METABOLIC CHANGES OF MANGO (*Mangifera indica* Linn.) SEEDS DURING DESICCATION AND GERMINATION

Thesis submitted to the
University of Calicut in partial fulfilment of the requirements
for the Degree of Doctor of Philosophy

JISHA MATHEW

DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
2006

Department of Botany

UNIVERSITY OF CALICUT

Dr. K.M. JAYARAM, Ph.D.
Lecturer

Division of Plant Physiology
and Biochemistry
Department of Botany
University of Calicut
Kerala, India – 673 635
Phone: 0494-2401144 * 406, 407
Fax : 0494 – 2400269
Grams: UNICAL
e-mail: jayaramkm@yahoo.co.in

15-03-2006.

CERTIFICATE

This is to certify that the thesis entitled **"Metabolic Changes of Mango [*Mangifera indica* Linn.] Seeds During Desiccation and Germination"** submitted by **Mrs. Jisha Mathew** in part fulfilment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, is a bonafide record of research work undertaken by her in this Department under my supervision during the period 2001-2006 and that no part there of has been presented before for any other degree.

Dr. K.M. Jayaram

'GAEA', P.O. KANHANGAD SOUTH, KASARAGOD DT., KERALA – 671 315 Phone: [redacted] - 205403

DECLARATION

I hereby declare that the thesis entitled **"Metabolic Changes of Mango [*Mangifera indica* Linn.] Seeds During Desiccation and Germination"** submitted by me in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, has not been submitted before for any other degree.

Jisha Mathew

Place: Calicut University Campus
Date: 15-03-2006

Acknowledgements

I convey my gratitude to ***Dr. K.M. Jayaram,*** *Lecturer, Department of Botany, University of Calicut for his guidance, co-operation and encouragement throughout this work.*

I express my profound gratitude to ***Dr. Nabeesa Salim,*** *Reader, Department of Botany for her whole hearted co-operation, guidance, support and constructive criticism throughout the course of my work, without which this would not have been possible.*

My thanks are due to ***Dr. Neelakandan*** *and* ***Dr. S. Nandakumar,*** *Former Heads, Department of Botany for providing necessary facilities and guidance for carrying out this work successfully.*

I am greatly indebted to ***Dr. P.V. Madhusoodanan,*** *Prof. & Head of the Department of Botany for providing facilities and constant encouragement for this completion of the work.*

My special thanks are to ***Ms. Shereena J, Ms. Vijayalakshmi K.M., Ms. Mini C.B,*** *Research Scholars, Department of Botany,* ***Ms. Sini S*** *&* ***Ms. Nisha V.M.*** *M.Phil students for the help rendered by them.*

Thanks are also due to ***Mr. Ratheesh Chandra, Mr. Gentle Jose, Mr. Abdul Salam A.K, Mr. Babu A.*** *and* ***Mr. Sanoj E.*** *Project fellows, Department of Botany for their help.*

I express my sincere thanks to FIP teacher fellows Physiology and Biochemistry laboratory for their co-operation and help.

I am grateful to ***Mr. K. Ajayakumar, Mr. Santhosh*** *and* ***Mr. Azeez,*** *Art & Photography unit for their assistance for the photographic work.*

I sincerely thank all those who have helped and encouraged me in one way or the other throughout the period of my work.

Finally I am grateful to my parents and husband for their love and encouragement.

Above all, I thank ***God,*** *the Almighty for the kindness and blessings he has been showering upon me.*

JISHA MATHEW

Dedicated to My

beloved Mother

CONTENTS

Introduction

Mangifera indica (Linn.), belonging to the family Anacardiaceae, is a large evergreen tree producing drupaceous fruits. The fruits are dark green in colour when developing on the tree and turns lighter green to yellow as they ripen. The mesocarp is the fleshy edible part of the fruit and when ripe its colour varies from yellow to orange with a smooth fibrous texture. Eventhough polyembryonic and monoembryonic seeds have been reported in mango (Morton, 1987), seeds used in the present investigation (Natumanga) are monoembryonic.

The chemical constituents of seeds of some commercial mango varieties have been studied (Diaz *et al.*, 1982; Lasztity *et al.*, 1988) and according to those authors, starch was more prominent. Studies on seed kernels of 3 mango varieties showed the presence of the essential amino acids (Augustin and Ling, 1987). Arogba *et al.*, (1998) investigated the polyphenol oxidase activity of filtered extract of ground kernel suspension of mango and found that the enzyme was most active at pH 6.0 and 25° C.

Chandra (1980) studied seed germination and biochemical analysis of fresh and aged seeds and observed cent percentage germination on zero and 30

days after harvest and declined there after. The germination studies of mango seeds collected from ripe fruits were done by Corbineau *et al.*, (1986) and those authors observed that the seeds possessed no dormancy, high moisture content (85%) and died quickly on dehydration. Fu *et al.*, (1989) studied the effect of desiccation, wet storage and cryopreservation on the viability of mango seeds. Those authors observed that the seeds have high moisture content and lose viability within 4-7 days when desiccated under open conditions and hence these seeds are recalcitrant.

Recalcitrant seeds are short-lived and hence they need to be planted immediately after collection. Many investigations have been undertaken to find out the storage longevity (storability) of recalcitrant seeds (King and Roberts, 1980; Farrant *et al.*, 1989; Tompsett, 1992; Xia *et al.*, 1992; Pritchard *et al.*, 1995). Moisture content of recalcitrant seeds comes under the range of 30-70% (Pammenter *et al.*, 1993) and the viability is lost if moisture content drops below a certain critical level before germination occurs (Farrant *et al.*, 1988, Pammenter *et al.*, 1994)

In accordance with the views of Pammenter and Berjak (1999) the most documented characteristic of recalcitrant seeds is that they are desiccation sensitive. Because germination is a continuum of development in recalcitrant seeds (Farrant *et al.*, 1988; Lin and Chen, 1995) and occurrence of very high moisture content at the time of maturation/shedding (Pammenter *et al.*, 1994), they are highly sensitive to desiccation. According to Pammenter and Berjak (1999) and Greggains *et al.*, (2001) desiccation sensitivity of recalcitrant seeds is due to mechanical damage caused by cell volume reduction impaired metabolism induced by dehydration and removal of water resulting in damage and denaturation of macromolecules.

A number of protective mechanisms associated with desiccation tolerance have been identified in recalcitrant seeds. According to Leprince *et*

al., (1993) and Sun and Leopold (1997) these mechanisms include changes in the composition of membrane, phase change of cytoplasm, accumulation of sucrose and oligosaccharides, synthesis of LEA proteins and increased ability to prevent free radical attack.

A common character of many earlier studies dealing with recalcitrant seeds is the consideration of germination as only an index or marker for assessment of viability, desiccation tolerance and/or storability. In the present study, however, not only germination was taken as a marker of viability and desiccation, but reserve mobilization during germination also was investigated.

Molecular mechanism of desiccation tolerance has been well documented in orthodox seeds (Leprince *et al.*, 1993; Bailly *et al.*, 2001). However, only limited information is available about the biochemical characteristics associated with desiccation sensitivity of recalcitrant seeds.

Desiccation tolerance in orthodox seeds is initiated when mass maturity is established and recalcitrant seeds on the other hand do not undergo maturation drying/desiccation and shed at high moisture and are more sensitive to desiccation. Mango seeds are desiccation sensitive and biochemical basis or metabolic changes during desiccation remains to be studied. In the present study the author focuses on the changes and distribution of metabolites in general and soluble carbohydrates in particular. Various responses of desiccation shown by mango seeds are considered in terms of distribution of moisture content and metabolites during desiccation until the viability is lost. This study is also aimed to ascertain whether the metabolic changes are responsible for desiccation intolerance and the potential for longevity is directly or indirectly related to development and/or maturation and ripening.

Since desiccation tolerance is known to be related to maturation, and premature harvesting causes more desiccation-associated changes (Hong and

Ellis, 1992) the present author embark on the venture of analyzing age-dependent changes in mango seeds during desiccation. Hence, mango seeds of 4 developmental stages were analyzed for desiccation and germination studies. A survey of literature revealed that studies have not been carried out to unveil the physiological and biochemical aspects of mango seeds during development. So the author is made an attempt to elucidate the physiological and biochemical changes of mango seeds during various stages of development such as young, mature, partly ripened and ripened.

Numerous cellular changes occur, associated with desiccation in recalcitrant seeds. Histochemical studies of insoluble polysaccharides and proteins facilitate the localization of these metabolites during desiccation and their distribution in various parts of seeds.

In addition to the physiological/biochemical aspects of desiccation tolerance/sensitivity of mango seeds *in vitro* culture of developing seeds also is proposed to include in the present investigation. The propagation of mango by tissue culture has been done by various authors inclusive of direct and indirect embryogenesis (Litz, 1985; Laxmi, *et a*l., 1999; Huang *et al.*, 2000; Chaturvedi *et al.*, 2004. Litz *et al.*, (1984, 1993) and De Wald *et al.*, (1989) standardized the protocol of different culture media. However, in the present study developing seeds are used as explants to standardize a protocol for the tissue culture of mango seeds.

Review of Literature

The terms 'orthodox' and 'recalcitrant' were introduced by Roberts (1973) to describe the behaviour of seeds during storage. Orthodox seeds can tolerate desiccation and freezing temperature; whereas recalcitrant seeds terminate growth activities, if the moisture content is reduced below certain critical value (12-31%). The life span of recalcitrant seeds is very short, which varies from few days to few months even when maintained in moist conditions.

Many of the plantation crops, fruit trees and timber species produce large recalcitrant seeds (Chin *et al.,* 1984). According to those authors plantation crops like rubber (*Hevea braziliensis*), cocoa (*Theobroma cacao)*, coconut (*Cocos nucifera*) and tea (*Camellia sinensis),* tropical fruit crops like mango *(Mangifera indica)*, jackfruit (*Artocarpus heterophyllus)*, mangostein (*Garcinea mangostene*) and rambutan (*Nephelium lappaceum*), tropical timber species belong to family Dipterocarpaceae and Auracaraiaceae and temperate representatives such as oak, chest nut and horse chest nut produce recalcitrant seeds.

Chin (1988) studied the recalcitrant behaviour of seeds and according to whom seeds of recalcitrant nature shed from parent plants with high moisture content. Because of their large size these seeds loss water at a slower rate than do orthodox seeds. The author came to a conclusion that large seed size may actually contribute to recalcitrant behavior.

Farrant *et al.*, (1988) classified recalcitrant seed types based on the rate of germination in the absence of additional water loss tolerated, into less recalcitrant seeds (eg. *Quercus* sp, *Araucaria* sp, *Podocarpus* sp.), moderately recalcitrant seeds (*Theobroma* sp, *Hevea* sp. and highly recalcitrant seeds (*Avicennia marina, Barrengtonia* sp, *Syzygium* sp.). Those authors considered *Coffea arabica* and *Citrus* sp., which were earlier grouped as recalcitrant, as intermediate between recalcitrant and orthodox species. According to them, desiccation sensitivity of recalcitrant seeds may be due to initiation of germination associated events in storage. They further reported that *Avicennia* seeds became increasingly sensitive to desiccation with germination related events. If the seeds of *Avicennia* were rapidly dried before beginning of germination, they can tolerate greater amount of water loss and thus survive to lower moisture content. Slowly dried seeds attain advanced stage of germination and so death occurs at high moisture content. Those authors also noticed that in *Avicennia* seeds initiation of sub-cellular changes similar to those of early germination occurred soon after shedding

There are several reports that loss of viability by desiccation was caused by membrane rupture and a rapid increase in leakage is considered as the main characters of recalcitrant seeds (Nautiyal and Purohit, 1985a; Farrant *et al.*, 1988 and Pammenter *et al.*, 1994). Kinetin is suggested to induce tolerance to desiccation-sensitive seeds by retaining the membrane integrity. Several reports (Wilson and McDonald, 1986; Hendry *et al.*, 1992 and Pammenter *et al.*, 1994) revealed that a close correlation exists between membrane permeability and

lipid peroxidation. In *Shorea robusta*, kinetin induced lowering of lipid peroxidation.

Finch-Savage, (1992a) characterized and discussed that the responses of *Quercus robur* seeds to desiccation are in relation to current knowledge of recalcitrant seed behavior. The author studied the relationship between viability and seed moisture content and found that this relationship was unaffected by rate of drying, year of harvest or presence of the pericarp. Desiccation sensitivity did not increase with storage. The author also noticed that excised embryonic axes survived with lower moisture content than intact seeds, however, in the intact seeds, loss of viability was appeared to be determined by critical moisture content in the cotyledons. Consequently, the level of desiccation tolerance within the axis attached to cotyledons was not determined by axis drying rate.

The absorption isotherm for excised embryonic axes of recalcitrant (i.e., Desiccation–sensitive) *Quercus robur* seeds were studied by Poulsen and Eriksen, (1992) to find out the relation between moisture content and water potential. From the studies these authors came to a conclusion that critical water potential for initiation of damage was –5Mpa, and that axes accumulated proline as a response to desiccation stress.

Desiccation studies of the embryonic axes of partially mature and mature seeds of jack fruit, tea and cocoa were conducted by Chandel *et al.*, (1995). Those authors came to a conclusion that embryonic axes of partially mature and mature jack fruit seeds could be desiccated to lower moisture levels of 14% and 7% respectively. They further observed that fully mature axes of tea seeds were also desiccation tolerant, but the axes of cocoa seeds could not survive when desiccated below 52% moisture content.

Studies have been conducted to correlate desiccation and viability of seeds of *Machilus thunbergii* (Lin and Chen, 1995) and *M. kusanoi* (Chien and Lin, 1997) and suggested that seeds of *M. thunrbergii* can be stored wet for 10 months at 4°C before any decrease of viability, nevertheless, the seeds at maturity entered shallow dormancy. Even without desiccation, seeds of *M. kusanoi* had short life span when stored under wet and cold condition i.e., at 5°C.

