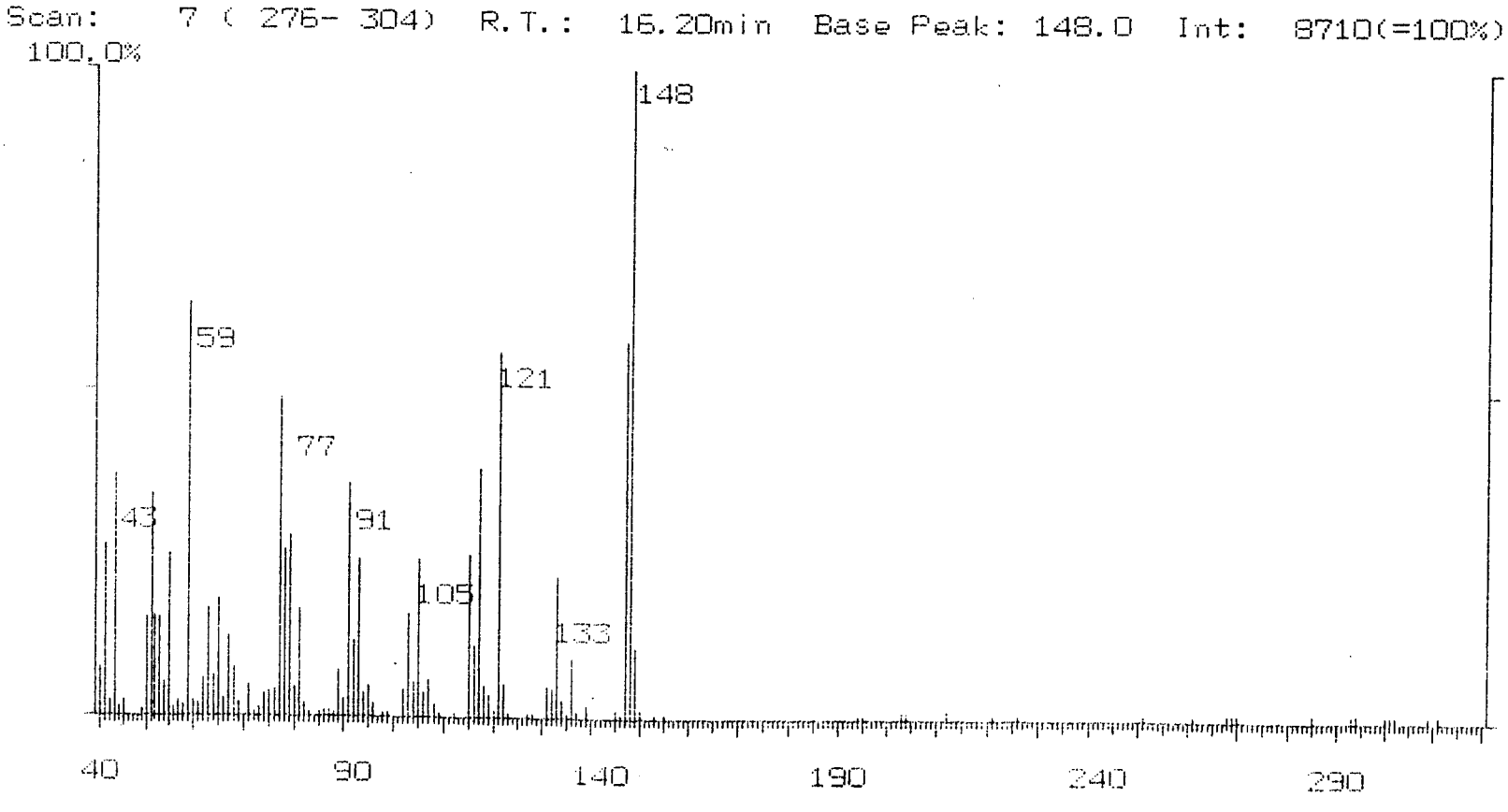


Fig. 4A$_{32}$. Mass Spectrum of unidentified compound

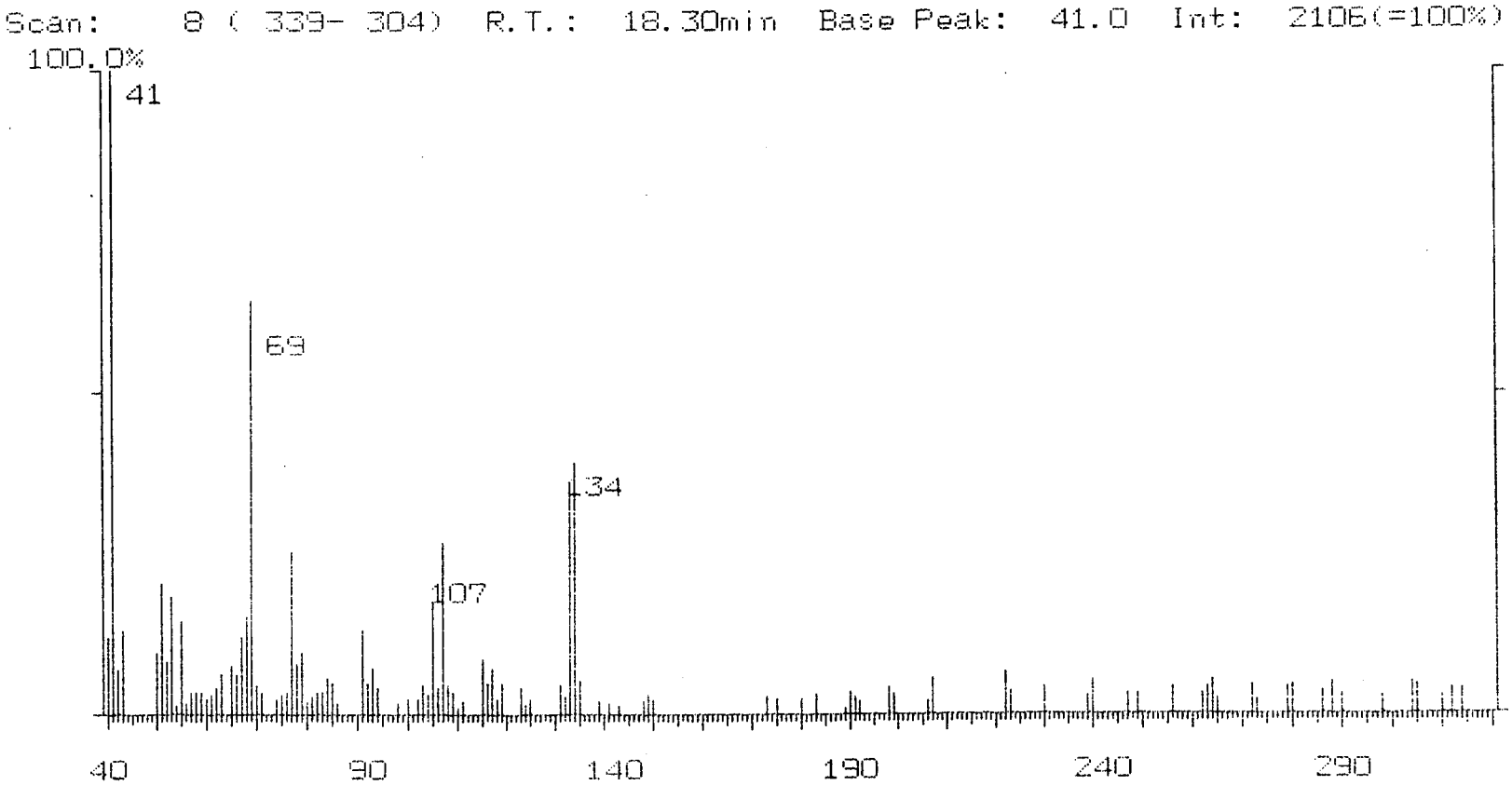


Fig. 4A$_{33}$. Mass Spectrum of Methyl cinnamate

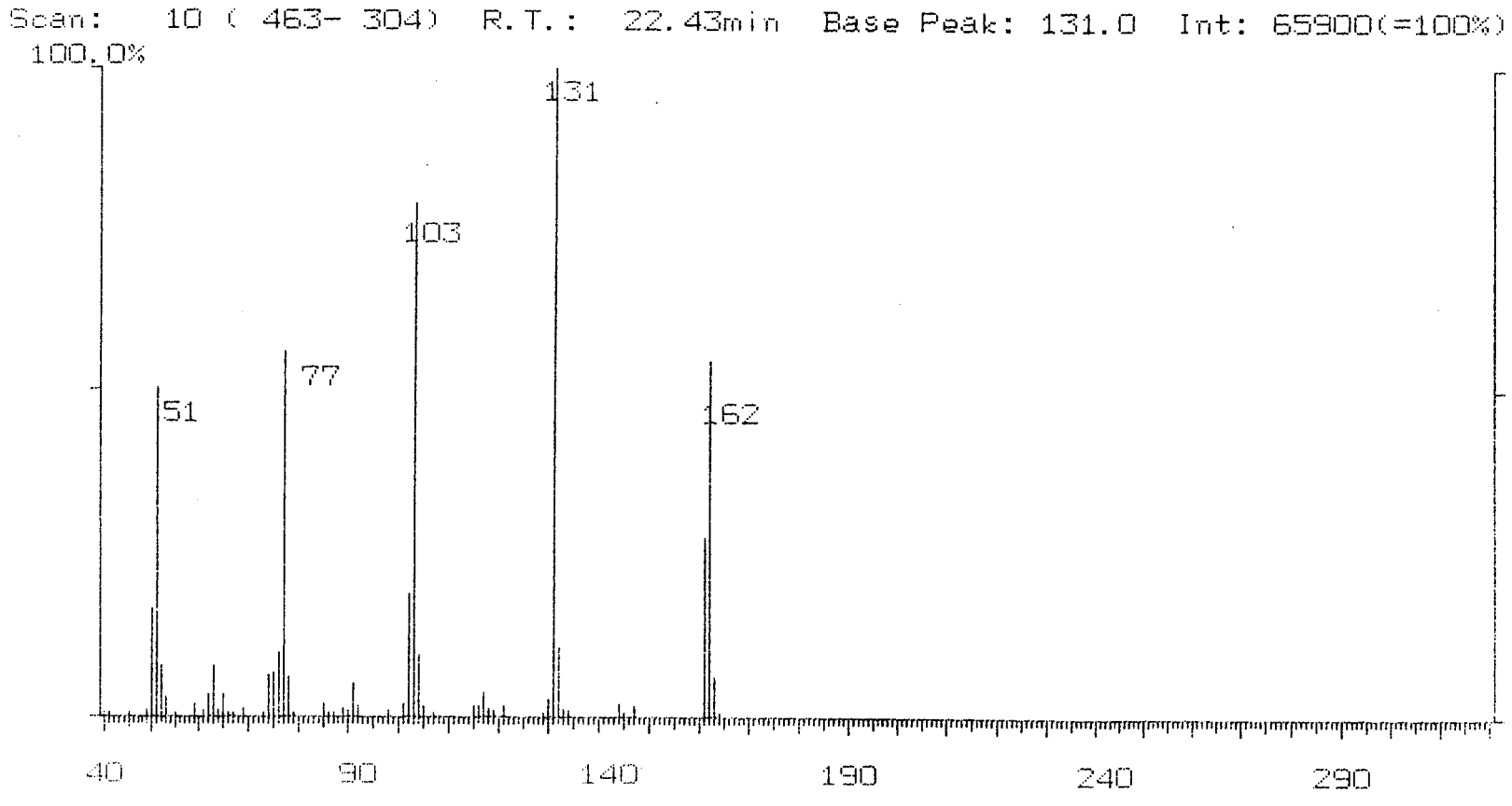


Fig. 4A$_{34}$. Mass Spectrum of unidentified compound

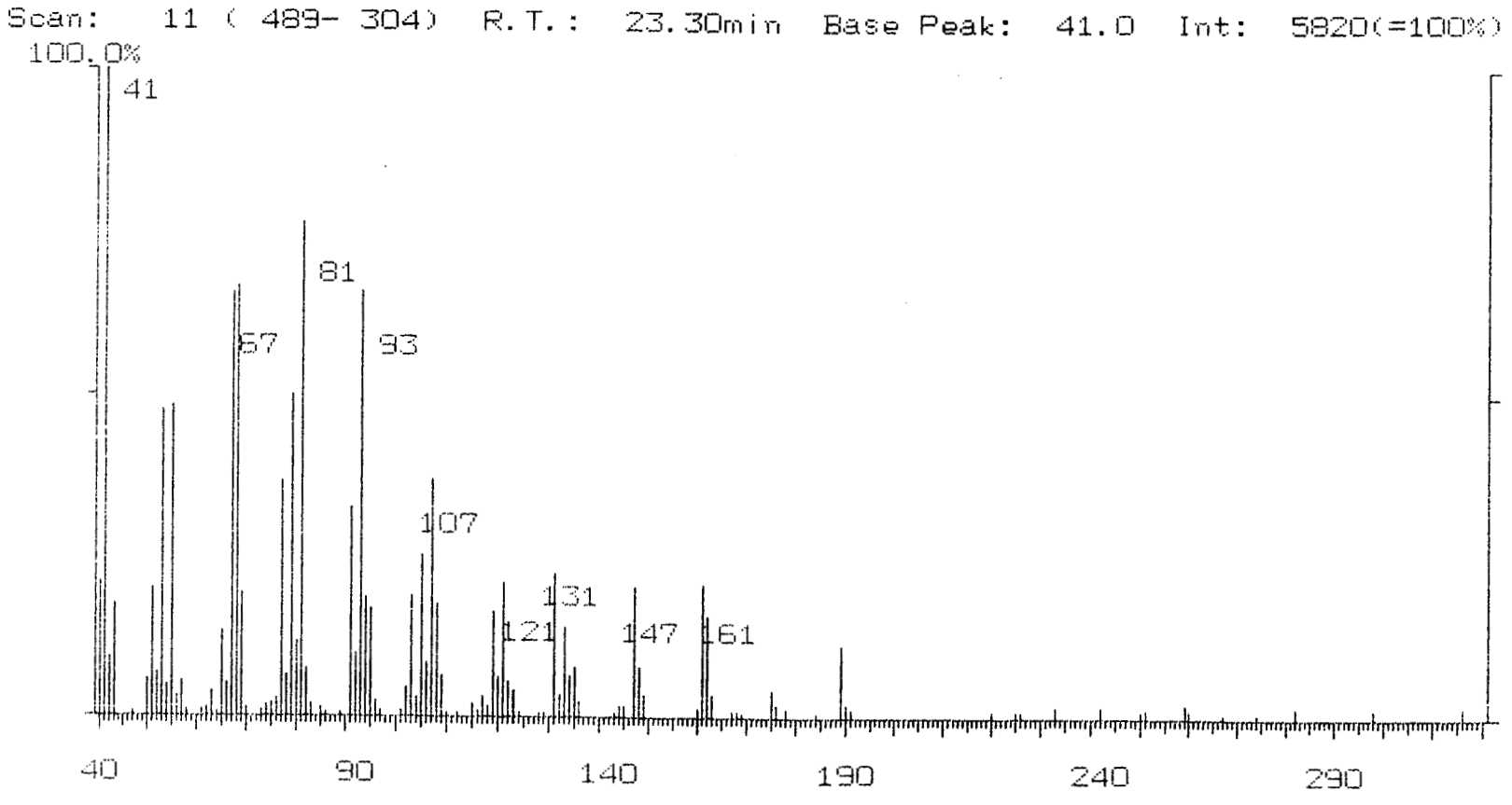


Fig. 4A$_{35}$. Mass Spectrum of β-caryophyllene

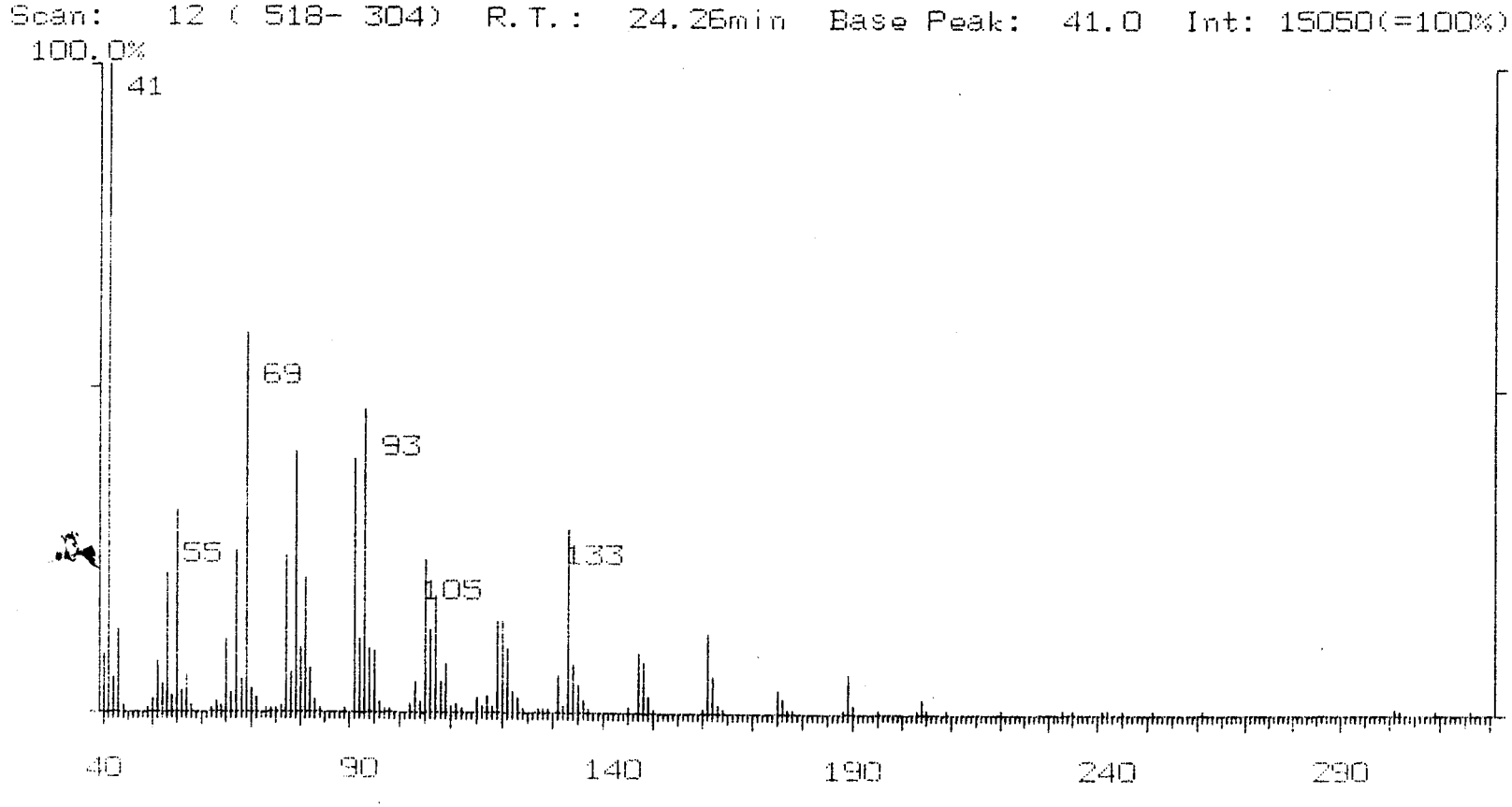


Fig. 4A$_{36}$. Mass Spectrum of Alloaromadendrene

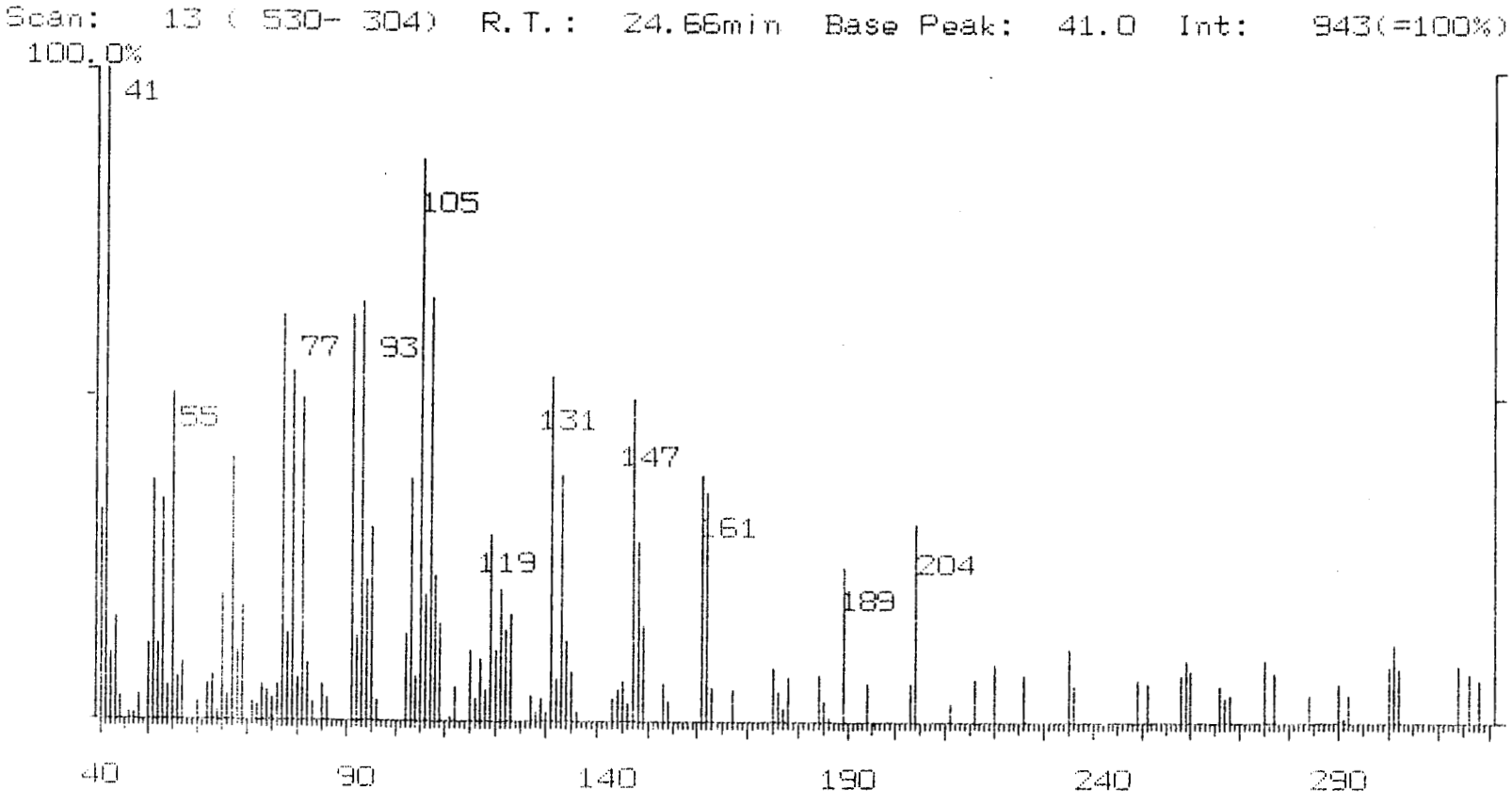


Fig. 4A$_{37}$. Mass Spectrum of α-humulene