Seeds of silver maple (*Acer saccharinum*) and red buckeye (*Aesculus pavia*) were air-dried at room temperature for 9-111 days. Like other recalcitrant seeds viability of these seeds was reduced or lost after drying. Silver maple seeds experienced a 50% reduction in viability by 5th day of drying and retained the moisture content of axes over 25%. Red buckeye seeds did not lose 50% viability upto 8 days and moisture content of the axes fell below 20% as the seeds dried (Connor and Bonner, 2001).

Davies and Pritchard (1998) conducted desiccation studies in seeds of some dry land plants *(Hyphaena thebaica, H. petersiana* and *Medemia argun*) and revealed that these seeds (not more than 15-60%) of dry land pants exhibited a sensitivity of desiccation to low RH. The response of recalcitrant seeds to desiccation and chilling depends on nature of seeds and varies from species to species

The water relations of desiccation sensitivity in embryonic axes and cotyledons of *Quercus robur* seeds using Differential Scanning Colorimetry (DSC) were investigated by Pritchard and Manger (1998). Those authors suggested that desiccation stress in recalcitrant seed axes is manifested at least two levels; firstly, a time-dependent, potentially reversible stress relating to the removal of vicinal water; and secondly, an irreversible injury resulting from the loss of bound water.

In desiccation sensitive seeds, the dehydration cause damages in three ways, 1) reduction in cell volume which can lead to mechanical damage 2) aqueous-based degradative process, probably consequent upon deranged metabolism at intermediate water contents. This is termed 'metabolism induced damage' and its extent depends upon the metabolic rate and the rate of dehydration; and 3) the removal of water intimately associated with macromolecular surfaces lead to denaturation: this is referred to as desiccation damage. The effects of drying rate and the maturity status of seeds are considered in relation to the responses to dehydration (Pammenter and Berjak, 1999).

Liang and Sun., (2000) investigated the effect of drying rate on desiccation tolerance of *Theobroma cacao* seed axes at 16°C. Rapid-drying at low relative humidity (RH) and slow-drying at high RH were more harmful to cocoa axes, because electrolyte leakage began to increase and axis viability began to decrease at high water contents. Maximum desiccation tolerance was observed with intermediate drying rates at RH between 88% and 91%, indicating the existence of an optimal drying rate or optimal desiccation duration. This maximum level of desiccation tolerance for cocoa axes (corresponding to a critical water potential of -9 MPa) was also detected using the equilibration method, in which axes were dehydrated over a series of salt solutions or glycerol solutions until the equilibrium is reached. These studies confirmed that the physiological basis of the optimal drying rate is related to both mechanical stress during desiccation and the length of desiccation duration during which deleterious reactions may occur. The optimal drying rate represents a situation where combined damages from mechanical and metabolic stresses become minimal.

In order to investigate the different degrees of desiccation sensitivity of nine African coffee species, parameters like the duration of seed development

and seed water content at maturity, were measured and the relationships between these parameters and some climatic characteristic of their specific native environments were investigated (Stephane *et al.,* 2000). Seed moisture content at maturity was not correlated with the level of seed desiccation tolerance. All these results are discussed on the basis of more detailed descriptions of the natural habitats of the coffee species studied.

Sacande *et al.*, (2000) studied the recalcitrant nature of Neem (*Azadirachta indica*) seeds. The seeds which have limited tolerance to desiccation are sensitive to chilling and imbibitional stress and showed intermediate storage behavior. The water content was considerably higher in axes than in cotyledons. Using differential scanning calorimetry, melting transitions of water were observed at water contents higher than 0.14g H_2O g/ dry weight in the cotyledons and 0.23g H_2O g/dry weight in the axes. Longevity during storage at very low water contents was limited. Dry neem seeds in the glassy state have great potential for extended storability at subzero temperatures.

When embryonic axes of tea (desiccation sensitive) and pea (desiccation tolerant) were dried at different rates or stored at different water contents to distinguish between damage associated with the immediate effects of water loss and the longer-term effects of a partially hydrated state, it was found that the recalcitrant seeds could not survive drying below critical water content, regardless of the drying rate (Walters *et al.,* 2001). According to those authors, rapid drying is required for accurate assessment of the critical water content. Slow drying leads to metabolic imbalance and artifactual assessment of the critical water content for desiccation damage. Both tea and pea seeds were susceptible to damage from metabolic imbalances, suggesting that the predominant stress from slow drying is ageing.

Neem seeds (42.2% seed moisture content) with 100% viability deteriorate when naturally desiccated to below 10.9% moisture content (Boby and Naithani, 2002). The desiccation–induced loss of viability was closely associated with over accumulation of superoxide anion and lipid peroxidation products both in the embryonic axes and cotyledons. The level of superoxide anion and lipid peroxidation products was higher in axes compared to cotyledons.

According to Lobos and Ellis (2002) seeds of *Fagus sylvatica* and *Fagus crenata* survived desiccation to about 3% moisture content (in equilibrium with 10% relative humidity at 20°C). Nevertheless, viability was reduced significantly and progressively by desiccation from 14% to 3% moisture content. During hermetic storage of large fraction of seeds of *F. sylvatica* and *F. crenata* at temperatures showed that (20 to -20°C in *F. sylvatica* and 10 to -20°C in *F. crenata* seeds) viability was lost more rapidly with reduction in moisture content below about 7.6-11.5% (40-71% relative humidity at 20°C). Thus, *F. sylvatica* and *F. crenata* exhibited intermediate seed storage behavior. Those authors concluded that optimum seed storage environments, within the range investigated, were provided by combining temperatures of -10 to -20°C with 7.8-11.5% (*F. sylvatica*) or 7.6-9.5% (*F. crenata*) moisture content.

Desiccation and storage experiments were performed on mahua (*Madhuca indica*) seeds to determine their storage behaviour, revealed that the mature seeds, which were shed at relatively high moisture content (53%), exhibited 100% viability initially (Varghese *et al.*, 2002). Drying of seeds to 39.4% moisture content (natural drying) had no adverse effect on germination but further drying to 37.7% moisture content (silica gel drying) reduced germination by 11%. The studies revealed that the seeds became non-viable when desiccated below 9.4% moisture content in both the drying conditions. A

strong positive correlation obtained between decline of viability and rate of dehydration. The undried and dried seeds of *M. indica* could not tolerate cold temperatures and were killed when exposed to 0^0C and -20^0C. The results suggest that the storage behaviour of *M. indica* seeds is true tropical recalcitrant.

Pammenter *et al.*, (2004) reported that the rehydration technique was influenced on the response of recalcitrant seed embryos to desiccation. The embryos or axes from seeds of three recalcitrant species (*Artocarpus heterophyllus, Podocarpus henkelii* and *Ekebergia capensis*) showed that excised material was rapidly dried to water contents within the range over which viability is lost during drying, and re-imbibed either rapidly, by plunching directly into water or slowly by placing the material on damp filter paper or exposing it to a saturated atmosphere for several hours. Although details of the response differed among species and developmental stage, in all cases direct re-imbibition of water resulted in higher (or similar, but never lower) survival than either of the slow rehydration techniques.

The effect of differential rates of drying on viability was examined in mature seeds of sal (*Shorea robusta*) during storage at ambient conditions (Varghese *et al.,* 2004). The initial seed moisture content (fresh weight basis) was 42.1% and the percentage of germination was determined after drying slowly (air dried naturally) and rapidly (over silica gel) compared to seeds held in vermiculate. Seeds become non-viable when desiccated to 19.5% and 9.8% moisture content in natural (slow) and silica gel (rapid) drying, respectively. Wet storage in vermiculate was not successful and eventually lead to loss of seed viability despite higher moisture content. Rapid drying of recalcitrant sal seeds to lower moisture levels excludes the possibility of desiccation induced damage compared to the naturally dried seeds, though the long term storage of these rapidly dried seeds at various temperatures was not possible.

Hilhorst *et al.,* (2004) investigated the desiccation sensitivity of seeds of *Inga vera* subsp. *affinis* at various stages of development, focusing on water relations and cell-cycle aspects, including DNA content and the microtubular cytoskeleton. The authors noticed that after a slight desiccation of immature seeds germination was increased, but further drying resulted in a quick decline of germinability. During seed development the desiccation sensitivity decreased slightly, but DNA content of the embryonic axis cells remained constant, suggesting no relation between those two parameters. The failure of germination of seeds after drying could not be attributed to cellular damage to DNA synthesis and mitosis, since the radicle protruded by means of cell elongation, without a need for cell division. However, the breakdown of microtubules during desiccation, and their subsequent inability to reassemble upon rehydration, may be related to the decreased germination, since microtubules are required for cell elongation.

The seed development in *Quercus robur* in relation to germinability and desiccation tolerance was studied by Finch-Savage (1992b). The author observed the changes in germination and desiccation sensitivity through the seed expansion phase of development in fruits of *Q. robur*. The onset of a reduction in desiccation sensitivity of seeds during development on tree coincided with acquisition of the capacity for germination on moist sand substrata. Tolerance of desiccation then increased throughout development and shedding, but viability was still lost at a fully desiccation-tolerant phase. The authors came to a conclusion that desiccation sensitivity in *Q. robur* seeds may have resulted from the premature term of development.

Eira *et al.,* (1999) studied the relationships among water content, relative humidity and temperature in seeds and excised embryos of *Coffea* sp. using water sorption isotherms. Isotherms were constructed at 5, 15 and 25°C and calculated for lower temperatures. There were no apparent differences in

sorption characteristics among whole seeds of several cultivars of *Coffea arabica* and among different other species of *Coffea*. Excised embryos of genetically diverse *Coffea* germplasm also exhibited similar sorption characteristics, though there were substantial differences observed between embryos and whole seeds. The shape of isotherms of coffee seed tissues was intermediate to the reverse sigmoidal shape observed for orthodox seeds and the monotonic shape observed for desiccation intolerant plant tissues.

Seed desiccation sensitivity was studied in nine species of the genus *Coffea* by measuring seed viability after equilibration over various saturated salt solutions (Dussert *et al.*, 1999). A quantal response model based on the logistic distribution was developed in order to describe the typical S-shaped patterns observed. The closeness of fit of the desiccation sensitivity model was shown, and the assumption that seed desiccation sensitivity follows a continuous distribution within species was verified.

Seed desiccation sensitivity among ten coffee species (*Coffea arabica*, *C. brevipes*, *C. canephora*, *C. eugenioides*, *C. humilis*, *C. liberica*, *C. pocsii*, *C. pseudo-zanguebariae*, *C. sessiliflora* and *C. stenophylla*) has been investigated by Chambrillange *et al.*, (2000). Among these species, the authors found that *C. liberica* and *C. humilis* were the most sensitive to desiccation and *C. pseudo-zanguebariae* the most tolerant. The authors also studied the sugar content in seeds of all species. But no significant relationship was found between seed desiccation sensitivity and: (i) the sugar content; (ii) the presence/absence of oligosaccharides; and (iii) the oligosaccharide: sucrose ratio.

The critical moisture content of recalcitrant seeds varies from species to species and in a relatively high value ranging from 12-31% even when the seeds stored at constant temperature ultimately experience the water limitation

and lose viability. Hor *et al*, (1984) reported that seeds of *Theobroma cacao* were damaged when desiccated below 27% moisture content.

Chin *et al.*, (1984) made viability studies of recalcitrant seeds of *Euphorbia longan, Nephelium maliensis* and *Artocarpus heterophyllus* and found that maintenance of high moisture content at ambient temperature were necessary for the retention of viability. Those authors noticed that seeds of *E. longan* and *N. maliensis* were dead at intermediate moisture level of 18% and 25% respectively. On drying *A. heterophyllus* seeds were found dead at a high moisture level i.e., 43% eventhough the seeds were equipped with a thin impermeable seed coat.

The effect of different drying methods on survival of excised embryonic axes of lychee (*Lychi chinensis*), longan (*Euphorbia longan*) and jack fruit (*Artocarpus heterophyllus*) seeds were investigated by Fu *et al.*, (1993). According to these authors excised embryonic axis of longan seeds can be dried down to relatively low moisture content (13%) for retaining viability, which was lower than that of lychee seeds. Only the embryonic axes of longan seeds with 18% moisture content could survive after 24 h. storage in liquid nitrogen.

According to Bonner (1996), in *Quercus nigra* (acorn) seeds, moisture content and germination decreased as the severity of desiccation increased. Leachate conductivity increased slightly but was not a sensitive indicator of loss of viability. The critical (lethal) moisture content was 10-15%. At 27°C any desiccation treatment that produced loss of 20-50 mg of moisture per gram of acorn dry weight per day should be suitable as a test for recalcitrance in these seeds.

The influence of moisture content on viability of seeds of four African tree species, from the Sahelian zone (*Boscia senegalensis)* and the Sudanian

zone (*Butyrospermum parkii, Cordyla pinnata* and *Saba senegalensis*), have been studied by Danthu *et al.*, (2000). Those authors suggested that all the species lost viability when moisture content dropped between 30-22% depending on the species. Seed longevity in wet and airtight storage did not exceed a few months. Temperatures close to zero elicited symptoms of chilling leading to rapid seed death. The optimum storage temperature was found as 15°C. These results allow the four species to be classified as recalcitrant. *Boscia senegalensis* is therefore an exception in arid zones where most species have orthodox seeds.

Anilkumar *et al.*, (2002) studied the relationship between desiccation and viability of *Myristica malabarica* seeds and observed that seeds remain viable only for a week under natural conditions. Fresh seeds with 27% moisture content showed 100% germination. On natural drying, the value of critical moisture content was found to be between 14 and 11% as the germination percentage decreased from 70 to 0% respectively. Eventually, the authors came to the conclusion that fresh seeds with 27% moisture content could be stored in polyethylene bags at 30^0 C/80% RH and those seeds retained their viability up to one year.

The morphology of *Eugenia dysenterica* (Myrtaceae) seeds and its germination response was investigated in relation to temperature, desiccation under different drying regimes and storage under the temperatures (Andrade *et al.,* 2003). Seeds with high moisture contents on shedding (47-53%), completely lost viability when moisture content was reduced to values below 18-22%. The authors observed a straight-line relationship between the probit of germination and moisture content percentage. These characteristics provide the evidence of recalcitrant storage behaviour of this species.

Hong *et al.*, (2000) reported that the viability of Norway maple seeds collected 21 days before mass maturity (68% moisture content, wet basis)

and at mass maturity (56% moisture content) was reduced from 52-85% to 0-7%, if dried rapidly (at 10-12% R.H. and 15-17° C for 3 days, then over silica gel) to 4-5% moisture content. Moist storage and/or subsequent desiccation affected stachyose, sucrose and to a lesser extent raffinose, concentrations. The oligosaccharide:total sugar ratio showed a similar pattern in relation to ability to germinate after desiccation to 4-5% moisture content among seeds collected on both intervals.

The decline of embryo moisture content from approximately 82 to 53% in 1997 and 56% in 1998 in recalcitrant seeds of *Acer saccharinum* during maturation was accompanied by decreased mitotic activity in the meristems and an increase in the percentage of cells in the G_1 phase of the cell cycle (Kozeko and Troyan, 2000). DNA synthesis and mitosis in the root apex ceased at approximately 53% embryo moisture content, and 67% of the cells were arrested in the G_1 phase. The authors further observed that during post-maturation drying, cell division in the shoot apex and embryonic leaves continued as long as the embryo moisture content was higher than 50 and 45%, respectively. Eventually they came to a conclusion that mitotic activity in the drying embryo may be controlled by its moisture level.

According to Merouani *et al.*, (2003) the reduction in moisture content of acorn (*Quercus ruber*), seed development until shedding differed greatly between acorn tissues with the pericarp and the embryo losing less water than cotyledons during the maturation process. Those authors found that the moisture content of cotyledons was found to be a good index of morphological maturity. The acorns aquired the ability to germinate early during maturation and reached their maximum capacity when the acorn moisture content decreased from 72% to 67%.

Storage studies of *Hevea braziliensis* seeds were done by Chin *et al.*, (1981) and suggested that *H. braziliensis* seeds can be successfully stored at

cold temperature of 7-10°C along with use of moist sterilized saw dust to maintain high moisture level. By electron microscopic studies the authors noticed some ultrastructural changes associated with loss of viability due to desiccation of seeds.

Corbineau and Come (1988) studied the effect of dry and wet storage on the survival of seeds of *Shorea roxburghii, Hopea odorata, Mangifera indica* and *Symphonia globulifera.* Germination was easier in all the seeds at relatively high moisture content and dies quickly upon dehydration. Wet storage of these seeds or seedlings, was difficult since chilling injury occurred if the temperature was too low. At temperatures that did not result in chilling injury, seedling growth is too fast to allow prolonged storage, except in the case of *Symphonia globulifera.*

Studies on the effects of desiccation, wet storage and cryopreservation on the viability of three species of recalcitrant seeds (*Mangifera indica, Litchi chiensis* and *Euphorbia longan*) revealed that seeds of all the three species had relatively high moisture content and die quickly (four to seven days) upon dehydration in open conditions (Fu *et al.*, 1990). These seeds when stored with optimal moisture content and temperature, viability was found maintained for 100 to 200 days or more and the excised embryonic axes of *M. indica* could be desiccated rapidly to 11.8% moisture content while retaining high viability and about 80% of the dehydrated excised axes of *E. longan* seeds with moisture content of around 19% can retain viability after storage in liquid nitrogen for 12 hours.

Xia *et al.*, (1992) investigated the optional and optimal temperature for germination of lychee (*Litchi chinensis*) and longan (*Euphorbia longan*) seeds and found that lychee seeds lost germinability when their moisture content reduced to about 27% and longan seeds almost lost viability when their moisture content declined to around 29%, but they regained 67% germination

at 30% moisture content and lost germinability completely at 22% moisture content. From the studies the authors concluded that the optional and optimal temperature for germination of lychee and longan seeds were 30°C and 25°C respectively.

Farrant *et al.,* (1992a) studied germinability and responses to storage dehydration throughout the development of the desiccation sensitive seeds of *Avicennia marina* and suggested that pre-mature seeds showed a germination lag that was equivalent to the period between harvest and full maturity but following short-term storage, this was reduced to that of mature seeds. From the studies those authors concluded that the seeds were unable to tolerate any dehydration prior to the acquisition of full germination capacity, but became tolerant to slight water loss once they became fully germinable, after which desiccation sensitivity was not influenced by the stage of development.

Hydrated recalcitrant seeds are metabolically active and undergo germination-associated changes in storage. Extensive vacuolation and increase in cell size imply a requirement for water additional to that present in the seed on shedding. In storage, recalcitrant seeds are exposed to an initially mild, but increasingly severe, water stress. Deleterious events associated with a water stress of considerable duration are suggested to lead ultimately to the death of the tissue (Pammenter *et al.*, 1994).

Drew *et al.*, (2000) conducted storage studies of recalcitrant seeds of *Trichilia dregeana* at 16 or 25°C, either at the water content at which they were shed or partially dried and observed that when the seeds were exposed to a short period (approximately 6 hours) at temperatures up to 30°C prior to storage, seeds at the original water content maintained viability for several weeks at 16°C. However, storage of undried seeds at 25°C was deleterious within 8 days, indicating a chemical basis for degeneration of hydrated recalcitrant seeds. Ultrastructural studies of 15 days storage seeds at 16°C

showed that the cells have retained little damage and to have been in an enhanced state of activity commensurate with ongoing development towards germination.

Sanjeev *et al.*, (2002) conducted the storage studies of neem seeds, using different containers to store the depulped seeds harvested at different stages of maturity. Maximum germination of neem seeds within different storage periods was recorded after 15 days, registering gradual decrease there after. The authors observed that seeds stored in earthern container exhibited superior germination over others with highest values. Eventually they concluded that the average germination values were obtained when the seeds were stored either in gunny bags or coated with clay.

Hermetic seed storage of arabica coffee at –20°C for 10.5 years provided evidence of a distinct optimum seed moisture content of 8.4% (in equilibrium with about 43% relative humidity at 20^0C) for longevity in storage (Hong and Ellis, 2002).

Seed germination studies of six wild collections of *Piper* species, after storage in liquid nitrogen as well as desiccation for one week were carried out by Decruse and Seeni (2003). The studies revealed that *Piper* seeds in general, performed best at moisture content (13%) equilibrated to ambient conditions (25 ± 5°C and 65% RH) for maximum survival after desiccation and liquid nitrogen exposure (Decruse and Seeni, 2003).

Metabolic changes during desiccation, storage and germination of the seeds of 4 species of *Quercus* such as *Q. alba, Q. nigra, Q. shumardii* and *Q. falcata* have been studied (Clutterbuck and Bonner, 1985). The studies showed a slow accumulation of starch in early stages of storage, a peak prior to emergence of radicle and decrease with radicle elongation. They concluded that the starch being broken down to simple carbohydrates for supply. Farrant

et al. (1992b) observed that in *Avicennia marina* seeds lower the amount of starch present, the more the chance for the soluble sugars to function principally as an immediate carbohydrate reserve in seed.

Ryszard *et al.,* (1997) studied the accumulation of oligosaccharides in relation to germinability during seed development and maturation in yellow lupin (*Lupinus luteus*). Seed fresh mass increased to a maximum at 35 days after flowering (DAF) followed by a decrease when axis and cotyledonary tissues changed color from green to yellow. Maximum seed fresh mass was corresponding to the maximum seed size. Seed dry mass continuously increased until 40 DAF. About 75% of mature seed dry mass was in cotyledons, 22% in testa, and 3% in axis. Maximum seed germinability occurred at 45 DAF after maximum seed dry mass and desiccation. Mature dry seeds contained 10.9% oligosaccharides and 1.5% sucrose. The highest rate of oligosaccharide accumulation appeared during seed desiccation and correlated with the acquisition of the ability to germinate.

Buitink *et al.*, (2000) studied the role of oligosaccharides in seed longevity. The authors came to a conclusion that longevity may indeed be regulated by cytoplasmic mobility and a decrease in longevity and oligosaccharide content while the sucrose content increased. No difference in the glass transition temperature was found between control and primed Impatiens seeds at the same temperature and water content. Oligosaccharides in seeds do not affect the stability of the intracellular glassy state, and that the reduced longevity after priming is not the result of increased molecular mobility in the cytoplasm.

Peterbauer and Richter (2001) investigated that raffinose family oligosaccharides are almost ubiquitous occurrence in plant seeds. They accumulate during seed development and disappear rapidly during germination.

The biosynthesis of raffinose, the first member of the series, proceeds by addition of a galactosyl unit to sucrose.

Changes in ascorbate content and enzymatic utilization pattern in embryonic axes and cotyledons of sal seeds were studied by Chaithanya *et al.*, (2000) and they observed that ascorbate levels were significantly higher initially in the embryonic axes (0.32mg/g fresh weight) and cotyledons (0.21mg/g fresh weight) of freshly mature, relatively hydrated (42.2% moisture content) and 100% viable sal seeds. It declined sharply as the tissues, embryonic axes and cotyledons, desiccated with absolutely no detectable amount in non-viable seeds (21% moisture content). Significantly strong correlation was obtained between desiccation of embryonic axes and cotyledon with loss of ascorbate levels and loss of germinability.

Proline metabolism is linked to phenolic metabolism by providing the precursors for the production of phenylalanine, the first substrate in phenyl propanoid biosynthesis (Hare and Cress, 1997). Phenolics produced by the phenyl propanoid pathway are powerful antioxidants in plant tissues. These compounds also serve as precursors to suberins and lignins which act as physical protectants for plants against cold temperatures and freeze/thaw conditions associated with spring conditions in *Brassica napus* (Fletcher and Kott, 1999).

An autoradiographic studies by leucine and thymidine incorporation into the meristematic root primordia and hypocotyl tips of seeds of the recalcitrant mangrove species, *Avicennia marina* showed that although there was a temporary reduction of protein synthesis at shedding, root primordia and surrounding hypocotyl cells of the axis never wholly cease incorporation of [^{3}H] leucine and regain pre-shedding levels of activity within a day (Boubriak *et al.*, 2000). Precursor studies using methyl-[^{3}H] thymidine showed that at shedding, there is a temporary cessation of incorporation into root meristem

nuclei that lasts no longer than 48 hr. Pre-shedding levels are regained in the meristem nuclei within a day. The authors concluded that the feature of continuous protein synthesis activity with only a temporary interruption in active cell cycling in *A. marina* root primordia helps to explain both the rapidity in seedling establishment and the extreme vulnerability to desiccation.

Quercus alba seeds (acorns) were subjected to desiccation to determine the effects of drying on lipids, proteins and carbohydrates of the embryonic axes and cotyledon tissues (Connor and Sowa 2003). Samples of fresh seed and seed dried for selected intervals were analyzed for water content and germination and for lipids, proteins and carbohydrates by Fourier transform-infrared (FT-IR) spectroscopy. Carbohydrates were further analyzed by gas chromatography (GC) and sucrose concentration in the embryonic axis was found increased dramatically after 5 days of drying. The most sensitive indicator of desiccation damage was the irreversible change in protein secondary structure in embryonic axes and cotyledon tissue. Gas chromatography observations indicated an abundance of sucrose in both the embryonic axes and the cotyledon tissue. Although sucrose concentrations in these tissues were initially similar, sucrose concentration in the embryonic axes became significantly greater than in the cotyledons as the seeds dried. The authors concluded that, acorns on drying increased concentration of sucrose does not prevent loss of viability, but acts as a glycoprotectant against cell collapse and cell wall membrane damage as water stress increases.

Leprince *et al.*, (1999) investigated the relation of axes and cotyledons of recalcitrant seeds of *Castanea sativa* exhibit responses of respiration to drying in relation to desiccation sensitivity. Oxidative damage originating from uncontrolled metabolism is thought to be responsible for the sensitivity to drying in recalcitrant seeds. They studied the comparison between the responses of respiration to drying and loss of membrane integrity in isolated

axes and cotyledons of the recalcitrant seed. Axes were found to be more tolerant to drying than cotyledons:membranes showed minor changes in their permeability during drying and 50% viability was retained in dried axes containing 0.12g/g. Plasma membranes in cotyledons lost their integrity below 0.6g/g, regardless of the drying rate. At the onset of drying, the rates of O_2 uptake declined rapidly in drying axes. Respiration in drying cotyledons sequentially increased to 1.4 fold at 1.2g/g then decreased concomitantly with the loss of membrane integrity. Desiccation sensitivity in recalcitrant seeds may be due to the inability to actively depress their metabolism during drying, there by increasing the chances of initiating peroxidative damage during drying.

The distribution of phenolics in Rap seed and its role in plant metabolism and physiology has been studied Yang and Shetty (1998). According to them there is a great diversity in the natural occurrence and distribution of phenolic compounds, reflecting differences in their role in plant metabolism and physiology. Cruciferous plants accumulate sinapic acid esters in their tissues; they are especially rich in sinapolcholine sinapine. It has been demonstrated that sinapine, during seed germination, can be a source of choline for membrane lipid synthesis. However, the presence of relatively high levels of sinapine may compromise the use of rap seed as animal feed.

Materials and Methods

Fruits of mango (*Mangifera indica* Linn.) used for the present study were collected from a tree (local variety, Natumanga) growing in the Botanical Garden, Calicut University during the months of April, May and June of 3 consecutive years -2001, 2002 and 2003.