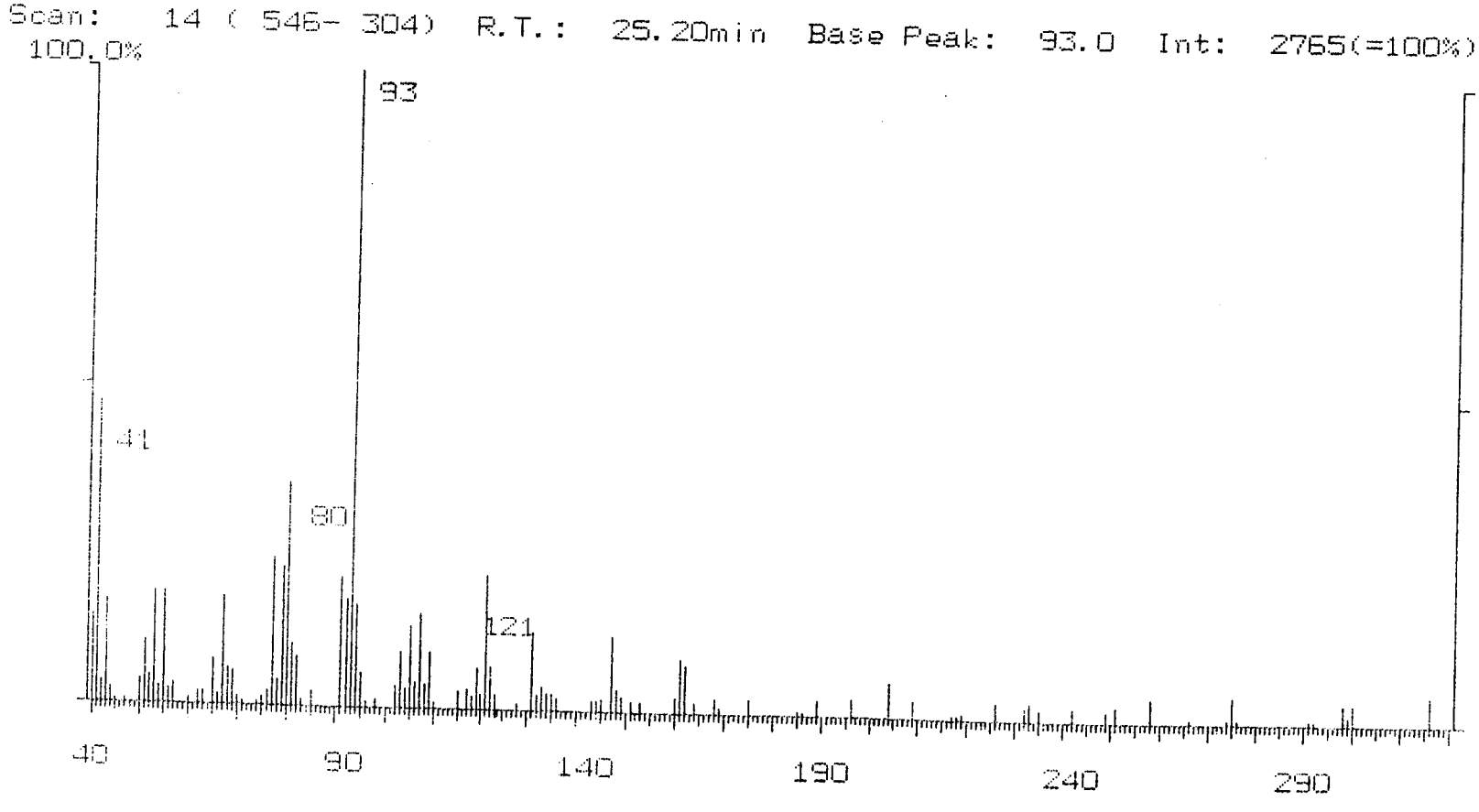


Fig. 4A$_{38}$. Mass Spectrum of Cubebene

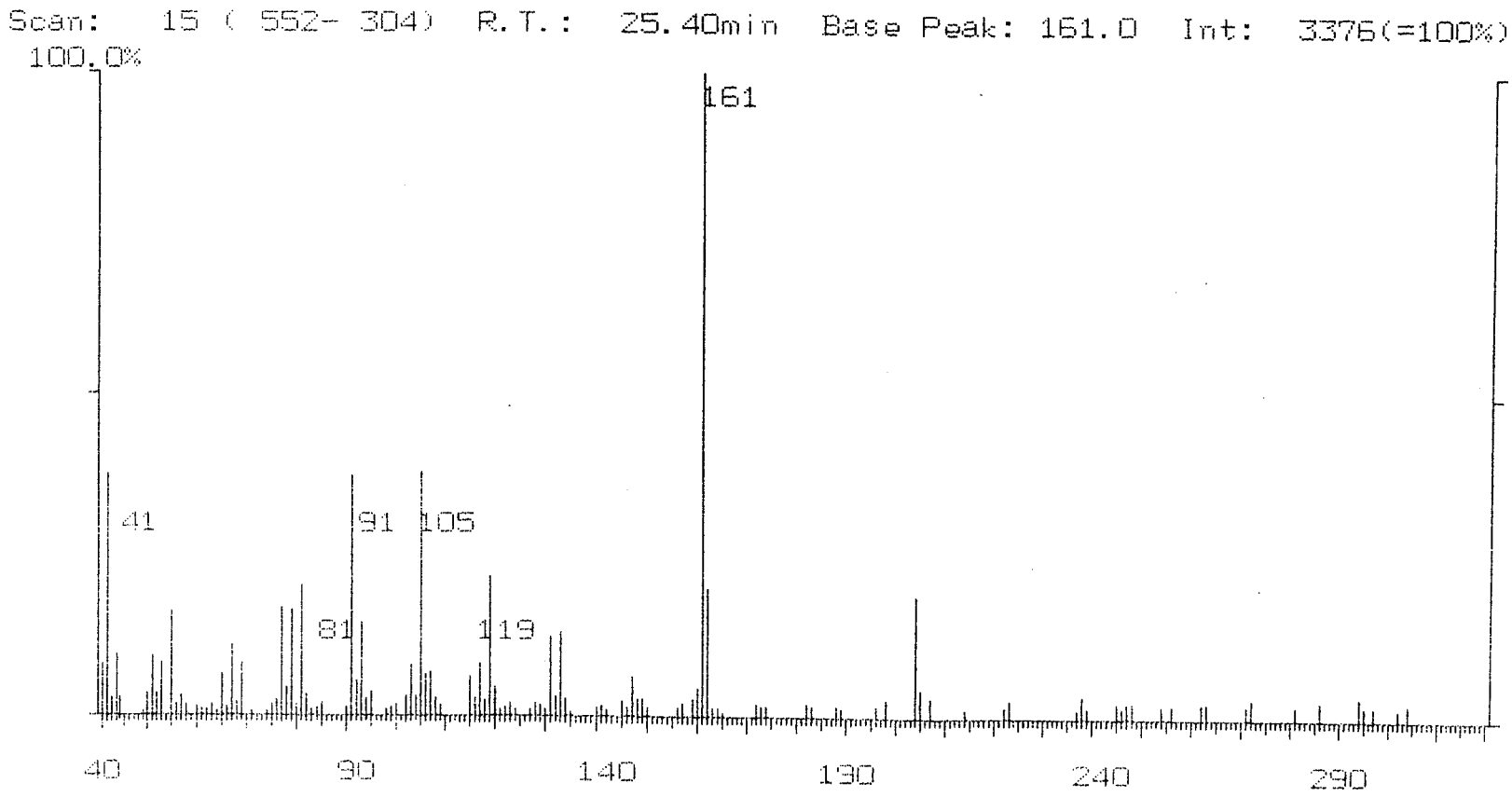


Fig. 4A$_{39}$. Mass Spectrum of Valencene

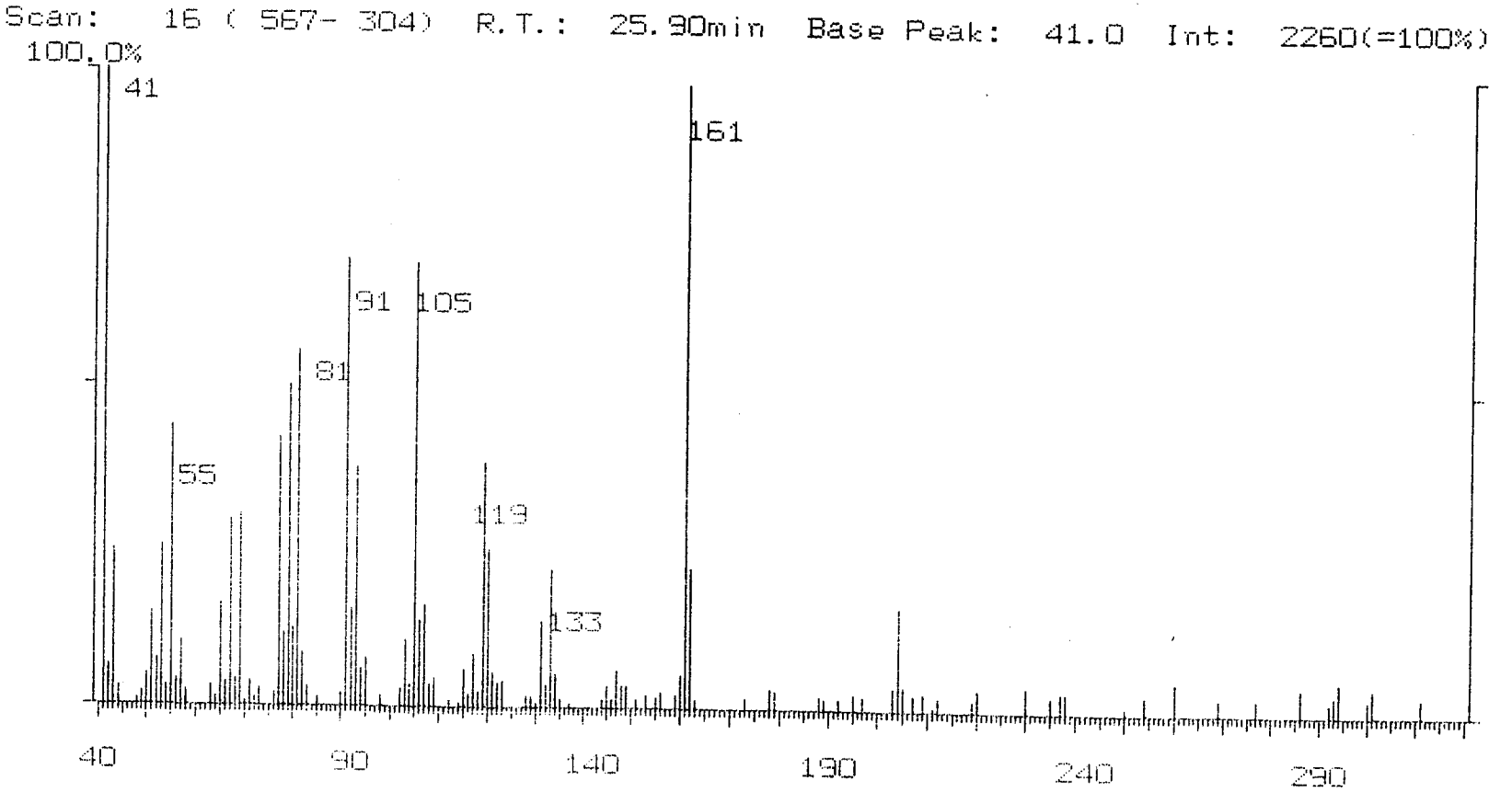


Fig. 4A$_{41}$. Mass Spectrum of α-himachalene

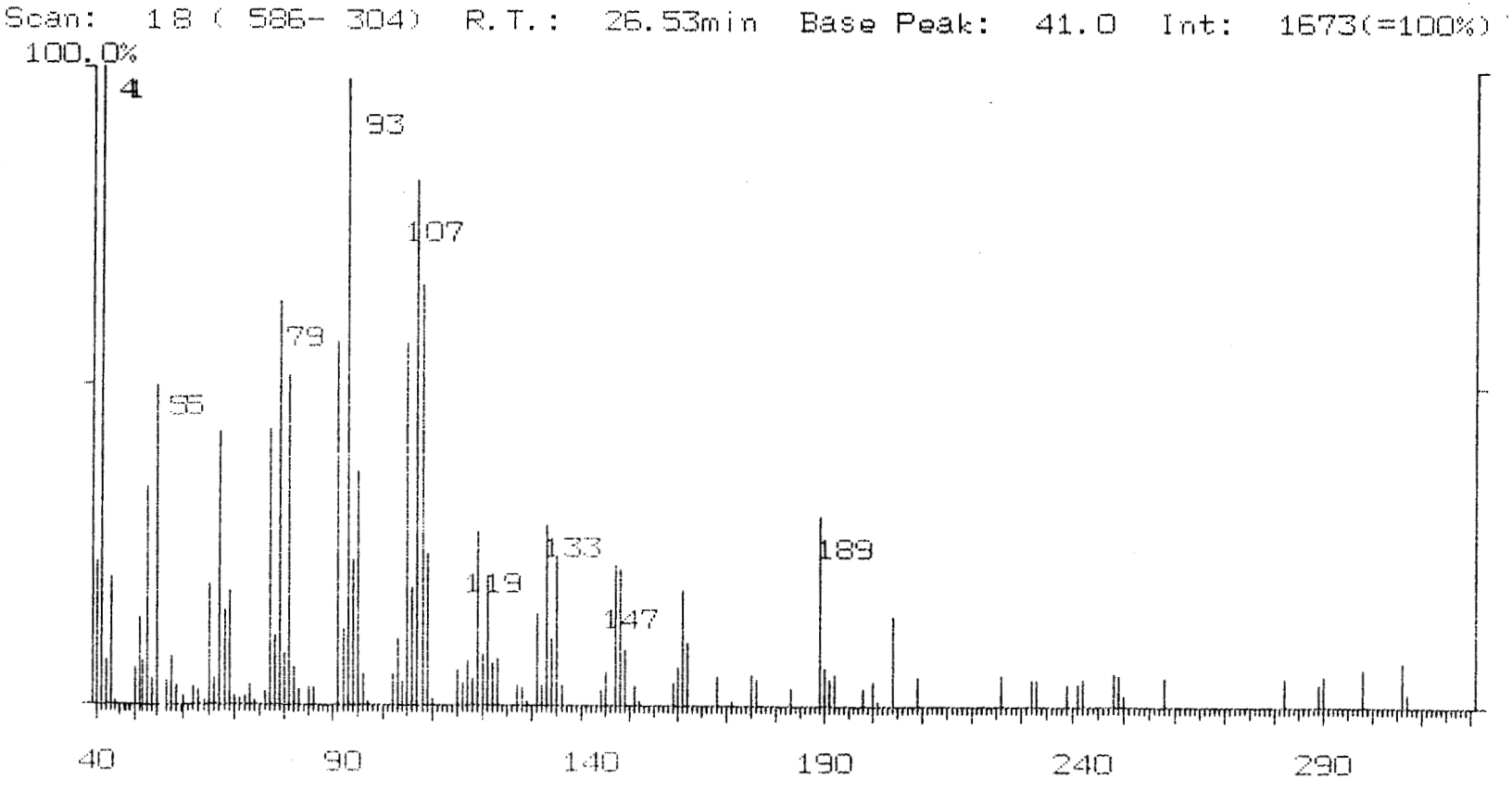
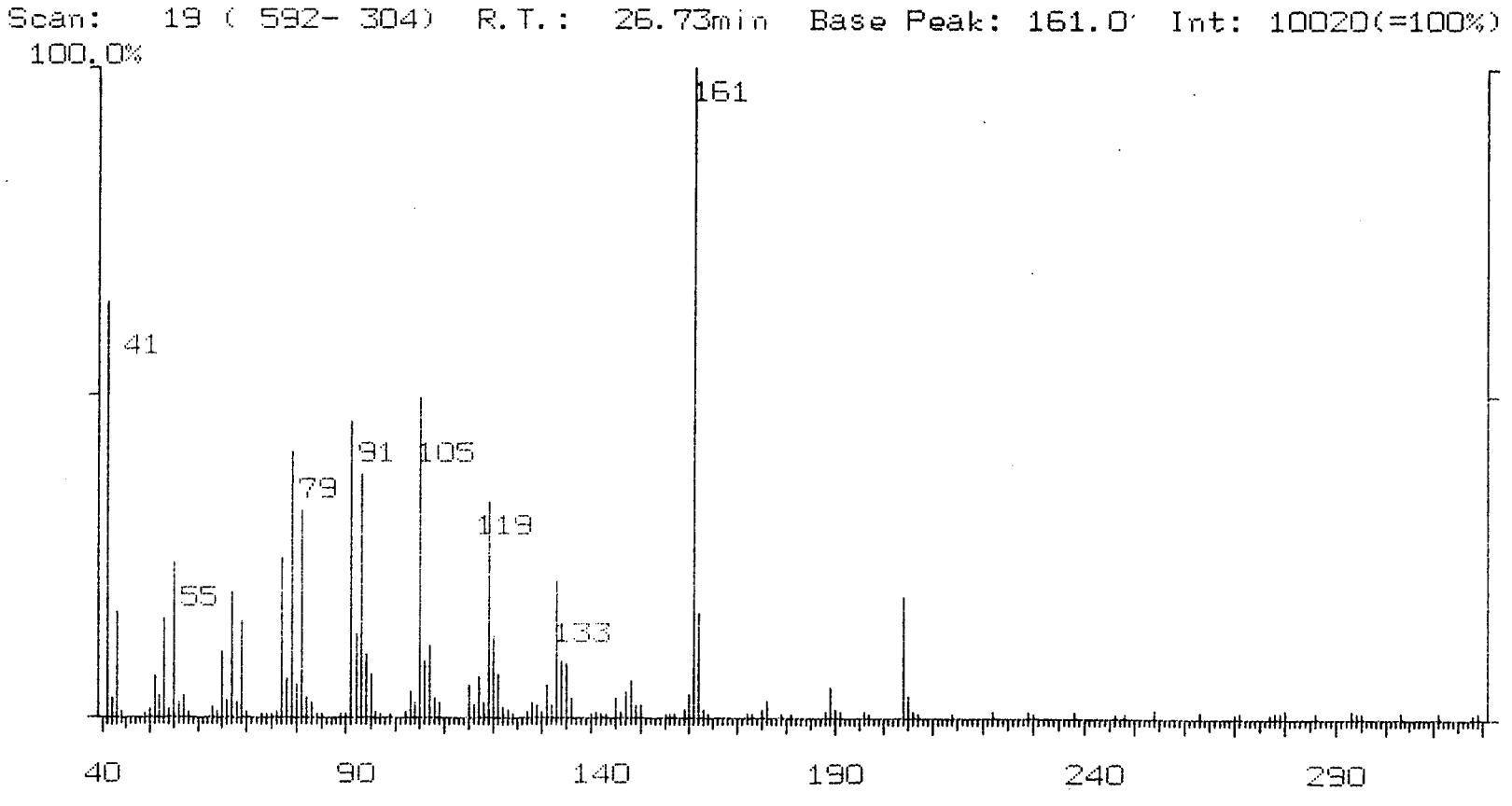


Fig. 4A$_{42}$. Mass Spectrum of β-gurjunene



O. basilicum L. cv. 'Fino verde'	Herb	Myrcene (0.31%) α-phellandrene (0.02%) α-terpinene (0.01%) Limonene (0.17%) 1,8-cineole (1.24%) (Z)-β-ocimene (0.06%) γ-terpinene (0.11%) (E)-β-ocimene (1.28%) p-cymene (0.06%) Terpinolene (0.15%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.01%) Octyl acetate (0.24%) α-copaene (0.13%) Camphor (0.44%) Linalool (49.48%) Octanol (0.14%) β-caryophyllene (6.74%) Methyl chavicol (0.5%) Neral (0.01%) α-terpineol (0.54%) α-borneol (0.16%) Geranyl acetate (1.93%) Geraniol (0.44%) Eugenol (16.19%) Chavicol (0.01%)	
cv. 'Sweet'	,,	α-pinene (0.12%) Camphene (0.02%) β-pinene (0.28%) δ-3-carene (0.17%) Myrcene (0.38%) α-phellandrene (0.02%) Limonene (0.17%) 1,8-cineole (3.37%) (Z)-β-ocimene (0.03%) γ-terpinene (0.07%) (E)-β-ocimene (0.70%) p-cymene (0.02%) Terpinolene (0.11%) (Z)-3-hexenyl acetate (0.02%) (Z)-3-hexenol (0.03%) Octyl acetate (0.07%) α-copaene (0.11%) Camphor (0.28%) Linalool (31.05%) Octanol (0.06%) β-caryophyllene (4.59%) Methyl chavicol (39.09%) Neral (0.05%) α-terpineol (0.56%) α-borneol (0.06%) Geranyl acetate (1.60%) Geraniol (0.12%) Eugenol (3.55%) Chavicol (0.23%)	Hasegawa *et al.*, 1997.

Fig. 4A$_{43}$. Mass Spectrum of Aromadendrene

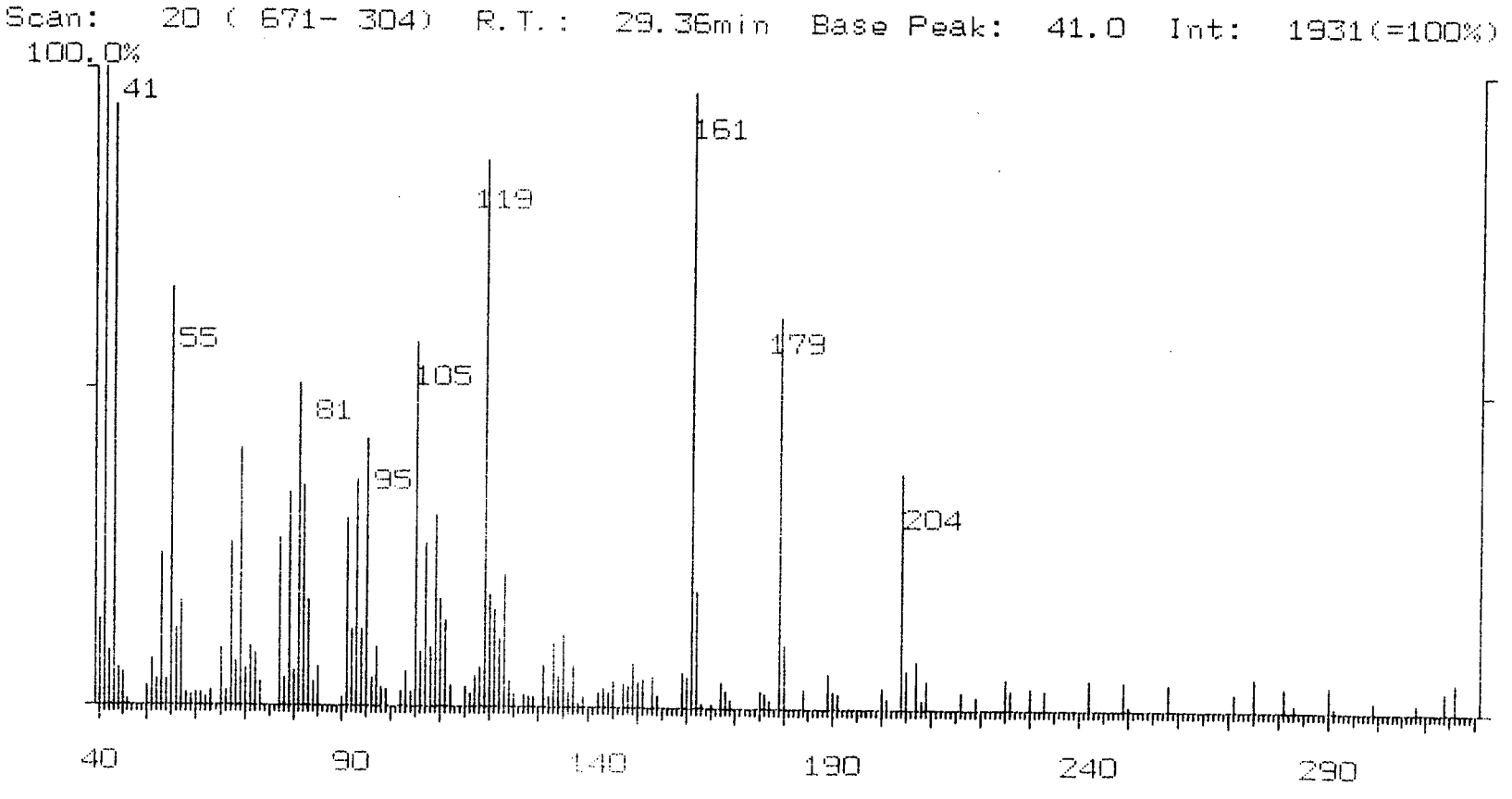
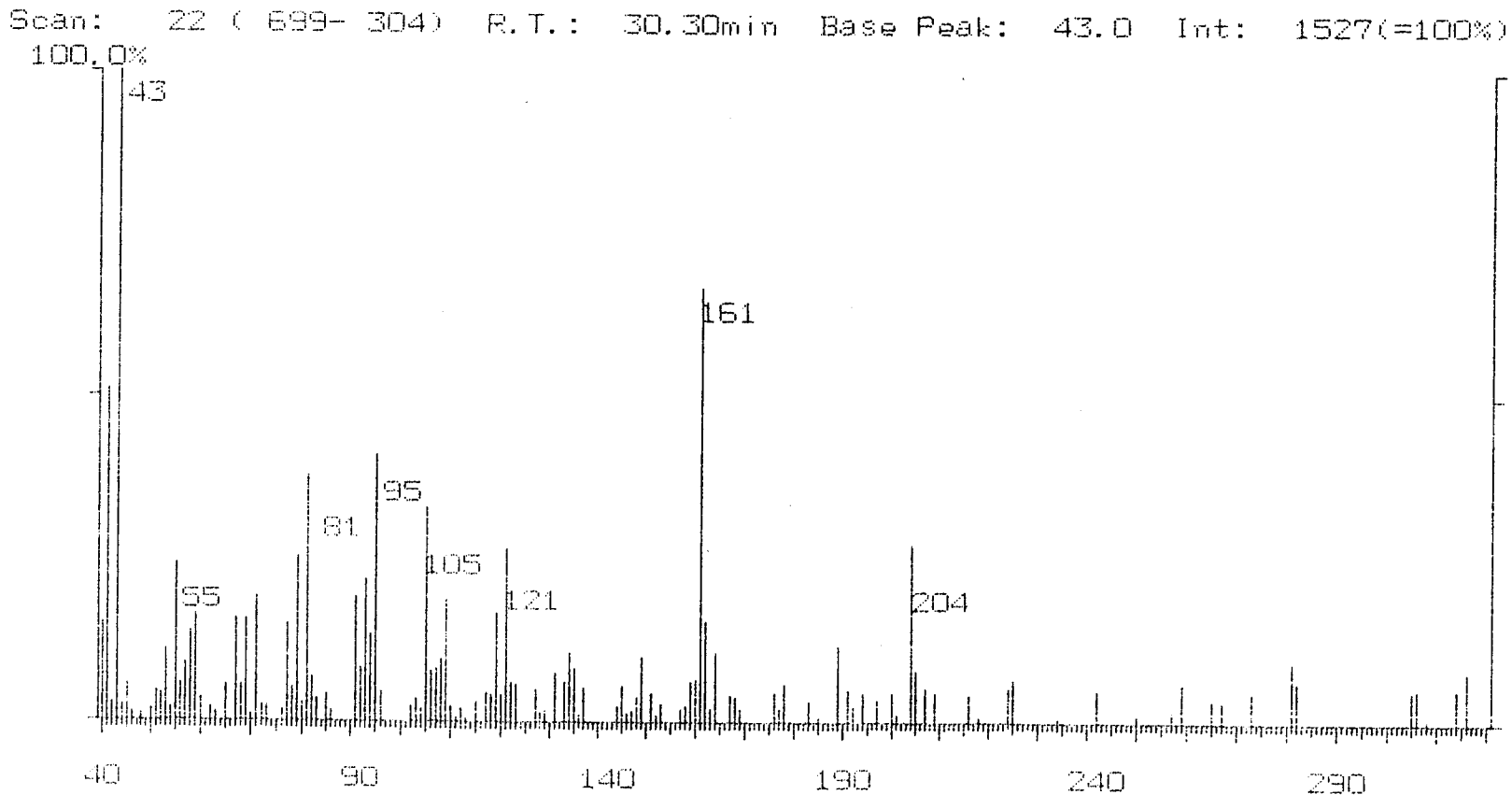


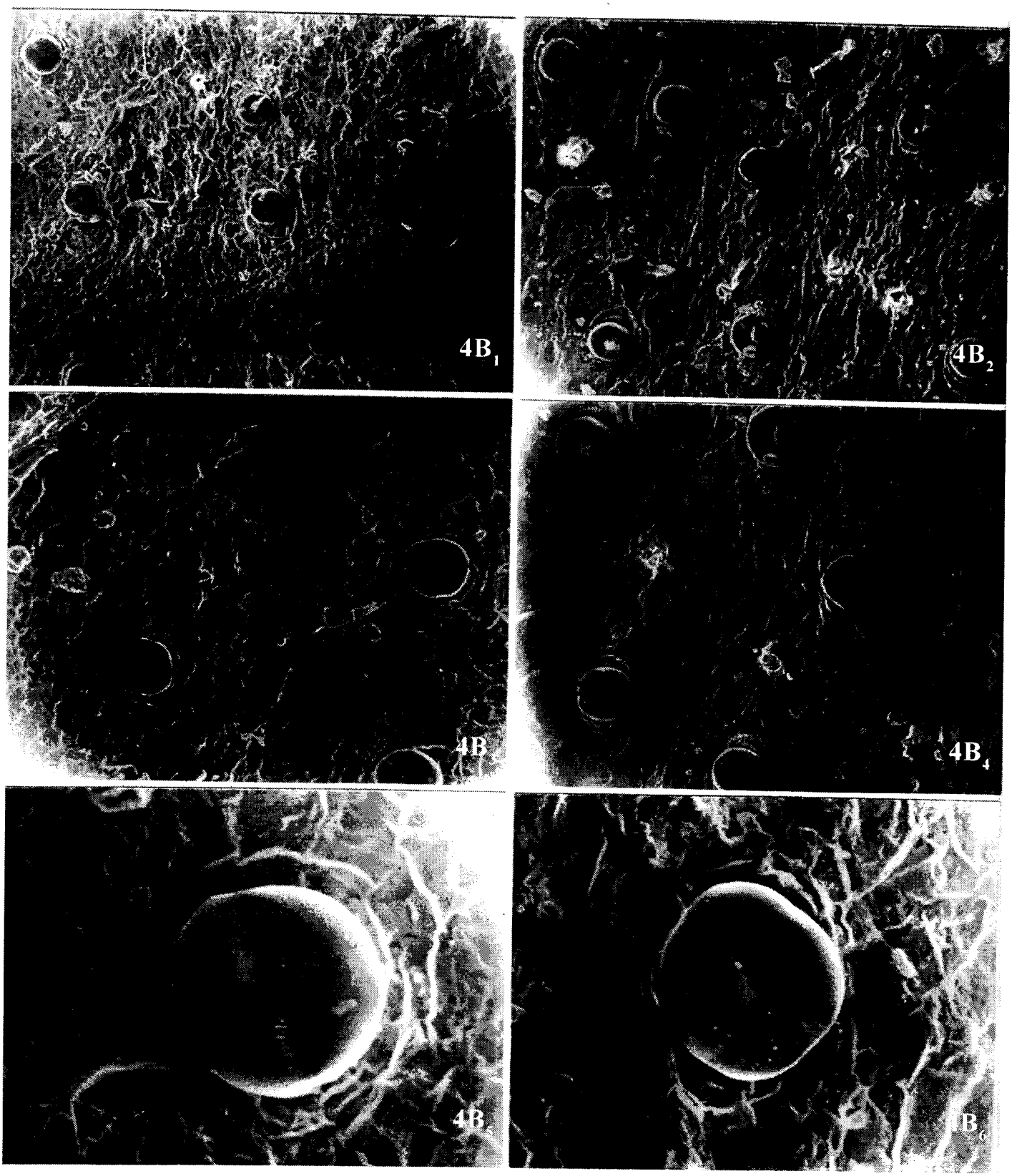
Fig. 4A$_{45}$. Mass Spectrum of unidentified compound



B. Scanning Electron Micrography (SEM)

Both secretory and non secretory trichomes are present on the leaves of *O. basilicum* L. var. *purpurascens* Benth. Two types of peltate glandular trichomes are seen, trichomes with two lobed head (Fig. $4B_5$) and trichomes with four lobed head (Fig. $4B_6$). Glandular hairs are often located in a depression of the leaf. Capitate glandular hairs are absent. The frequency of occurrence of glandular trichomes is higher on the adaxial surface of the leaf than the abaxial surface. Non glandular trichomes are uniseriate, simple and unbranched (Fig. $4B_3$).

Somaclonal variant (Figs. $4B_2$ & $4B_4$) did not show any variation in the structure and occurrence of trichomes with the somatic one (Figs. $4B_1$ & $4B_3$).



Figs. $4B_1$-$4B_4$. Scanning Electron Micrographs of glandular trichomes present in the leaf of *O. basilicum* L. var. *purpurascens* Benth.($4B_1$-60x & $4B_3$-90x) and its somaclonal variant ($4B_2$-60x & $4B_4$-90x).

Fig. $4B_5$. Two lobed glandular trichome (300x).
Fig. $4B_6$. Four lobed glandular trichome (300x).