For a comparative study of seed development and subsequent germination, fruits were collected at 4 different stages of development. Developing fruits were tagged immediately after anthesis to note the number of days taken for each stage of development and denoted as days after anthesis (DAA). Fruits of various stages were collected as detailed below:

(1) **Young fruits**: Fruits with just hardened seed coat and the flesh almost white in colour (55 ± 5 DAA).

(2) **Mature fruits**: Fruits with hard seed coat and the flesh white in colour (80 ± 5 DAA).

(3) **Partly ripened fruits**: Fruits with very hard seed coat and the flesh slightly yellowish in colour towards the centre (95 ± 5 DAA)

(4) Ripened fruits: Fruits with yellow colour, soft flesh and very hard seed coat (105 ± 5 DAA).

Tagged fruits of various developmental stages (one stage of development at a time) were harvested and depulped without any injury to the seeds. Minimum 100 fruits of the same developmental stage were collected at a time and pooled before sampling. All sample collections were made during 3 consecutive years to repeat all experiments.

1. DESICCATION STUDIES

Freshly harvested fruits of each developmental stage were depulped and the seeds were washed and after blotting the seeds were kept for desiccation in open trays at room temperature (30 ± 3°C).

1.1. Sampling

Desiccated and control seed samples were harvested at an interval of 5 days upto 30 days, constituting 7 samples each, in each developmental stage. Fresh seeds of each developmental stage served as the control for desiccated seeds.

1.1.1. Germination studies

Forty seeds each in triplicate from the control and desiccated were sampled as described earlier and sown for germination in polyethylene bags filled with washed sand. Daily count of germinated seeds was taken, germination rate, percentage of germination and seed vigour index was calculated as given below:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seed Vigour Index

The Seed Vigour Index (SVI) was calculated according to (Copeland and McDonald 1995), using the formula given below:

$$SVI = \frac{\text{No. of seeds germinated on first count}}{\text{Day of first count}} + \ldots + \ldots + \frac{\text{No. of seeds germinated on last count}}{\text{Day of last count}}$$

1.1.2. Seedling Vigour

Seedling vigour of desiccated and control seedlings of all developmental stages was calculated using biomass method. Seedlings after 10 days of germination were uprooted carefully and washed in running tap water to remove the sand particles. The seedlings with cotyledon were blotted and weighed using electronic balance (Shimadzu). Then the weighed samples were kept in hot air oven at 100°C for 1 hour and kept at 60°C until constant weight was obtained. The seedling vigour was expressed as biomass per seedling.

1.1.3. Moisture content determination

Five seeds each in triplicates of desiccated and control seeds from each developmental stage were taken and fresh weights were determined using electronic balance. Then the weighed seeds were kept in hot air oven at 100°C for 1 hour and at 60°C until constant weights were obtained. Moisture content (MC) was expressed as g $g.^{-1}$

1.1.4. Biochemical studies

The control and desiccated seeds were sampled at different intervals as described earlier. Ten seeds in duplicate were decoated, and the entire embryos were cut into small pieces and pooled. Samples for biochemical studies were taken from this pooled tissue.

1.1.4.1. Dry weight determination of tissues

A known amount of fresh tissue was taken and the weighed tissue was kept in hot air oven at 100°C for 1 hour and then at 60°C until constant weight was obtained. Percentage of dry weight was calculated as explained by International Seed Testing Association (1985). Separate samples were taken for biochemical estimation of starch, sugars, total protein, free amino acids and total phenolic content.

1.1.4.2. Estimation of starch

The method of Pucher *et al.*, (1948) described by Whelan (1955) was used to estimate the starch in seed samples.

One gram of tissue was weighed and homogenized in a glass mortar and pestle in double distilled water and heated to break amyloplast. After cooling to room temperature, perchloric acid was added to a final concentration of 30% (v/v). The homogenate was then centrifuged for 5minutes and the supernatant was collected. The residue was again homogenized and re-extracted 4 times with 30% (v/v) perchloric acid, centrifuged and the supernatant was collected. The final volume of combined supernatant was noted. A known volume of extract was pipetted from combined supernatant and an equal volume of freshly prepared iodine-potassium-iodide reagent was added and kept for 10-20 minutes and centrifuged for 10 minutes. The supernatant was decanted off. The precipitate was then washed with alcoholic sodium chloride to remove excess iodine reagent. After centrifugation the blue precipitate was treated with alcoholic sodium hydroxide till blue color was discharged and then centrifuged to remove the supernatant. The residue was again washed with alcoholic sodium chloride. The precipitate was dissolved in a known volume of 10% (v/v) sulfuric acid by heating in a boiling water bath and centrifuged for 10 min.

Estimation of starch was done according to Montgomery (1957). To 1ml of the aliquot 0.1ml 80% (w/v) phenol was added and shaken well. Five ml of concentrated sulfuric acid was added quickly from a burette and allowed to cool. The optical density of the solution was measured at 540 nm (No. 4 green filter) using Systronics colorimeter. Soluble starch was used as standard.

1.1.4.3. Estimation of sugars

The quantitative and qualitative analyses of sugars were done using Thin-Layer Chromatography (TLC).

1.1.4.3.1. Preparation of thin layer plates

Sodium acetate solution of 0.02M was mixed with 30g silica gel G and spread on clean glass plates and 250μm thickness plates were prepared. The glass plates with silica gel were kept at room temperature for drying.

1.1.4.3.2. Preparation of sample

One gram tissue of control and desiccated seed samples of various developmental stages were ground with 80% ethyl alcohol in a glass mortar and pestle. The homogenate was taken in round bottomed flask fitted with vertical condenser and refluxed continuously for 4 hours. The suspension was centrifuged and supernatant was collected. Extraction and centrifugation were repeated 4 times and the supernatants were combined and evaporated about to dryness over a bath of boiling water. The precipitated sugars were then eluted in 3 ml distilled water.

1.1.4.3.3. Separation and estimation of sugars by TLC

One micro liter of the extract was applied on TLC plates and resolved using Chloroform- Methanol (60:40) solvent system according to Stahl (1969).

Visualization of sugars was carried out using anisaldehyde-sulfuric acid reagent of Stahl (1969).

1.1.4.3.4. Preparation of Anisaldehyde-Sulfuric acid reagent

One ml concentrated sulfuric acid was added to a solution of 0.5ml anisaldehyde in 50ml acetic acid. The reagent was freshly prepared for each experiment.

1.1.4.3.5. Treatment after spraying Anisaldehyde-Sulfuric acid reagent

The plates were heated at 100-105°C until the spot attain maximum colour intensity. The pink background was bleached by exposure to steam (water bath).

1.1.4.3.6. Quantitative analysis

The algebraic method of Purdy and Truter (1962) was used for quantitative analysis of sugars. In this method the solution containing the unknown sample was diluted precisely. The solution containing the standard sugar, diluted and undiluted sample solutions were chromatographed on the same thin layer plate and the spot area was measured. The results were calculated using the following equation:

$$\log W = \log Ws + \left(\frac{\sqrt{A} - \sqrt{As}}{\sqrt{Ad} - \sqrt{A}} \right) \times \log d$$

where,

d = dilution factor.

W = amount of the analysis material.

Ws = amount of the standard applied.

As = spot area of the standard.

A = spot area of the material for analysis.

Ad = spot area of diluted material for analysis

1.1.4.4. Estimation of total protein

The method of Lowry *et al.*, (1951) was followed to estimate the total protein, as modified by Khanna *et al.*, (1969).

Protien extraction was done with aqueous medium but water soluble protein was very low and various extraction methods were tried and finally it was found that alkali soluble protein content was present in considerable quantity.

Two grams tissue was ground in 2 N sodium hydroxide using glass mortar and pestle. The suspension was centrifuged and the supernatant was collected. A known volume from the supernatant was pipetted out and mixed with equal volume of cold 10% (w/v) trichloroacetic acid and the precipitate was allowed for flocculation for 30 minutes in an ice bath. The protein precipitate was collected by centrifugation for 10 min. and the supernatant was decanted off. The residue was washed twice with cold 2% (w/v) trichloroacetic acid, followed by washing with 80% (v/v) acetone and then with anhydrous acetone, for removing pigments. The precipitate obtained after centrifugation was digested in known volume of 0.1 N NaOH by heating in a bath of boiling water for five minutes. The suspension was clarified by centrifugation. To 1 ml of the supernatant 5 ml of alkaline copper reagent was added and shaken well. After 10 min., 0.5 ml of 1N Folin-Ciocalteau reagent was added, immediately shaken well and kept in dark for 30 min. The blue colour developed is due to the reduction of phosphomolybdic-phosphotungstic reagent by copper treated protein. The optical density was read at 750 nm using Spectronic 21 (Bausch and Lomb) Spectrophotometer. Bovine Serum Albumin (BSA) fraction V powder was used as standard.

1.1.4.5. Estimation of free amino acids

The quantitative analysis of amino acids was done using Thin-Layer Chromatography. Thin layer-plates of 250μm thickness were prepared using silica gel G and distilled water.

1.1.4.5.1. Preparation of sample

One gram tissue of each control and desiccated seeds of all developmental stages were ground with 80% ethyl alcohol using glass mortar and pestle. The homogenate was taken in round bottomed flask fitted with vertical condenser and refluxed on boiling water bath for 2 hours. The suspension was centrifuged and supernatant was collected. The residue was re-extracted 3 times with 80% alcohol and after each centrifugation the supernatant was combined with original extracts. The combined extract was evaporated to dryness over a boiling water bath. The precipitated amino acids were then eluted in 3 ml 10% iso-propanol.

1.1.4.5.2. Separation and estimation of amino acids by TLC

One micro litre of the extract was applied on TLC plates and resolved using 2-dimensional TLC. The solvent system for first direction was n-butanol-acetic acid-water (80:20:20). Then these thin layer plates were dried at room temperature and the second direction was resolved using the solvent chloroform–methanol-17% ammonia (40:40:20). Visualization of separated spot was carried out using Ninhydrin reagent (Stahl, 1969).

1.1.4.5.3. Preparation of Ninhydrin reagent

The Ninhydrin reagent was prepared by dissolving 0.2g Ninhydrin in 100ml ethyl alcohol.

After spraying Ninhydrin reagent the plates were heated at 100- 105°C until the spot attain maximum colour intensity. The pink background was bleached by exposure to steam (water bath).

1.1.4.5.4. Quantitative analysis

The algebraic method of Purdy and Truter (1962) was used for quantitative analysis. In this method the solution containing the unknown sample was diluted precisely. The solution containing the standard amino acids, diluted and undiluted sample solutions were chromatographed on the same thin layer plate and the spot area was measured. The results were calculated using the equation:

$$\log W = \log Ws + \left(\frac{\sqrt{A} - \sqrt{As}}{\sqrt{Ad} - \sqrt{A}} \right) \times \log d$$

Where,

d = dilution factor.

W = amount of the analysis material.

Ws = amount of the standard applied.

As = spot area of the standard.

A = spot area of the material for analysis.

Ad = spot area of diluted material for analysis.

1.1.4.6. Estimation of phenolics

Estimation of phenolics was done by using Folin–Denis Reagent (Folin and Denis, 1915).

1.1.4.6.1. Preparation of sample

One gram tissue was ground in a clean glass mortar and pestle by adding 80% ethyl alcohol. This mixture was refluxed for two hours over a

steam bath. After refluxing the homogenate was cooled and filtered under suction. The tissue was ground again and refluxed for 1 hr and the supernatant was collected and combined with the original extract.

1.1.4.6.2. Preparation of Folin-Denis reagent

To 750 ml of distilled water, 100gm sodium tungstate, 20 gm phospho molybdic acid, and 50 ml ortho phosphoric acid was added. This mixture was refluxed for 2 hrs, cooled and diluted to 1 litre. The reagent was titrated against standard alkali and made upto 0.25 N.

1.1.4.6.3. Estimation of phenolics

A known volume of the extract was taken in a test tube. The volume was made upto 2ml by adding distilled water. Two ml Folin-Denis reagent was added, and mixed. After three minutes 2 ml sodium carbonate was added. The tubes were kept for an hour and absorbance was measured at 725 nm using Spectronic 21 (Bausch and Lomb) Spectrophotometer. Tannic acid was used as standard.

1.1.5. Histochemical studies

Seed parts such as cotyledons of seeds of all developmental stages subjected to various intervals of desiccation and control samples as described earlier were collected and fixed for histochemical studies.

I.1.5.1. Tissue preparation

Samples were fixed in FAA, dehydrated through alcohol-TBA series, infiltrated and embedded in paraffin wax (Johanson, 1940). Using a rotary microtome (LEICA, modal RM 2125RT) the individual blocks were cut at 10μ thickness and the sections were mounted on glass slide using Haupt's adhesive

and used for histochemical staining. The sections were deparaffinised, hydrated and stained for localization of insoluble polysaccharides and proteins.

1.1.5.2. Localization of insoluble polysaccharides

Localization of insoluble polysaccharides was done according to Berlyn and Miksche (1976) using periodic acid-Schiff's reagent.

1.1.5.2.1. Preparation of Schiff's reagent

Two gram of basic fuchsin and 3.8g of potassium meta bisulfite were added to 200ml of 0.15 N HCl in a conical flask and shaken on a mechanical shaker for 2hrs. One gram of activated charcoal was added to the mixture and stirred for 15min., so as to decolorize the solution. The mixture was filtered using a Buchner funnel, side armed flask, vacuum pump and Whatman No.1 filter paper. The stain was kept at 4°C.

1.1.5.2.2. Procedure of staining

The hydrated sections were placed in 0.5% (w/v) periodic acid solution at 23°C for 15 min. and the sections were washed using running tap water for 10 min. The sections were then placed in Schiff's reagent for 10 min. at 4°C and washed in tap water for 20 sec. After washing in tap water the sections were placed in 2% Sodium bisulfite for 2 min. and washed again in tap water for 5-10 min. The sections were dehydrated through alcohol series, cleared in xylene and were mounted in DPX.