DISCUSSION

Fig. 4A$_{39}$. Mass Spectrum of Valencene

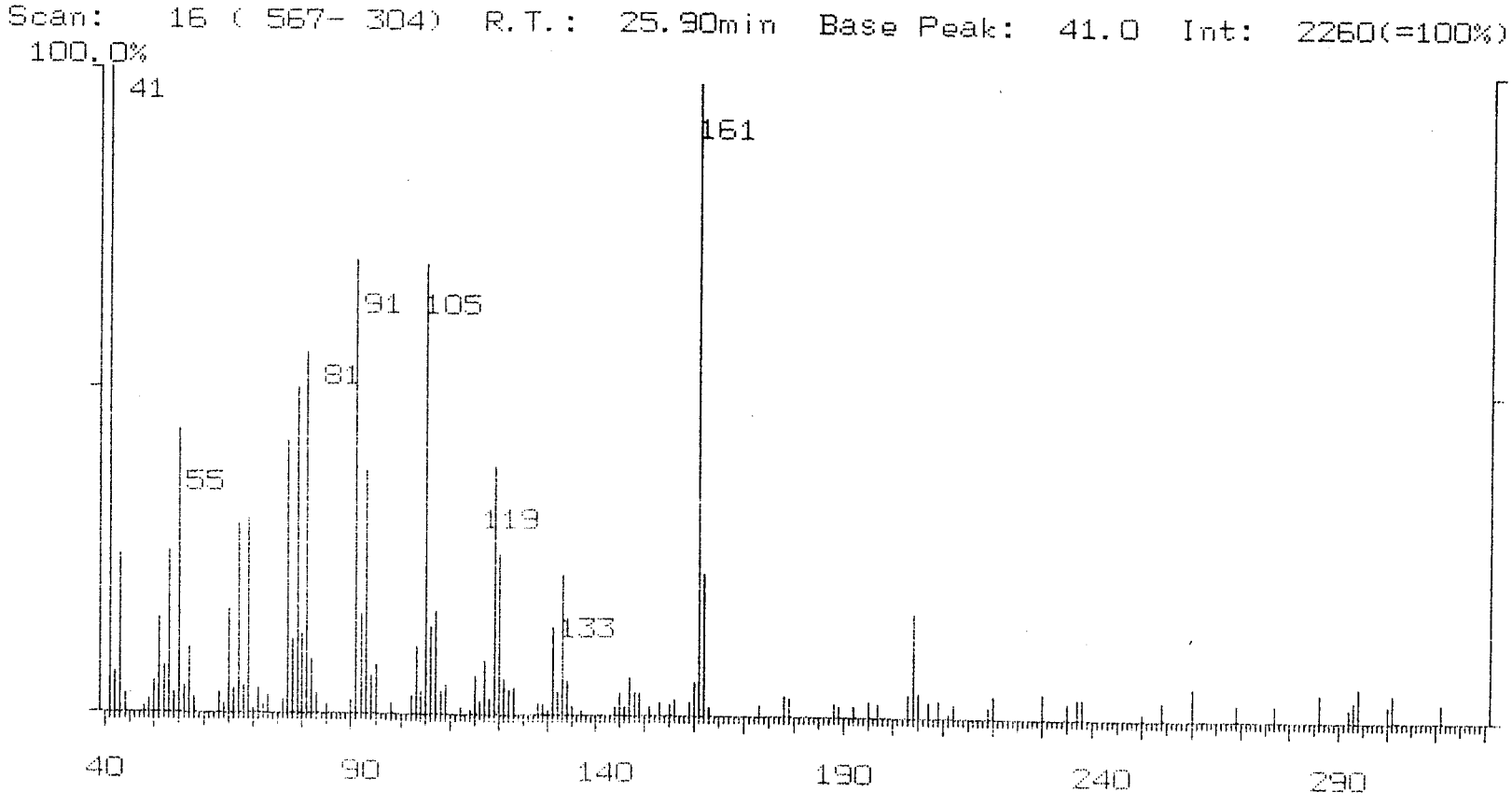


Fig. 4A$_{40}$. Mass Spectrum of γ-caryophyllene

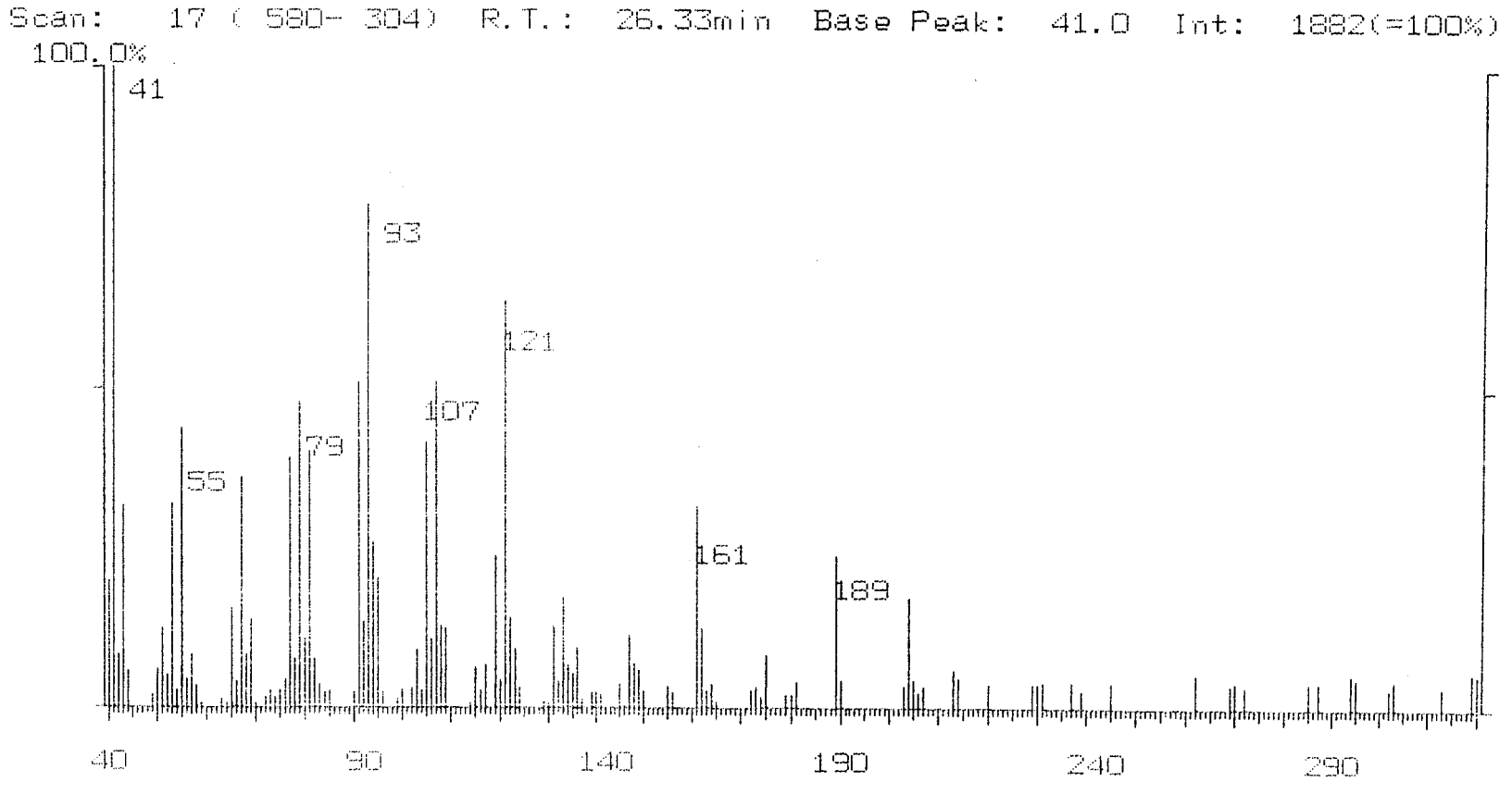


Fig. 4A$_{43}$. Mass Spectrum of Aromadendrene

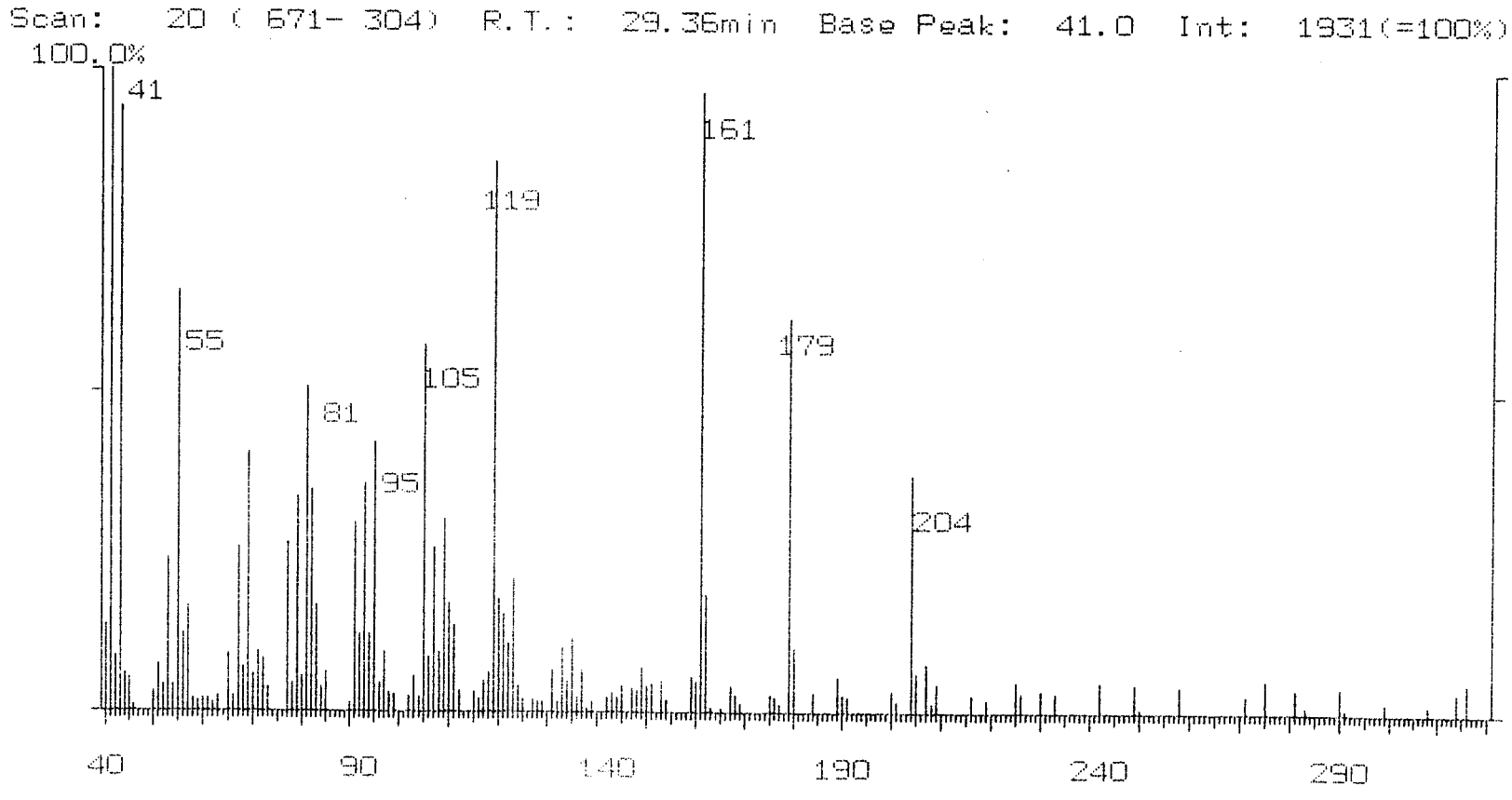
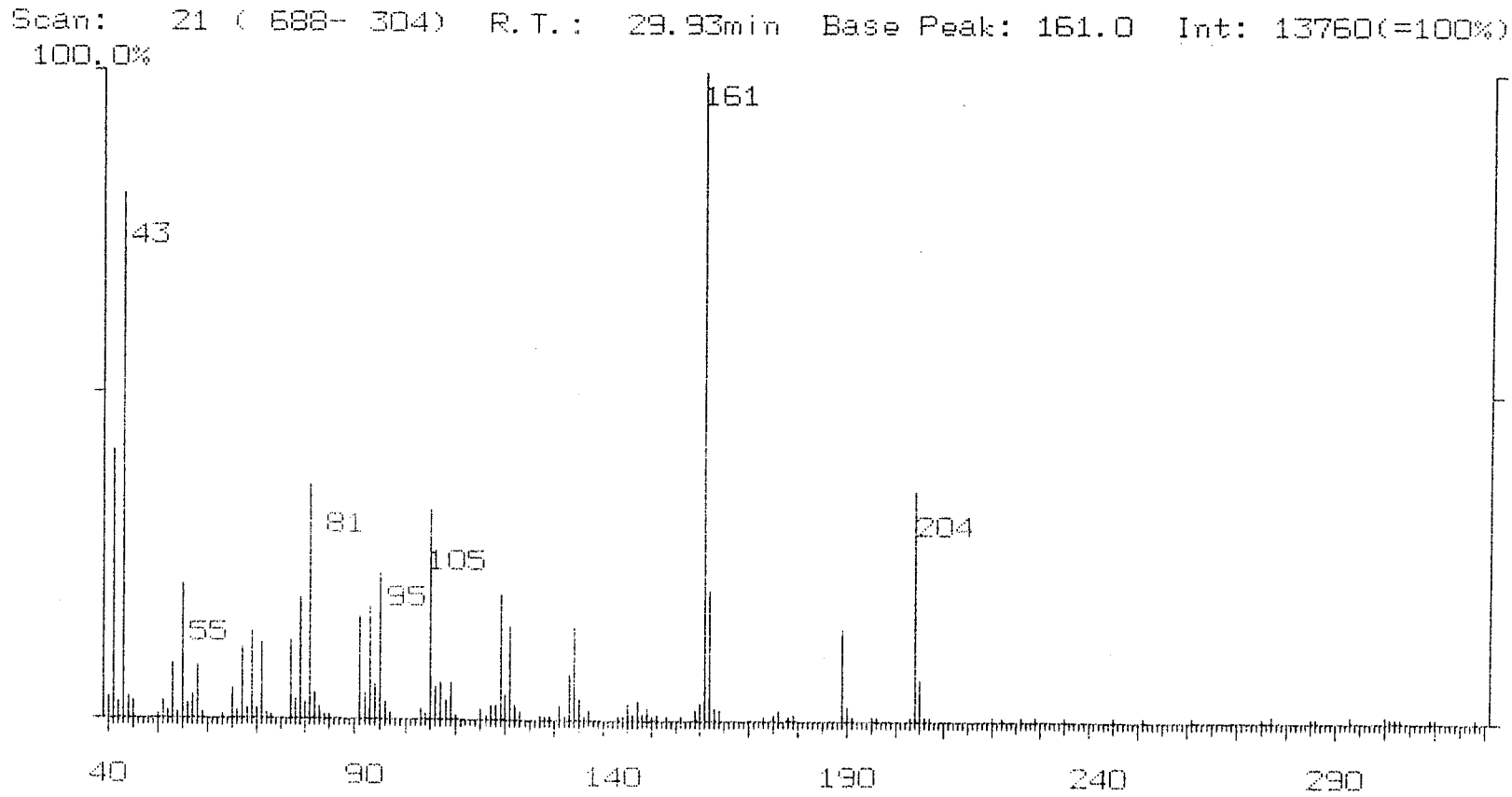


Fig. 4A$_{44}$. Mass Spectrum of unidentified compound



1. *In vitro* multiplication

The past 20 years of research on plant tissue culture, regenerated plants and progenies of the regenerated plants have revealed a rich array of genetic variants. The genetic behaviour of these variants generally appears similar to that of naturally occurring mutants. However, the frequency of various classes of mutants derived from plant tissue culture is elevated far beyond that expected in nature. Among the various methods developed to micropropagate plants, enhanced axillary branching culture has become the most important propagation method. This method is especially advantageous because propagation is simple and its rate is high.

Plants regenerated from relatively undifferentiated callus cultures possess a vast array of genetic changes. Such variations can result in useful agricultural and horticultural products. The presence or absence of variation depends upon the source of explant and the method of regeneration (Larkin *et al.*, 1989).