I.1.5.3. Localization of total proteins

For the localization of total protein sections were stained with Coomassie Brilliant blue stain (Khasim, 2002).

1.1.5.3.1. Preparation of Brilliant Blue stain

The stain was prepared by dissolving 250 mg Coomassie Brilliant blue in 100 ml 70% acetic acid.

I.1.5.3.2. Procedure of staining

The hydrated sections were placed in the stain for 3 minutes at 30° C and rinsed in 7% acetic acid solution, dehydrated in 95% and absolute alcohol in 5 minutes. Finally the sections were mounted in glycerin containing 55 % acetic acid.

2. GERMINATION STUDIES

Control and all experimental samples of desiccated seeds, sampled as described earlier were used for germination studies. Seed samples were planted in polyethylene bags filled with washed sterilized sand and kept for germination. Samples were collected after 10 days of germination. Biochemical estimation of starch, sugars, total proteins, free amino acids and phenolics were done as described earlier. Histochemical localization of insoluble polysaccharides and proteins also were done using periodic acid Schiffs' reagent and Coomassie Brilliant blue stains respectively as detailed under desiccation studies.

Observations were made and photomicrographs were taken using Nikon microscope (ECLIPSE E 400) and Nikon Digital Camera (DXM 1200F) attached with digital image analyzer.

Statistical analysis

All experiments starting from sampling were repeated minimum of 5 times and mean value was taken and standard deviation and standard error were calculated. Data was analyzed using Fisher's 't' test of significance.

3. IN VITRO STUDIES

An attempt was made for the *in vitro* propagation of somatic embryos from developing seeds (20-25 DAA) of *Mangifera indica* Linn. local variety Natumanga, grown in the Calicut University Botanical Garden.

3.1. Medium

Murashige and Skoog (1962) medium (MS medium) was used as the basal medium for the present study. Semisolid and suspension media were used and semisolid medium was solidified with 0.8% (w/v) agar (Merck India Ltd., Mumbai), but liquid medium was not supplemented with agar. Basal medium was modified by the addition of different growth regulators viz. 2, 4-D, NAA, BA, Kn, CW at different concentrations either singly or in combination depending on the aim.

3.1.1. Media preparation

The stock solutions of macro and micronutrients, iron chelates, and vitamins of MS medium were prepared separately. Iron chelate stock solution was stored in an amber coloured bottle. Plant growth regulators used in the present study were prepared separately by dissolving in respective solvents and made to the final volume with double distilled water. The stock solutions of the media were stored in a refrigerator (4°C).

Appropriate quantities of different stock solutions were pipetted out and after the addition of sucrose and growth regulators, the final volume of the medium was made up with double distilled water. The pH of the medium was adjusted prior to autoclaving in between 5.6 and 5.8 using 1N NaOH or 1N HCl solution. About 15 and 25 ml of the medium were dispensed in the culture tubes and conical flasks (100 ml) respectively. For suspension cultures, 25 ml of the medium was transferred to each conical flask. The semisolid

medium was solidified using 0.8 % agar (w/v). The media were sterilized at a pressure of 1.06 kg/cm^2 (121^{0}C) in autoclave for 20 min and were stored in the culture room.

3.2. Explants and surface sterilization

Young fruits (20-25 DAA) of mango local variety Natumanga, were used as the source of explants. The fruits were washed under running tap water followed by a dilute solution of Extran (5% v/v) for 5 min. After repeated wash with distilled water, fruits were kept in cotton plugged conical flask and brought to the work area of pre-sterilized laminar airflow cabinet. All the further operations were carried out in sterile conditions of laminar airflow. The fruits were surface sterilized with 0.1% mercuric chloride for 7 min and rinsed with sterile distilled water. The fruits were cut longitudinally without damaging the developing seed. Embryos were removed and bisected along the longitudinal axis. The seed halves without embryos were used as explants and were cultured on sterile media. All the cultures were incubated at a temperature of 25±2^{0}C with 16/8 h photoperiod provided by white fluorescent tubes (25 μmol m^{-2} s^{-1}).

3.3. Subculture

Initiated callus and embryos were subcultured on fresh medium of the same or varied growth regulator concentrations after 70 days. Subsequent transfers were carried out to fresh medium at an interval of 30 days for multiplication, maturation and rooting. Suspension cultures were made by transferring approximately 250 mg friable callus to conical flasks with liquid containing medium and were kept on a rotary shaker at 100 rpm. Suspension cultures were kept in dark.

3.4. Determination of total phenolics in suspension cultures

The total phenolics of mature somatic embryos and that excreted into the medium (MS basal suspension medium) after 60 days were extracted with 80% alcohol and the total phenolics was determined according to Folin-Denis (1915) as described in earlier section

3.5. Histological studies

The embryos at different developmental stages were examined by taking serial sections (10μ) using microtome (LEICA-RM 2125RT). The sections were stained using Delafield's hematoxylin as described by Johanson (1940).

Results

1. Germination Studies

1. 1. Germination percentage

Mango seeds are readily germinable and cent percent germination was obtained when fresh seeds were sown (Table 1, Fig. 1). All the four types of seeds except ripened exhibited cent percent germination in the control as well as samples desiccated upto 20 days. In young and ripened seeds germination percentage was reduced significantly after 20 days ($p<0.01$ in ripened seeds), these seeds showed 60% germination on 25^{th} day. The mature and partly ripened seeds showed reduction in germination only after 25 days. But the fully ripened seeds exhibited reduction in germination percentage after 15 days of desiccation and germinability of these seeds lost completely after 25^{th} days of desiccation.

1.2. Seed vigour index

Young control seeds desiccated for 5 days showed a significant increase ($p<0.01$) in seed vigour index (Table 2, Fig. 2). After that, in these seeds more or less same value was maintained by 15 and 20 days desiccated seeds.

Table 1: Effect of desiccation on germination of *Mangifera indica* seeds

Samples (seeds)	Number of days of desiccation						
	0	5	10	15	20	25	30
	Germination %						
Young	100	100	100	100	100	57.5 ± 5.12	33.8 ± 4.87
Mature	100	100	100	100	100	100	72.5 ± 3.22
Partly ripened	100	100	100	100	100	100	77.5 ± 5.12
Ripened	100	100	100	100	87.5 ± 4.22	62.5 ± 5.23	00

Table 2: Effect of desiccation on seed vigour index of *Mangifera indica* seeds

Samples (Seed)	Days of desiccation						
	0	5	10	15	20	25	30
	seed vigour index						
Young	1.019 ± 0.015	1.225 ± 0.012	1.067 ± 0.012	1.132 ± 0.013	1.104 ± 0.013	0.619 ± 0.012	0.359 ± 0.012
Mature	1.021 ± 0.030	1.106 ± 0.041	1.217 ± 0.011	1.205 ± 0.004	1.188 ± 0.012	1.107 ± 0.017	0.776 ± 0.038
Partly Ripened	1.835 ± 0.016	1.803 ± 0.021	1.603 ± 0.011	1.305 ± 0.021	1.396 ± 0.041	1.452 ± 0.033	1.088 ± 0.045
Ripened	2.215 ± 0.12	1.658 ± 0.014	1.619 ± 0.011	1.554 ± 0.034	1.499 ± 0.016	0.745 ± 0.036	–

Figure 1: Effect of desiccation on germination of *Mangifera indica* seeds

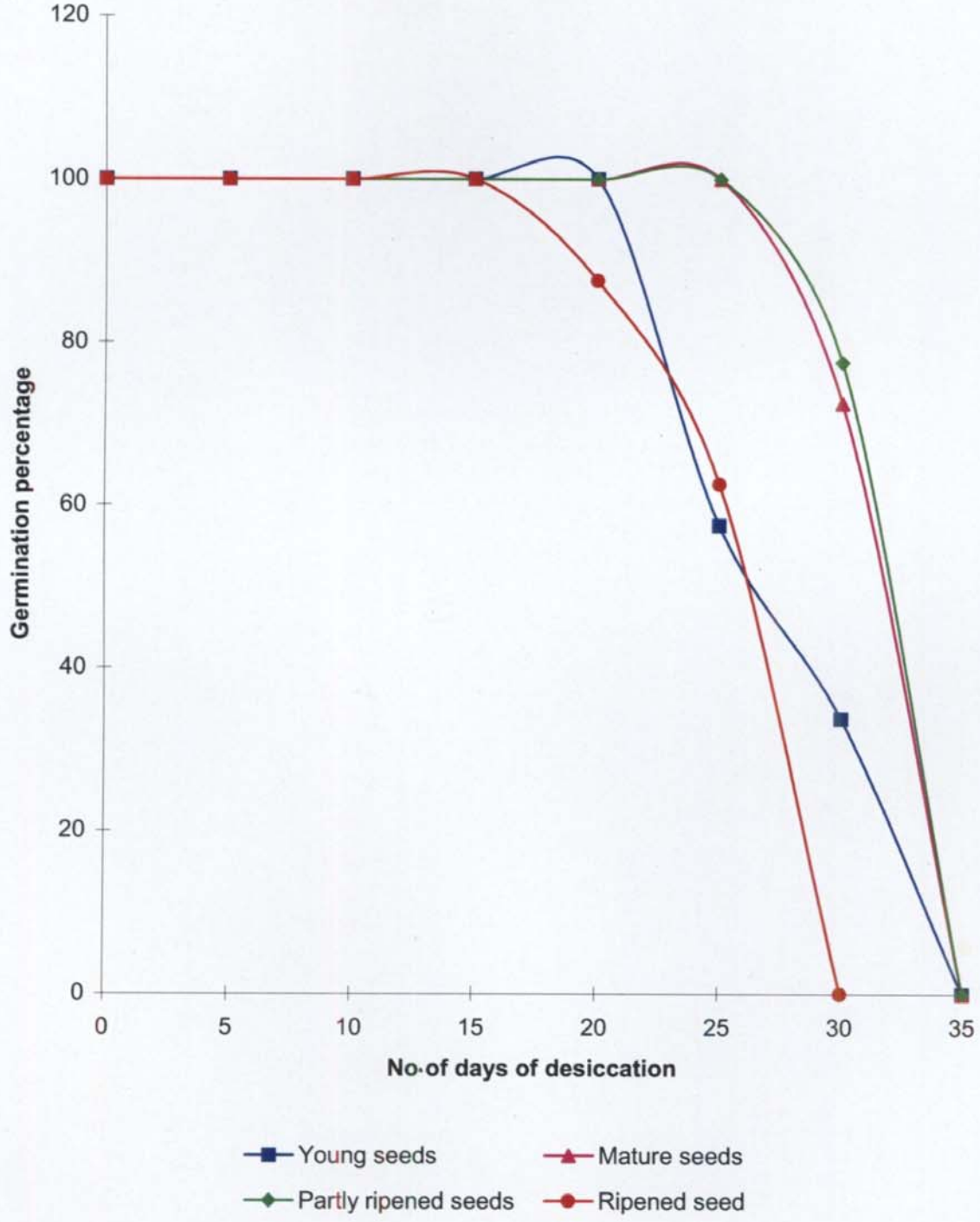
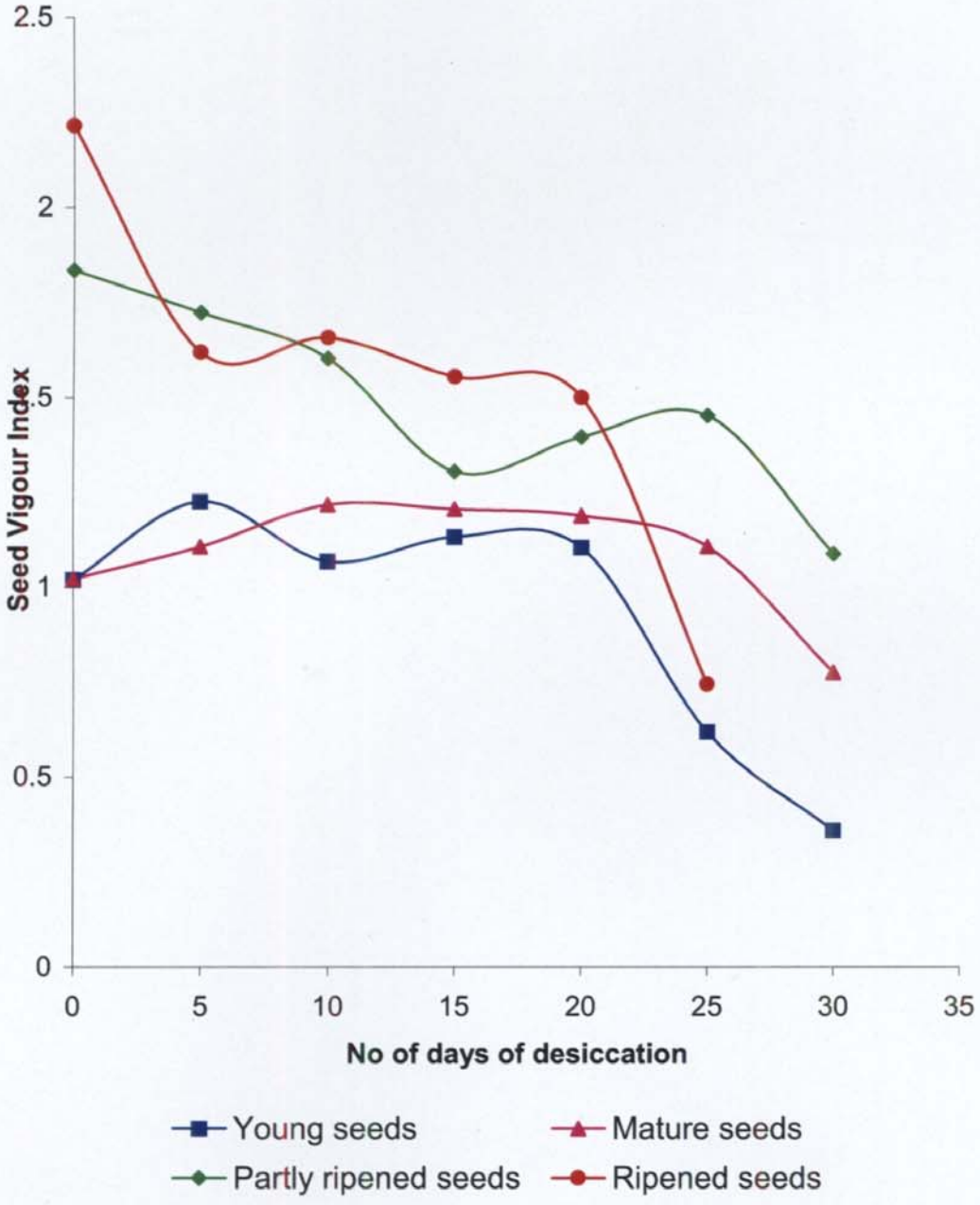


Figure 2: Effect of desiccation on seed vigour index of *Mangifera indica* seeds



Afterwards the significant reduction in seed vigour index was noticed ($p<0.01$). Mature seeds desiccated for 10, 15 and 20 days showed almost same seed vigour index. Partly ripened control seeds showed the maximum seed vigour index compared to desiccated seeds. From 15 days onwards seed vigour index was not changed significantly. Ripened control seeds showed the maximum seed vigour index compared to other seed treatments. Seeds desiccated for 5 days showed significant reduction ($p<0.01$) in seed vigour index and the same was maintained up to 20 days. Among ripened seeds the 25 days desiccated seeds showed minimum seed vigour index.