For the present study, nodal segments of *O. basilicum* L. var. *purpurascens* Benth. were used. This method is considered to be a low risk method for genetic instability (Pierik, 1991; Schoofs, 1992) because organized meristems are considered to be more resistant to genetic changes than unorganized callus (Shenoy & Vasil, 1992). Notwithstanding these considerations, there are numerous reports on the incidence of somaclonal variation among micropropagated plants (Swartz, 1990; Maynard *et al.*, 1991; Grenan, 1992; Martinelli, 1992; Moore *et al.*, 1992; Schoofs, 1992; Rani *et al.*, 1995; Hashmi *et al.*, 1997).

The present results indicate that the combination of BAP and IAA was practically effective in inducing multiple shoots in *O. basilicum* L. var. *purpurascens* Benth. The morphogenic response of explant was dependent on the type and concentration of growth hormones. There was maximum multiplication in the medium with 2 mg/l BAP + 0.3 mg/l IAA (Fig. 1.2). With KIN the result was unsatisfactory with regard to multiplication though shoot elongation was noticed. BAP shows more activity than KIN for shoot proliferation (Lundergan & Janic, 1980; Rahman & Blake, 1988; Sen & Sharma, 1991). Three different auxins, namely 2,4-D, NAA and IAA with BAP or KIN were used for shoot multiplication. There was no significant synergistic effect of any auxin treatment except in the set of IAA with BAP. Of the two cytokinins tested (BAP and KIN), BAP was found to be comparatively more effective than KIN in inducing multiple shoots from nodal segments (Figs. 1.1 & 1.2).

To overcome the apical dominance of the terminal shoot bud and to enhance the branching of the lateral buds from axils, the role of cytokinins have been understood. It is

known that BAP is the most effective synthetic cytokinin for stimulating axillary shoot proliferation for different plant systems (Bhojwani, 1980; Hasegawa, 1980; Kitto & Young, 1981; Welander *et al.*, 1989; Nadel *et al.*, 1991; Devi *et al.*, 1994; Gangopadyay *et al.*, 1998). The stimulatory effect of BAP on multiple shoot formation was reported in *O. sanctum* by Shahzad and Siddiqui (2000). The stimulatory effect of BAP in the present investigation in multiple shoot formation was in consonance with the reports on *Ocimum* (Ahuja *et al.*, 1982; Pattnaik & Chand, 1996; Sahoo *et al.*, 1997; Phippen & Simon, 2000).

The wide spread occurrence of somaclonal variation has been extensively documented in many species from cereals to trees (Bajaj, 1990; Phillips *et al.*, 1994). However, among the essential oil yielding plants a little effort has been made so far to obtain somaclonal variants (Sreenath & Jagadishchandra, 1991; Sahoo & Debata, 1995; Patnaik *et al.*, 1999).

In the current study it is observed that the combination of KIN with NAA produced phenotypically distinct somaclonal variant (Figs. 1.11 & 1.12). These variants produced three leaves from each node instead of the normal two opposite leaves. Plants isolated from tissue culture have been reported to be variant in many cases. Plantlets of sugarcane isolated through tissue culture technique have shown variations in morphology, chromosome number and enzyme system (Skirvin, 1978). Morphological variation in somaclones of *Lathyrus* was also reported (Tiwari *et al.*, 1995).

Plants regenerated from tissue or organ culture could diverge from the donor plants for one or more phenotypic traits as a result of different mechanisms of somaclonal variations which include changes in chromosome number and structure, dominant and recessive mutations, changes in chloroplasts and mitochondria (Bingham & Mc Coy, 1986). Stress induced by tissue culture process (eg. hormone effects, nucleotide pool imbalance etc.) causes alterations in DNA. These alterations could affect the expression of specific genes (Kaeppler & Phillips, 1993). Conditions in the artificial environment of cell culture may enhance the spontaneous mutation rate. The mutation rate may also be enhanced by leakage of toxic by-products and exudates from the calli into the surrounding medium (Olhoft & Phillips, 1999). It has already been recognized that the nature of growth regulators used in the medium may result in the occurrence of somaclonal variation (Patel & Berlyn, 1982). One of the possible mechanisms suggested to explain somaclonal variation is the activation of different classes of mobile genetic elements, such as those reported during tissue culture of maize (Peschke & Phillips, 1991), tobacco (Hirochika, 1993) and rice (Hirochika *et al.*, 1996). Transposable element activation has been shown to be induced by genomic shock (Mc Clintock, 1984). Epigenetic variation is another important cause of somaclonal variation in plants. Epigenetic aspects of somaclonal

variations involve mechanisms of gene silencing or gene activation that were not due to chromosomal aberrations or sequence change. (Kaeppler *et al.*, 2000).

2. Cytological Analysis.

The plant cells growing in an artificial culture environment show numerous genetic changes. These alterations are manifested as increased frequencies of single gene mutations, chromosome breakages, transposable element activations, quantitative trait variations and modifications of normal DNA methylation patterns (Kaeppler & Phillips, 1993). Variations could have been induced by *in vitro* process or by added biochemicals and stresses (Swartz, 1990) and therefore understanding the events, which give rise to such variations need more study.

Tissue culture induced variation has usually been based on phenotypic differences in regenerated plants and their progeny. However, genomic changes appear to be the basis for the phenotypic alterations (Phillips *et al.*, 1994).

In the present investigation, the chromosome number of both *O. basilicum* L. var. *purpurascens* Benth. (Fig. 2.1A_2) and its somaclonal variant (Fig. 2.2A_2) was found to be 2n=48, which is in consonance with majority of the earlier reports (Table A). However the somaclonal variant showed karyotypic variations (Figs. 2.1C & 2.2C). Variations in karyotypes involved change in centromeric position and total length of the chromosomes (Tables 5 & 6). The change in chromosome length may be the aftermath of cryptic changes, probably duplication, which may arise due to *in vitro* stress produced in the altered culture environment. Moreover, retrotransposon activation and insertions may significantly contribute to the change in physical size of the genome (Olhoft & Phillips, 1999). Similar reports are available in *Allium cepa* (Sekerka, 1977b) and *Papaver somniferum* (Bajwa & Wakhlu, 1986). Reports on karyotypic variations are also available in *Triticum durum* (Gupta & Ghosh, 1983) and *Haplopappus gracilis* (Singh, 1981). Chennaveeraiah and Habib (1966) reported the structural rearrangements of chromosomes in cultures of *Capsicum annuum*.

The chemical composition of the culture medium has been shown to affect the cytogenetic behaviour of plant cells *in vitro* (Bennici *et al.*, 1970; Singh, 1972; Singh & Harvey, 1975; Bajwa & Wakhlu, 1986; Karp, 1992). Mineral deficiencies, chelating agents, some heavy metal ions and anaerobic conditions have been implicated in inducing chromosomal breakage and rearrangements in plants (Steffensen, 1961). The hormone itself can potentially be toxic to the cell thereby directly causing chromosomal aberrations.

The concentration and type of hormone in the culture medium influence the pattern of methylation in cultured *Daucus carota* L. genomes. (LoSchiavo *et al.*, 1989; Arnholdt-Schmitt *et al.*, 1991). Singh (1976) reported chromosomal changes induced by KIN in *Haplopappus gracilis*. The effects of cytokinins and auxins in inducing chromosomal changes were studied in tissue culture of *Nicotiana* (Ronchi *et al.*, 1976). The majority of reports agree that the chromosome aberrations are generated during culture by growth regulators such as 2,4-D, IAA, NAA and KIN (Singh, 1993). Singh (1986) reported that the chromosomal variation in callus cultures of barley was possibly due to media components. The significance of nutrient medium in chromosomal behaviour of *Allium* and *Capsicum* cultures was studied by Nair *et al.* (1993) and Nair and Kumar (1998).

Chromosome rearrangements are frequently found in plants regenerated from tissue cultures. The alterations, which are the result of chromosome breakage events, have been well characterized in maize and oats (Benzion *et al.*, 1986). A variety of mutational types have been explained which most likely, are responsible for the observed phenotypic variation. These changes include cytological aberrations, which are primarily the result of chromosome breakage, single base changes, changes in the copy number of repeated sequences and alterations in DNA methylation pattern. Chromosome breakage followed by the reunion of the broken ends leads to translocations, inversions, duplications and deletions (Benzion & Phillips, 1988; Lapitan *et al.*, 1988; Phillips *et al.*, 1994). Among these abnormalities translocations were the most frequently observed (Kaeppler *et al.*, 2000).

A genetic change commonly observed in tissue culture is the alteration of chromosome number, which has also been included among the types of genetic events responsible for some of the commercially important plant chimeras (Marcotrigiano & Gouin, 1984). In this study, the *O. basilicum* variant did not show any sign of instability in chromosome number but showed structural rearrangements. However, these results require molecular evidences for genomic variation. The examination of solid-stained chromosomes can only reveal changes in number and in gross morphology. Molecular approaches such as DNA finger printing were recently used to analyze the somaclonal variants (Isabel *et al.*, 1996; Piccioni *et al.*, 1997; Yang *et al.*, 1999).

3. Random Amplified Polymorphic DNA (RAPD) Analysis

The tissue culture environment may cause a general disruption of cellular controls, leading to numerous genomic changes present in tissue culture regenerants (Phillips *et al.*, 1994). As far as the genetic stability of the proliferated tissue is concerned, RAPD markers

are an efficient tool for the detection of somaclonal variation in tissue culture. Genetic molecular markers were considered to be reliable in monitoring variability in the DNA sequence of plants (Goto *et al.*, 1998). Direct analysis of DNA by use of RAPD markers proved a very sensitive technique for evaluating genetic changes after *in vitro* culture (Piccioni *et al.*, 1997). RAPD analysis using arbitrary 10-mer oligonucleotide primers was employed in order to investigate the genetic variability of somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth.

Two types of polymorphisms were observed in this study, such as band intensity differences and the absence of fragments (Figs. 3.1-3.3). *O. basilicum* L. var. *purpurascens* Benth. (Somaclonal variant) produced three additional bands with primer OPX 04, seven additional bands with OPX 15 and three additional bands with OPX 16 when compared to the donor plant. The presence of additional bands in the somaclonal variant supports the increase in chromosome length in it, as evidenced by the CHIAS studies. The presence of RAPD markers in two genotypes indicates a high level of sequence homology at that site. In the case where the marker was present in one genotype but not in the other, there is the certainty of sequence difference (Williams *et al.*, 1990; Yang *et al.*, 1999). Failure of amplification of different lines may be due to a single base change or completely different sequences (Vierling & Nguyen, 1992). According to Rani *et al.* (1995) the polymorphisms in amplification products represent changes in the sequence of the primer binding site (eg. point mutations) or changes which alter the size or prevent the successful amplification of a target DNA (eg. insertions, deletions, inversions). Differences in band intensity were observed and have been reported in celery (Yang & Quiros, 1993) and in peach (Hashmi *et al.*, 1997). Sequence variation arising through the culture process has been detected in several different ways including genome scanning using RAPDs (Kaeppler *et al.*, 1998; Olhoft & Phillips, 1999; Kaeppler *et al.*, 2000).

From the present study it can be concluded that somaclonal variation at DNA level is present in the variant of *O. basilicum* L. var. *purpurascens* Benth. (Figs.3.1-3.3). Similar somaclonal variations were reported in *Triticum aestivum* (Brown *et al.*, 1993), *Lolium* (Wang *et al.*, 1993), poplar (Rani *et al.*, 1995), beet (Munthali *et al.*, 1996) and peach (Hashmi *et al.*, 1997) using RAPDs. These results also suggest that RAPDs are useful for establishing the genetic basis for somaclonal variation. This is the first information on the molecular basis of variation detected by RAPD in micropropagated plants of *Ocimum*.

4. Essential oil Analysis

A. Gas Chromatography-Mass Spectrometry (GC-MS)

Tissue culture generated plants may vary from the parent plant for its morphological characters, as a result of somaclonal variation induced by culture stresses. Morphological variations are sometimes associated with useful agronomic characters such as oil yield, oil content etc. (Patnaik *et al.*, 1999).

In most cases, study of somaclonal variations has been limited to phenotypic variation and has been associated with changes in chromosome number and structure. Somaclonal variations in yield and quality characters have utmost importance in crop improvement. The present analysis showed that there is a significant change in the essential oil content and composition of somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth. (Figs. $4A_1$ & $4A_{24}$). The essential oil components detected in both the donor plant as well as its somaclonal variant is in agreement with many of the previous reports (Table B).