1.3. Rate of germination

Young control seeds started germination on 37th day of sowing (Table 3) and spread over was during a period of six consecutive days. Maximum germination was observed on 39th day. Young seeds desiccated for 5 days showed rapid germination than control seeds. These seeds started germination on 31st day and spread over was almost similar to that of control seeds. But maximum germination was noted during initial days. Seeds desiccated for 10 days started germination on 36th day. These seeds showed a delayed germination. Seeds desiccated for 15 days exhibited earlier germination i.e., on 33rd day. In the case also the spread over was maximum on 35th day of germination. Twenty days desiccated seeds showed a comparatively delayed germination. The maximum spread over was shown during first three days of germination. Young seeds desiccated for 25 days start germination on 36th day and spread over were only for a period of 4 days and germination percentage was only 50%. Seeds desiccated for 30 days showed only 30% germination, which started only on 35th day.

Germination of mature mango seeds was started after 37 days and the spread over was during a period of six consecutive days (Table 4). Maximum number of seeds germinated on 39th day. Mature seeds desiccated for 5 days

Table 3: Effect of desiccation on rate of germination of young *Mangifera indica* seeds

Number of days of desiccation	Total seeds planted	Days of Germination													Non Viable seeds
		31	32	33	34	35	36	37	38	39	40	41	42	43	
		No. of seeds germinated													
0 (control)	40							4	8	12	8	4	4		0
5	40	10	12	7	4	4	3								0
10	40						7	9	12	8	2	2			0
15	40			4	8	10	8	6	4						0
20	40					10	15	11	2	2					0
25	40						5	12	4	2					17
30	40					4	4	3	2						27

Table 4 : Effect of desiccation on rate of germination of mature *Mangifera indica* seeds

Number of days of desiccation	Total seeds planted	Days of Germination													Non viable seeds
		30	31	32	33	34	35	36	37	38	39	40	41	42	
		No. of seeds germinated													
0 (control)	40								4	8	12	10	3	3	0
5	40					3	10	13	7	4	3				0
10	40		7	12	8	6	4	3							0
15	40		6	10	9	5	4	4	2						0
20	40			8	13	10	4	2	2	1					0
25	40					8	10	7	4	5	4	2			0
30	40							8	10	5	4	2			11

showed germination earlier than the control seeds and spread over was six days as in control. These seeds started germination on 34th day after planting. Seed samples desiccated for 10 days and 15 days showed early germination compared to the control i.e., on the 31st day, but the spread over period was 6 and 7 consecutive days respectively. The same trend of germination rate and spread over period of 7 days was occurred in 20 and 25 days desiccated seeds but these seeds started to germinate only on 32nd and 34th day respectively. Mature seeds desiccation beyond 10 days showed an increase in spread over period from 6 days to 7 days. In the case of seeds desiccated for 30 days germination started only on 36th day and spread over period was limited to 5 days.

Partly ripened control seeds started germination on 20th day and spread over was 6 consecutive days. Maximum seeds were germinated on the 22nd day (Table 5). The same pattern in rate of germination and spread over period was seen in 5 days desiccated seeds. Further desiccation did not affect the spread over period but delayed germination. Seeds desiccated for 10 days started germination on 23rd day but it further delayed to 27th day for seeds of all other periods of desiccation. Seeds desiccated for 15 days showed maximum germination on the 4th day of germination. The 20, 25 and 30 days desiccated seeds showed maximum germination on 2nd day of germination.

The ripened control seeds started germination on 16th day and spread over period was seven consecutive days (Table 6). The seeds desiccated for 5 days started to germinate only on 22nd day and the spread over period was reduced to 6 consecutive days. Control, 10, 15 and 20 days desiccated seeds, showed the maximum germination on 2nd day and other samples registered maximum germination on 3rd day. Seeds desiccated for 25 days started germination only on 28th day. Seeds desiccated for 30 days were nonviable.

Table 5: Effect of desiccation on rate of germination of partly ripened *Mangifera indica* seeds

Number of days of desiccation	Number of seeds planted	Days of germination													Non viable seeds
		20	21	22	23	24	25	26	27	28	29	30	31	32	
		No. of seeds germinated													
0 (control)	40	7	9	13	6	3	2								0
5	40	5	8	10	9	4	4								0
10	40				6	9	11	8	4	2					0
15	40								2	4	8	14	7	5	0
20	40								8	14	7	5	4	2	0
25	40							8	13	9	4	4	2		0
30	40								7	11	6	4	2	1	9

Table 6: Effect of desiccation on rate of germination of ripened *Mangifera indica* seeds

Number of days of desiccation	No. of seeds planted	Days of germination																Non viable seeds
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
		No. of seeds germinated																
0 (Control)	40	7	10	8	5	4	4	2										0
5	40							4	8	12	10	4	2					0
10	40								8	12	10	4	3	2	1			0
15	40									9	12	7	5	4	2	1		0
20	40										8	10	11	6	5			0
25	40													4	8	10	3	15
30	40				-	-	-	-	-	-								40

Among the four different stages of seeds, the ripened seeds germinated quite earlier than others and the seeds could not withstand desiccation beyond 20 days compared to other seeds of different developmental stages.

1.4. Seedling vigour

Young seeds germinated after 5 days of desiccation showed a rapid increase in seedling vigour and upto 15 days more or less same seedling vigour was maintained, followed by a gradual reduction leading to half when the seeds were desiccated for 30 days (Table 7, Fig. 3).

Similar pattern of change in seedling vigour was exhibited by mature seeds also. When mature seeds were desiccated for 30 days the seedling vigour was reduced to one third of that of the control seeds.

Partly ripened seeds also exhibited the same pattern of seedling vigour as that of young and mature seeds. But 30 days of desiccation resulted in the reduction of seedling vigour to almost half compared to the control seeds.

Ripened seeds showed a varied pattern in seedling vigour in comparison with the seeds of other treatments. In these seed lot the seedling vigour showed a gradual reduction during desiccation period. After 25 days of desiccation, the seedling vigour was reduced to 60% compared to the control seeds. Comparatively maximum seedling vigour was exhibited by control seeds.

1.5. Moisture content

Young seeds contained maximum moisture content (Table 8, Fig. 4). During desiccation, these seeds showed gradual but insignificant reduction in moisture content up to 15^{th} day, and on 20^{th} day the reduction in moisture content was significant compared to other stages. Only half of the moisture content was retained after 30 days.

Table 7: Effect of desiccation on seedling vigour of *Mangifera indica* seeds (g^{-1} dry weight)

Samples (Seed)	Days of desiccation						
	0(control)	5	10	15	20	25	30
	seedling vigour (g^{-1} dry weight)						
Young	13.54 ± 4.23	21.84 ± 2.42	18.74 ± 1.12	18.96 ± 2.15	14.38 ± 2.11	9.68 ± 2..22	6.45 ± 1.01
Mature	22.95 ± 2.54	26.65 ± 2.13	20.78 ± 1.54	17.61 ± 2.13	14.73 ± 1.32	11.21 ±1.45	8.95 ±1.54
Partly Ripened	31.06 ± 1.48	33.08 ± 4.20	25.57 ±1..45	23.23 ± 3.32	17.87 ± 115	15.24 ± 1.15	13.79 ± 1.42
Ripened	34.06 ± 3.42	27.76 ± 2.21	25.24 ± 1.23	24.86 ± 1.22	23.64 ± 1.30	21.34 ± 1.02	-

Table 8: Effect of desiccation on moisture content distribution in *Mangifera indica* seeds ($g\ g^{-1}$)

Samples (seeds)	Number of days of desiccation						
	0	5	10	15	20	25	30
	Moisture content in $g\ g^{-1}$						
Young	0.814 ± 0.040	0.724 ± 0.042	0.655 ± 0.021	0.632 ± 0.024	0.461 ± 0.043	0.415 ± 0.033	0.396 ± 0.023
Mature	0.764 ± 0.010	0.748 ± 0.012	0.729 ± 0.013	0.642 ± 0.015	0.552 ± 0.042	0.484 ± 0.032	0. 463 ± 0.045
Partly ripened	0.716 ± 0.010	0. 693 ± 0.015	0.625 ± 0.012	0.589 ± 0.033	0.547 ± 0.033	0.433 ± 0.035	0.385 ± 0.043
Ripened	0.586 ±0.042	0.487 ± 0.012	0.439 ± 0.015	0.403 ± 0.013	0.390 ± 0.014	0.352 ± 0.043	0.320 ± 0.021