The reports on somaclonal variants of essential oil yielding plants such as *Mentha piperita* (Holm *et al*, 1989; Nadaska *et al.*, 1990), *M. arvensis* (Kukhreja *et al.*, 1992), *Cymbopogon winterianus* (Mathur *et al.*, 1988) and *C. martinii* (Patnaik *et al.*, 1999) revealed favourable variation in oil content. A significant genetic variation was reported in variation with physiological and biochemical traits in different somaclones of *Lathyrus* (Tiwari *et al.*, 1995).

Chemical investigation on essential oils of *O. basilicum* L. var. *purpurascens* Benth. and its somaclonal variant showed that the analyzed plant materials may probably belong to methyl chavicol-methyl cinnamate-camphor chemotype. In both samples the major components remained unchanged even if there is marked variation in the other constituents. This indicates that the genetic changes due to culture stresses did not affect the biosynthetic pathway of major components. The absence of some constituents (Table 8) in the essential oil of somaclonal variant may be due to some hindrance to the biosynthesis of these compounds. The phenolics, sesquiterpenes and monoterpenes in *Ocimum* have separate biopathways (Khosla *et al.*, 1989). Almost all the secondary metabolites, monoterpenes, sesquiterpenes and phenyl propenes arise from one of the three biosynthetic pathways or from a combination of two or more of these pathways. These are known as the acetate, mevalonate and shikimate pathways (Waterman, 1993).

So the change in one pathway leads to variation in the chemical composition of the essential oil.

In the present investigation there is a change in composition of minor components (Table 8). Volatile oils are chemically complex mixtures often containing in excess of 100 individual components. Most oils have one to several major components, which impart characteristic odour, taste, but the many minor constituents also play their part in the final product (Waterman, 1993).

The biosynthesis of secondary plant products is controlled by genetic factors (Franz, 1989). Because of the close connection between differentiation and developmental process and secondary metabolism in plants, the growth regulators may influence the formation of secondary metabolites (Petri *et al.*, 1989). The genetic basis of biosynthesis of monoterpenoids and sesquiterpenoids in *Mentha* (Lincoln *et al.,* 1986) and *Ocimum* (Khosla *et al.*, 1985) were already proved. In the present investigation the essential oil variation may be the after-effect of genetic changes revealed by chromosome studies and RAPD analysis.

B. Scanning Electron Micrography (SEM)

Differences in trichome densities are a probable factor associated with oil yield as observed earlier in the different cultivars of Patchouli (Sugimura *et al.*, 1990). In the leaf, all the volatile oil is contained in the trichomes (Croteau & Johnson, 1984). In the present study SEM conducted on the leaves showed that there is no significant correlation between variation in essential oil yield and trichome densities of *in vivo* grown plant and the somaclonal variant. The trichome density, the type and the size of the glands remain more or less the same in both the donor plant and its somaclonal variant. So it can be concluded that the quantitative and qualitative differences observed among *O. basilicum* L. var. *purpurascens* Benth. and *O. basilicum* L. var. *purpurascens* Benth. (Somaclonal variant) are not due to any differences either in the trichome density or in the trichome morphology but due to the genetic factors as evidenced by cytological and RAPD analyses.

Study of somaclonal variations is relevant to applications such as *in vitro* plant propagation and plant transformation. Barbara Mc Clintock (1984), in her Nobel lecture said, " Some responses to stress are especially significant for illustrating how genome may modify itself when confronted with unfamiliar conditions. Changes induced in genomes when cells are removed from their normal locations and placed in tissue culture surroundings are outstanding examples of this. The establishment of a successful tissue culture from animal cells, such as those of a rat or mouse, is accompanied by readily observed genomic restructuring. None of this animal tissue culture has given rise to a new animal. This, the significances of these changes for the organism as a whole is not directly testable. The ability to determine this is a distinct advantage of plant tissue culture."

All the genetic modifications that occur in nature are also manifested in tissue culture, but at an accelerated rate. Somaclonal variations may be the avenue to learn more about the behaviour of genetic material when exposed to tissue culture environment. Specific genomic alterations associated with tissue culture variations have been well characterized, but the mechanism leading to these changes is not well understood. Revealing the mechanisms of mutation will lead to a better understanding of genomic change in response to stress, factors contributing to genomic stability and methods to control variation among tissue culture regenerants. Any step made towards understanding the basis of tissue culture induced genetic variation should be helpful in developing a more stable and manipulable somatic cell systems.

The purpose of the present work is to describe the phytochemical variations found among the tissue culture regenerants of *O. basilicum* L. var. *purpurascens* Benth. and then relate this to the genetic basis of variation. Detailed studies such as gene identification, gene sequencing and gene expression on this aspect are needed for the utilization of such variations in crop improvement by site directed mutagenesis and genetic engineering. *O. basilicum* L. var. *purpurascens* Benth. being a pharmaceutically and commercially important plant of international status, any attempt to improve this essential oil crop will have vital as well as commercial importance.

SUMMARY

The family Lamiaceae is a very large natural group of aromatic and medicinal plants. *Ocimum* is an important member of the Lamiaceae family. Sweet basil (*Ocimum basilicum* L.) is a perennial shrub, which grows in several regions all over the World and is a widely cultivated species. It is indigenous to lower hills of Punjab and cultivated throughout India. The essential oil of *O. basilicum* L. is used in pharmaceutical industries and traditional medicines. Fresh herb is also used as a condiment and spice.

Attempts have been made in past to distinguish between varieties of O. *basilicum* L. Eventhough different varieties were reported the total number of varieties is still obscure.

In this work, efforts have been directed at presenting the cytogenetical aspects and the related phytochemical after-effects of somaclonal variation in *O. basilicum* L. var. *purpurascens* Benth. Cytogenetical assays include Chromosome Image Analysis System (CHIAS) and Random Amplified Polymorphic DNA (RAPD) analyses. Whereas, the phytochemical studies include Gas Chromatography-Mass Spectrometry (GC-MS) and Scanning Electron Micrography (SEM).

1. *In vitro* multiplication

For the induction of multiple shoot regeneration, nodal explants were inoculated in the MS medium with combinations of cytokinins and auxins and cytokinin alone. The multiple shoot induction was noticed in the medium with 1 mg/l KIN+0.2 mg/l NAA. The frequency of shoot induction and percentage of initiation was higher in the medium containing 2 mg/l BAP+0.3 mg/l IAA. The nodal cultures in the medium containing KIN and NAA produced morphologically variant plantlets with three leaves at each node. These plants were used for further analysis.

2. Cytological Analysis

The ploidy levels of both, the donor plant and the somaclonal variant were invariably tetraploid (2n=4x=48). Neither chimeral nor aneuploid variations were found. Chromosome morphology of the regenerated plant showed slight variation. Some of the chromosomes were not the exact replica of the standard chromosomes of the donor plant and exhibited structural changes, thereby change in basic

karyotype also. The somaclonal variant showed increase in total chromosome length and average chromosome length. Changes in DI, VC and TF% were also noticed.

3. Random Amplified Polymorphic DNA (RAPD) Analysis

To obtain more information on genetic diversity between somaclonal variant and the donor plant, RAPD analysis was carried out using 15 primers of arbitrary sequence. Approximately 100 bands were amplified for each sample. Of the 15 primers used, 14 successfully amplified the extracted DNA with consistently reproducible banding. One of the 15, OPX 02 had to be excluded from the final comparison because of the inconsistent amplification. The number of bands resolved per primer ranged from a minimum of 3 to maximum of 11. The size of amplification product also differed and ranged from 564 bp to 4277 bp.

The RAPD fingerprints of the somaclonal variant differed from that of the donor plant with three primers used. Although both genotypes gave unique products with every primer, some primers OPX 04, OPX 15 and OPX 16 revealed additional bands for somaclonal variant. Altogether these primers generated 13 new RAPD bands in the somaclonal variant that were absent in the donor plant fingerprints, while all the bands that were present in the donor plant fingerprints were scored in the somaclonal variant. In many cases intensity differences between corresponding bands were pronounced.

4. Essential oil Analysis

A. Gas Chromatography-Mass Spectrometry

The essential oils of *O. basilicum* L. var *purpurascens* Benth. and its somaclonal variant were analyzed quantitatively and qualitatively. The oil yield of somaclonal variant (0.9%) was higher than that of the donor plant (0.6%). The essential oils were analyzed by GC-MS and 22 compounds were identified which included monoterpenes, sesquiterpenes and phenols. The GC-MS pattern of somaclonal variant essential oil was distinctly different when compared with the pattern of the donor plant oil.

The quantitative and qualitative differences observed in the essential oil directly influenced the colour of the oil distilled. The oil from the *in vivo* grown plant have light yellow colour, whereas essential oil of *in vitro* grown plant showed slight dark yellow.

There is a clear difference between the compositions of two oils analyzed. The analysis of the oil samples revealed a range of variation in their constituents. The major components identified from both oils were same (methyl chavicol, methyl cinnamate and camphor), showing variation in percentage composition of methyl chavicol (37.9% & 38.6%) and camphor (17.5% & 21.8%). The percentage of methyl cinnamate (27.4%) is the same in both oils.

2-butanol (0.5%), camphene (0.6%), limonene (3.3%), γ-terpinene (tr.) and β-bisabolene (tr.) were present in the essential oil of *in vivo* grown plant, whereas these components were absent in the somaclonal variant. The somaclonal variant is characterized by the presence of cis-sabinene hydrate (0.6%) and alloaromadendrene (tr.), which were absent in the donor plant. In addition to these, two unidentified components were found to be present in the essential oil of somaclonal variant.

B. Scanning Electron Micrography (SEM)

Differences in the trichome densities are a probable factor associated with oil yield from the essential oil yielding plants. The present studies showed that there is no significant correlation between variation in essential oil yield and trichome densities of *O. basilicum* var. *purpurascens* Benth. and its somaclonal variant.

For the present study nodal segments of *O. basilicum* L. var. *purpurascens* Benth. were used. This method is considered to be a low risk method for genetic instability because organized meristems are considered to be more resistant to genetic changes than unorganized callus. Notwithstanding these considerations, there are numerous reports on the incidence of somaclonal variation among micropropagated plants.

The wide spread occurrence of somaclonal variation has been extensively documented in many species from cereals to trees. However, among the essential oil yielding plants a little effort has been made so far to obtain somaclonal variants.

In the current study it is observed that the combination of KIN with NAA produced phenotypically distinct somaclonal variant. These variants produced three leaves from each node instead of normal two opposite leaves. Plants regenerated from tissue or organ culture could diverge from the donor plants for one or more phenotypic traits as a result of different mechanisms of somaclonal variations which include changes in chromosome number and structure, dominant and recessive mutations, changes in chloroplasts and mitochondria.

Tissue culture induced variation has usually been based on phenotypic differences in regenerated plants and their progeny. However, genomic changes appear to be the basis for the phenotypic alterations. The chemical composition of the culture medium has been shown to affect the cytogenetic behaviour of plant cells *in vitro*. Chromosome aberrations, especially duplications are frequently found in plants regenerated from tissue cultures, which may complement to the increase in their chromosome size. However these assumptions require molecular evidences.

Direct analysis of DNA by using RAPD markers proved to be a very sensitive technique for evaluating genetic changes after *in vitro* culture. Two types of polymorphisms were observed in this study, such as band intensity differences and the absence of fragments. The presence of RAPD markers in two genotypes indicates a high level of sequence homology at that site. In the case where the marker was present in one genotype but not in the other, there is the certainty of sequence difference. Failure of amplification of different lines may be due to a single base change or completely different sequences.

In most cases, study of somaclonal variations has been limited to phenotypic variation and has been associated with changes in chromosome number and structure. Somaclonal variations in yield and quality characters have utmost importance in crop improvement. The present analysis showed that there is a significant change in the essential oil content and composition of somaclonal variant of *O. basilicum* L. var. *purpurascens* Benth. The biosynthesis of secondary plant products is controlled by genetic factors. Because of the close connection between differentiation and developmental process and secondary metabolism in plants, the growth regulators may influence the formation of secondary metabolites. In the present investigation the essential oil variation may be the after-effect of genetic changes revealed by chromosome studies and RAPD analysis.

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