Figure 3: Effect of desiccation on sedling vigour of *Mangifera indica* seeds

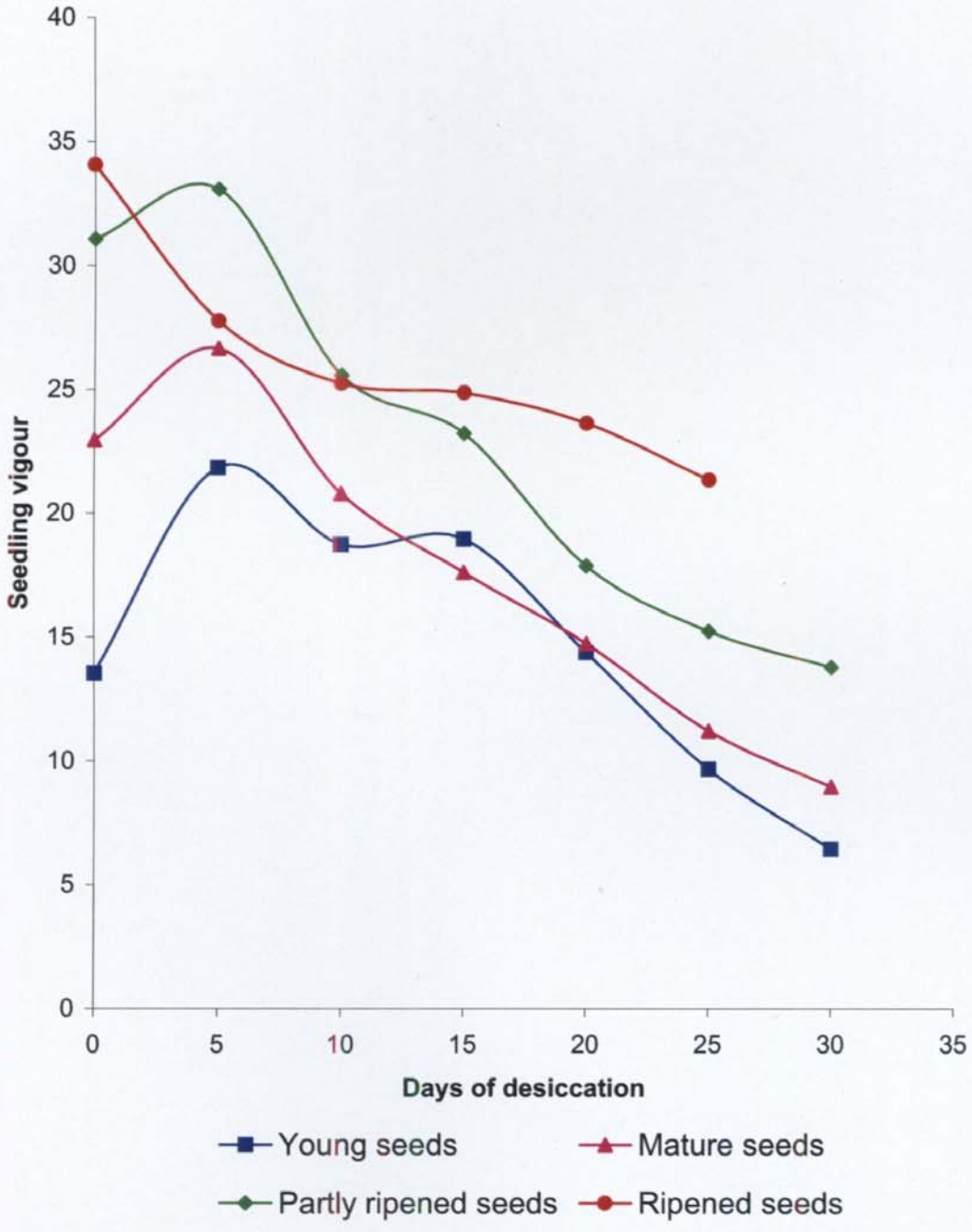
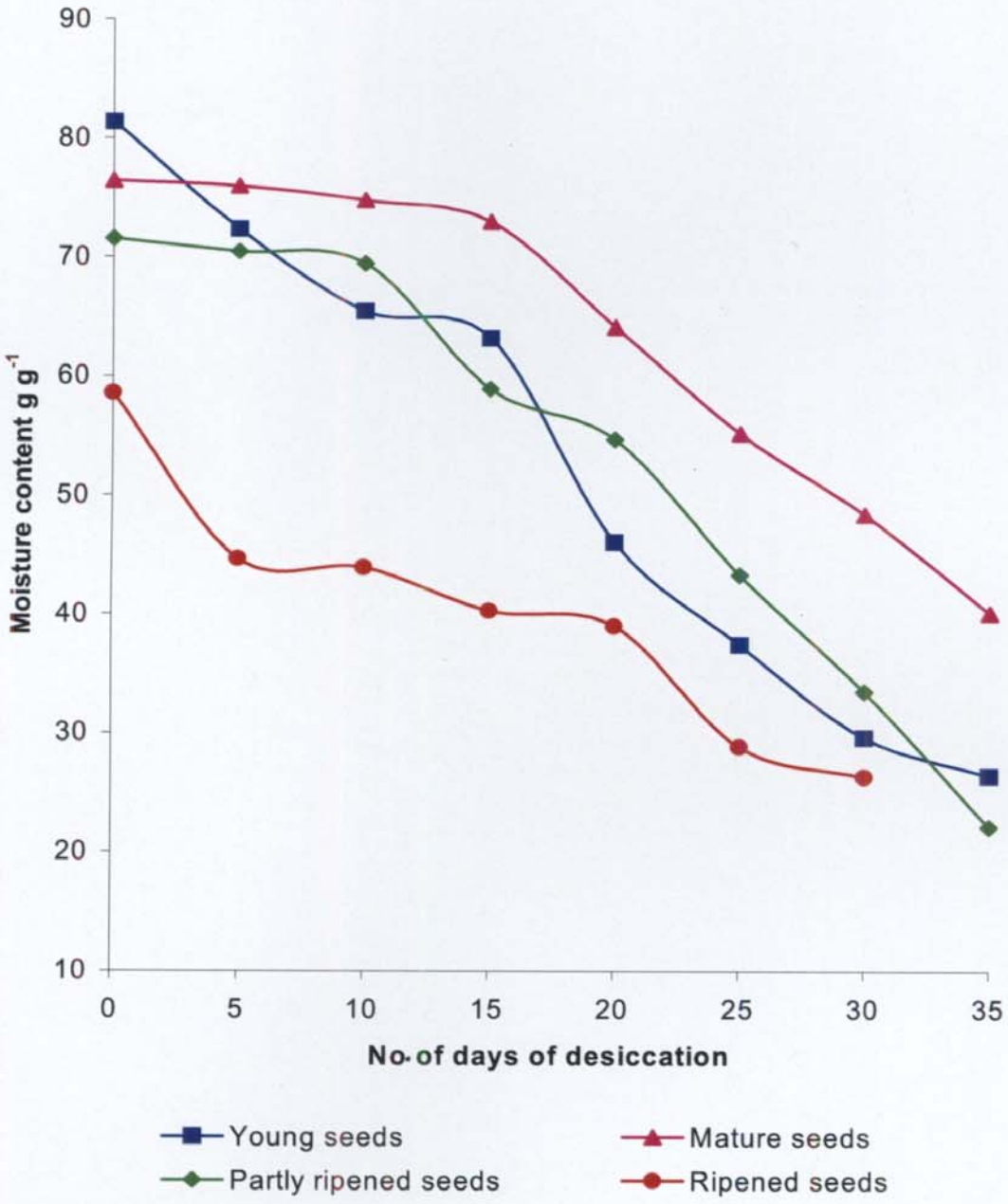


Figure 4: Effect of desiccation on moisture content distribution in *Mangifera indica* seeds(g g^{-1})



R3C

Mature seeds had almost same moisture content as that of young seeds. During desiccation up to 25 days a significant reduction in moisture content was noted. But the moisture content was reduced to 60% on 30th day of desiccation compared to control.

Partly ripened seeds showed the characteristic reduction in moisture content as that of young seeds. A sudden lowering of moisture content was observed in ripened seeds during early days of desiccation. Thereafter the reduction was gradual. These seeds retained only half the moisture content of the control seeds during later days of desiccation.

2. Biochemical studies of desiccated seeds

2.1. Dry weight determination of seed tissue

Tissue dry weights of control and desiccated seeds of all samples are given (Table 9, Fig. 5). Young control seeds showed only 11% dry weight. During the entire period of desiccation, the tissue dry weight gradually increased and attained maximum on 30th day.

Mature seeds also showed increased dry weight, which increased gradually and significantly during desiccation. A doubling of the tissue dry weight was occurred on 20 days of desiccation. These seeds attained maximum dry weight during final days of desiccation.

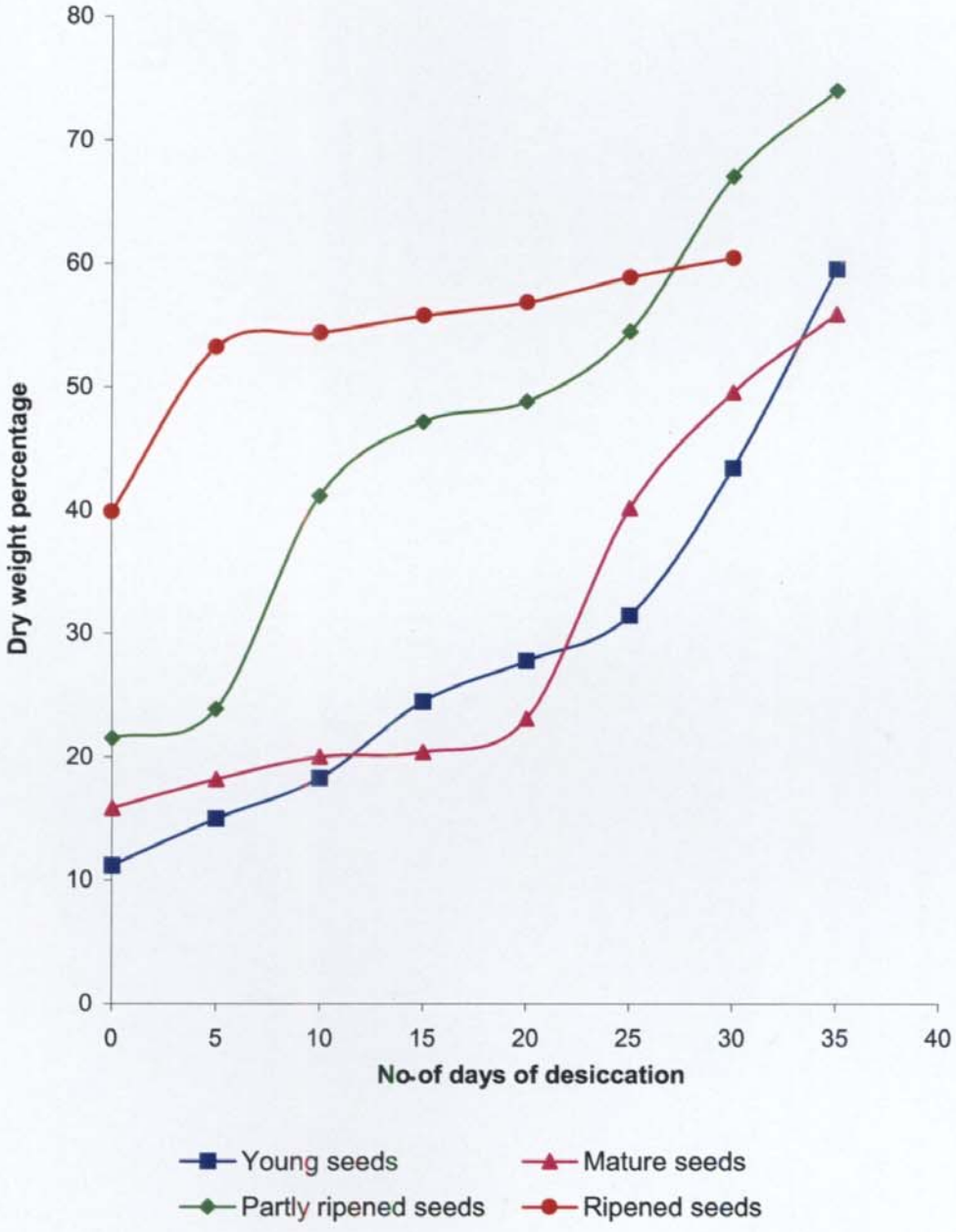
Partly ripened seeds showed a gradual increase in dry weight during 5th day of desiccation. Seeds desiccated for 10 days exhibited two-fold increase. The seeds desiccated for 30 days showed maximum tissue dry weight.

Ripened seeds showed a sharp increase in dry weight on 5th day of desiccation. Seeds desiccated for 10, 15 and 20 days maintained more or less same tissue dry weight. Then there was a gradual increase in tissue dry weight

Table 9: Effect of desiccation on dry weight content in *Mangifera indica* seed tissues

Samples (seeds)	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Dry weight %						
Young	11.21 ± 2.18	15.00 ± 2.23	18.28 ± 2.18	24.49 ± 2.28	27.77 ± 2.18	31.45 ± 4.48	43.45 ± 4.68
Mature	15.86 ± 2.11	20.00 ± 1.61	23.12 ± 1.02	28.90 ± 1.66	34.61 ± 1.28	40.17 ± 5.16	49.58 ± 3.16
Partly ripened	21.51 ± 1.16	23.90 ± 1.11	41.16 ± 4.62	47.17 ± 3.86	48.82 ± 2.84	54.49 ± 4.13	67.07 ± 5.10
Ripened	39.87 ± 4.16	53.22 ± 1.04	54.36 ± 1.01	55.76 ± 1.22	56.85 ± 1.61	58.90 ± 2.11	60.45 ± 1.68

Figure 5: Effect of desiccation on dry weight % of *Mangifera indica* seeds



reaching a maximum on 30th day. Among the control seeds, ripened seeds showed maximum dry weight and minimum by young seeds.

2.2 Distribution of starch content

Starch content of mango seeds, of different developmental stages was estimated during the desiccation period of 30 days and given in Table 10 (Fig. 6). Young control seeds showed only 11% starch content which was found to increase regularly during the entire period of desiccation. After 30 days of desiccation. The seeds contained about 26% of starch.

A slight increase in starch content was occurred in mature control seeds. On the 5th day of desiccation the starch content was increased by 4% and again increased gradually upto 15 days. The starch content was doubled when seeds desiccated for 20 days. During 25-30 days of desiccation the starch content was found increased regularly.

Partly ripened control seeds contained 13% of starch. During the period of desiccation, these seeds showed the same pattern of increase as in the previous cases. On 30 days of desiccation these seeds showed 35 % of starch content, which was the maximum amount of starch, compared to all the control as well as other treatments.

Among control seeds maximum starch content was occurred in ripened seeds. A sudden increase of starch content was observed after 5 days of desiccation and during the later stages, the seeds showed a regular and gradual increase upto a maximum of 25% in seeds samples desiccated for 30 days.

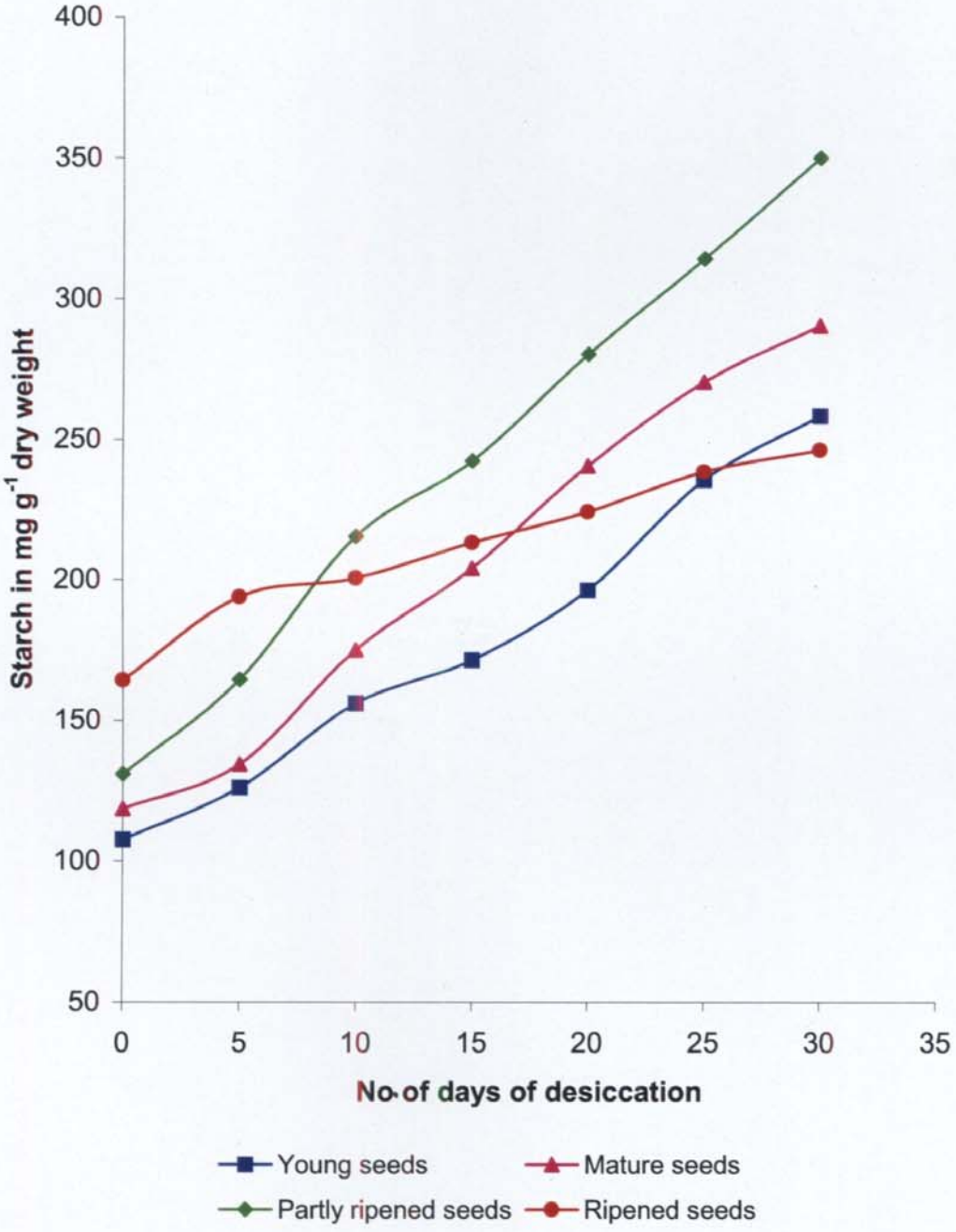
2.3. Distribution of sugar content

The sugar content of different seeds during desiccation was shown in Table 11a and 11b, (Fig. 7, 8 and 9). Except partly ripened and ripened seeds

Table 10: Effect of desiccation on starch content in *Mangifera indica* seeds (mg g^{-1} dry weight)

Samples (Seed)	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Starch in mg g^{-1} dry weight						
Young	107.69 ± 6.47	126.15 ± 7.25	156.05 ± 5.47	171.41 ± 8.36	196.34 ± 6.87	235.44 ± 6.58	258.28 ± 2.48
Mature	118.54 ± 6.58	134.46 ± 8.56	174.99 ± 7.58	204.15 ± 7.78	240.53 ± 6.36	270.34 ± 8.25	290.34 ± 8.45
Partly Ripened	130.90 ± 5.64	164.51 ± 6.42	215.38 ± 8.32	242.31 ± 7.56	280.21 ± 7.62	314.15 ± 8.48	350.00 ± 6.47
Ripened	164.34 ± 6.23	193.85 ± 5.25	200.55 ± 4.58	213.17 ± 5.45	224.15 ± 4.35	238.45 ± 5.57	246.15 ± 4.41

Figure 6: Effect of desiccation on starch content in *Mangifera indica* seeds (mg g^{-1} dry weight)



of all treatment contained maltose along with other sugars fructose, glucose, raffinose, rhamnose and stachyose.

Young control seeds consisted of higher amount of fructose than other sugars. During desiccation upto 15 days fructose content was found to increase. After 15 days there was a gradual reduction upto 30 days. Glucose and maltose contents of young seeds were found to increase during entire period of desiccation. Raffinose initially showed an increase and then decreased after 20 days of desiccation. An increase in stachyose was noticed during desiccation only upto 20 days and there after stachyose was not detected. A gradual reduction in rhamnose content was observed throughout the period of desiccation. Sucrose content was found to increase significantly from young control seeds to 30 days desiccated seeds.

Mature control seeds contained more glucose compared to other sugars. Fructose was lesser than in young seeds but showed a gradual increase during desiccation, whereas the glucose content was found to decrease gradually during desiccation. Maltose and rhamnose contents of mature seeds were increasing gradually during desiccation. Raffinose content was found to increase significantly upto 20 days of desiccation ($p<0.01$) and beyond that it sharp reduction was occurred. Stachyose was gradually increased during desiccation upto 20 days and then showed a decline. The sucrose content increased significantly upto 30 days of desiccation.

In partly ripened control and desiccated seeds, maltose was absent. A regular increase of fructose, glucose, stachyose and sucrose during desiccation period was exhibited by partly ripened seeds. A significant increase of raffinose content was noted during 25 days of desiccation ($p<0.01$). Then it showed a sharp decrease and again increased in 30 days desiccated seeds. The increase in stachyose content was maximum in 20 days desiccated seeds. During 25 days of desiccation the stachyose content was declined and

Table 11a: Effect of desiccation on sugar content in *Mangifera indica* seeds (mg g $^{-1}$ dry weight)

Samples (seed)	Sugar	Number of days of desiccation						
		0 (control)	5	10	15	20	25	30
		Sugar in mg g $^{-1}$ dry weight						
Young	Fructose	4.29 ± 0.015	4.63 ± 0.02	4.76 ± 0.06	4.88 ± 0.04	3.89 ± 0.05	3.32 ± 0.02	3.25 ± 0.04
	Glucose	2.14 ± 0.06	2.25 ± 0.01	2.30 ± 0.03	2.41 ± 0.05	2.53 ± 0.02	2.62 ± 0.02	2.72 ± 0.01
	Maltose	1.34 ± 0.015	1.46 ± 0.05	1.75 ± 0.04	1.85 ± 0.02	2.10 ± 0.02	2.17 ± 0.03	2.29 ± 0.02
	Raffinose	0.02 ± 0.001	0.2 ± 0.03	0.54 ± 0.02	0.35 ± 0.03	0.54 ± 0.04	0.18± 0.04	0.067 ± 02
	Rhamnose	1.57 ± 0.02	1.18 ± 0.03	1.23 ± 0.04	1.14 ± 0.04	0.92 ± 0.03	0.52 ± 0.03	0.34 ± 0.02
	Stachyose	0.01 ± 0.001	0.10 ± 0.01	0.27 ± 0.05	0.35 ± 0.03	0.41 ± 0.02	ND	ND
	Sucrose	0.214 ± 0.04	1.19 ± 0.03	3.11 ± 0.03	3.31 ± 0.02	3.47 ± 0.03	3.96 ± 0.02	4.39 ± 0.04
	Total	**9.584**	**11.72**	**14.29**	**14.29**	**13.86**	**12.77**	**13.057**
Mature	Fructose	4.03 ± 0.03	4.16 ± 0.04	4.80 ± 0.04	5.05 ± 0.03	5.17 ± 0.03	5.27 ± 0.05	5.34 ± 0.03
	Glucose	4.68 ± 0.04	4.54 ± 0.04	4.21 ± 0.02	4.03 ± 0.03	3.43 ± 0.05	3.23 ± 0.004	2.40 ± 0.02
	Maltose	1.62 ± 0.05	1.81 ± 0.03	1.90 ± 0.02	2.17 ± 0.03	2.30 ± 0.06	2.24 ± 0.06	2.17 ± 0.05
	Raffinose	0.046 ± 0.02	0.171 ± 0.03	0.501 ± 0.02	0.786 ± 0.04	1.15 ± 0.03	0.15 ± 0.03	0.086 ± 0.04
	Rhamnose	2.74 ± 0.02	2.88 ± 0.25	2.97 ± 0.35	3.17 ± 0.08	3.26 ± 0.12	3.41 ± 0.50	4.77 ± 0.07
	Stachyose	0.025 ± 0.005	0.085 ± 0.005	0.25 ± 0.04	0.39 ± 0.03	0.57 ± 0.04	0.07± 0.02	0.12 ± 0.01
	Sucrose	0.35 ± 0.05	0.70 ± 0.05	3.83 ± 0.03	3.93 ± 0.02	5.01 ± 0.04	5.71 ± 0.05	5.52 ± 0.02
	Total	**13.47**	**14.346**	**18.896**	**18.896**	**20.89**	**15.08.**	**15.406**
Partly Ripened	Fructose	4.62 ± 0.05	4.84 ± 0.04	4.97 ± 0.07	5.15 ± 0.04	5.42 ± 0.06	5.51 ± 0.01	5.57 ± 0.02
	Glucose	2.08 ± 0.05	2.18 ± 0.06	2.24 ± 0.07	2.39 ± 0.03	2.47 ± 0.04	2.55± 0.06	2.67 ± 0.05
	Maltose	ND	ND	ND	ND	ND	ND	ND
	Raffinose	0.27 ± 0.02	0.51 ± 0.03	0.82 ± 0.02	0.94 ± 0.03	1.54 ± 0.04	1.59 ± 0.03	1.18 ± 0.04
	Rhamnose	0.68 ± 0.06	4.58 ± 0.02	3.55 ± 0.02	0.89 ± 0.04	2.38 ± 0.02	2.55 ± 0.01	2.82 ± 0.04
	Stachyose	0.13 ± 0.03	0.25 ± 0.04	0.41 ± 0.05	0.47 ± 0.04	0.77 ± 0.04	0.29 ± 0.02	ND
	Sucrose	3.01 ± 0.04	3.96 ± 0.02	5.41 ± 0.03	5.69 ± 0.05	4.58 ± 0.06	4.64 ± 0.03	3.94 ± 0.05
	Total	**10.79**	**16.32**	**17.40**	**14.46**	**17.16**	**17.13**	**16.18**
Ripened	Fructose	4.67 ± 0.04	5.03 ± 0.02	5.47 ± 0.03	5.84 ± 0.02	5.88 ± 0.04	5.96 ± 0.02	6.01 ± 0.01
	Glucose	3.21 ± 0.09	3.18 ± 0.08	3.24± 0.03	3.29 ± 0.01	3.34 ±0.02	3.04 ±0.06	3.45 ± 0.06
	Maltose	ND	ND	ND	ND	ND	ND	ND
	Raffinose	0.135 ± 0.03	0.24 ± 0.01	0.26 ± 0.01	0.36 ± 0.02	0.54 ± 0.03	0.15 ± 0.03	0.07 ± 0.02
	Rhamnose	2.79 ± 0.04	1.58 ± 0.03	3.79 ± 0.04	0.59 ± 0.03	ND	ND	ND
	Stachyose	0.067 ± 0.07	0.12 ± 0.04	0.13 ± 0.06	0.10 ± 0.04	0.027 ± 0.02	0.075 ± 0.003	0.07 ± 0.03
	Sucrose	2.93 ± 0.03	2.38 ± 0.03	2.40 ± 0.08	1.66 ± 0.03	3.14 ± 0.04	1.60 ± 0.02	2.17 ± 0.01
	Total	**11.707**	**12.53**	**15.29**	**15.29**	**11.84**	**14.025**	**12.01**

ND –Not Detected

Table 11b: Effect of desiccation on sugar content in *Mangifera indica* seeds (mg g $^{-1}$ dry weight)

Samples (seeds)	Sugar	Number of days of desiccation						
		0 (control)	5	10	15	20	25	30
		Sugar content in mgg^{-1} dry weight						
Young	Sucrose	0.214 ± 0.04	1.19 ±0.03	3.11 ± 0.03	3.31 ± 0.02	3.47 ± 0.03	3.96 ±0.02	4.39 ± 0.04
	Raffinose	0.02 ± 0.001	0.2 ± 0.03	0.54 ± 0.02	0.35 ± 0.03	0.54 ± 0.04	0.18 ±0.04	0.067 ± 0.02
	Oligosaccharides	6.43	6. 88	7. 06	7. 29	6.42	5. 94	5. 97
	Oligosaccharide: sucrose	0.14	0. 25	0. 26	0. 21	0. 27	0.04	0.01
	Sucrose:raffinose	10 : 1	9 : 1	6 : 1	10: 1	6 :1	22 : 1	65 : 1
Mature	Sucrose	0.35 ± 0.05	0.70 ± 0.05	3.83 ± 0.03	3.93 ± 0.02	5.01 ± 0.04	5.71 ±0.05	5.52 ± 0.02
	Raffinose	0.046 ±0.02	0.171±0.03	0.501± 0.02	0.786 ± 0.04	1.15 ± 0.03	0.15 ±0.03	0.086±0.04
	Oligosaccharides	8.71	8.70	9.01	9.08	8.60	9.23	9.73
	Oligosaccharide: sucrose	0.07	0.26	0.19	0.29	0.34	0.14	0.03
	Sucrose:raffinose	17 : 1	4 : 1	7 : 1	5 : 1	4 : 1	5 : 1	10 : 1
Partly ripened	Sucrose	3.01 ± 0.04	3.96 ±0.02	5.41 ± 0.03	5.69 ± 0.05	4.58 ± 0.06	4.64± 0.03	3.94±0.05
	Raffinose	0.27± 0.02	0.51 ±0.03	0.82 ± 0.02	0.94 ± 0.03	1.54± 0.04	1.59± 0.03	1.18 ± 0.04
	Oligosaccharides	6.70	7. 02	7. 21	7. 54	7. 89	7. 06	8. 24
	Oligosaccharide: sucrose	0.13	0.19	0.22	0.24	0.50	0.3	0. 29
	Sucrose:raffinose	11 : 1	7 : 1	7 : 1	6 : 1	3 : 1	3 : 1	21 : 1
Ripened	Sucrose	2 .93 ±0.03	2.38 ±0.03	2.40 ± 0.08	1.66 ± 0.03	3.14 ± 0.04	1.60 ±0.02	2.17 ±0.01
	Raffinose	0.135± 0.03	0.24 ± 0.01	0.26 ± 0.01	0.36 ± 0.02	0.54 ± 0.03	0.15 ±0.03	0.07 ±0.02
	Oligosaccharides	7.79	8. 21	8.71	9.13	9.22	9.00	9.46
	Oligosaccharide: sucrose	0.22	0.15	0.16	0. 27	0.18	0.14	0.06
	Sucrose:raffinose	7 : 1	10 : 1	10 : 1	5 : 1	6 : 1	31 : 1	30 : 1

Figure 7: Effect of desiccation on sucrose content in *Mangifera indica* seeds (mg g^{-1} dry weight)

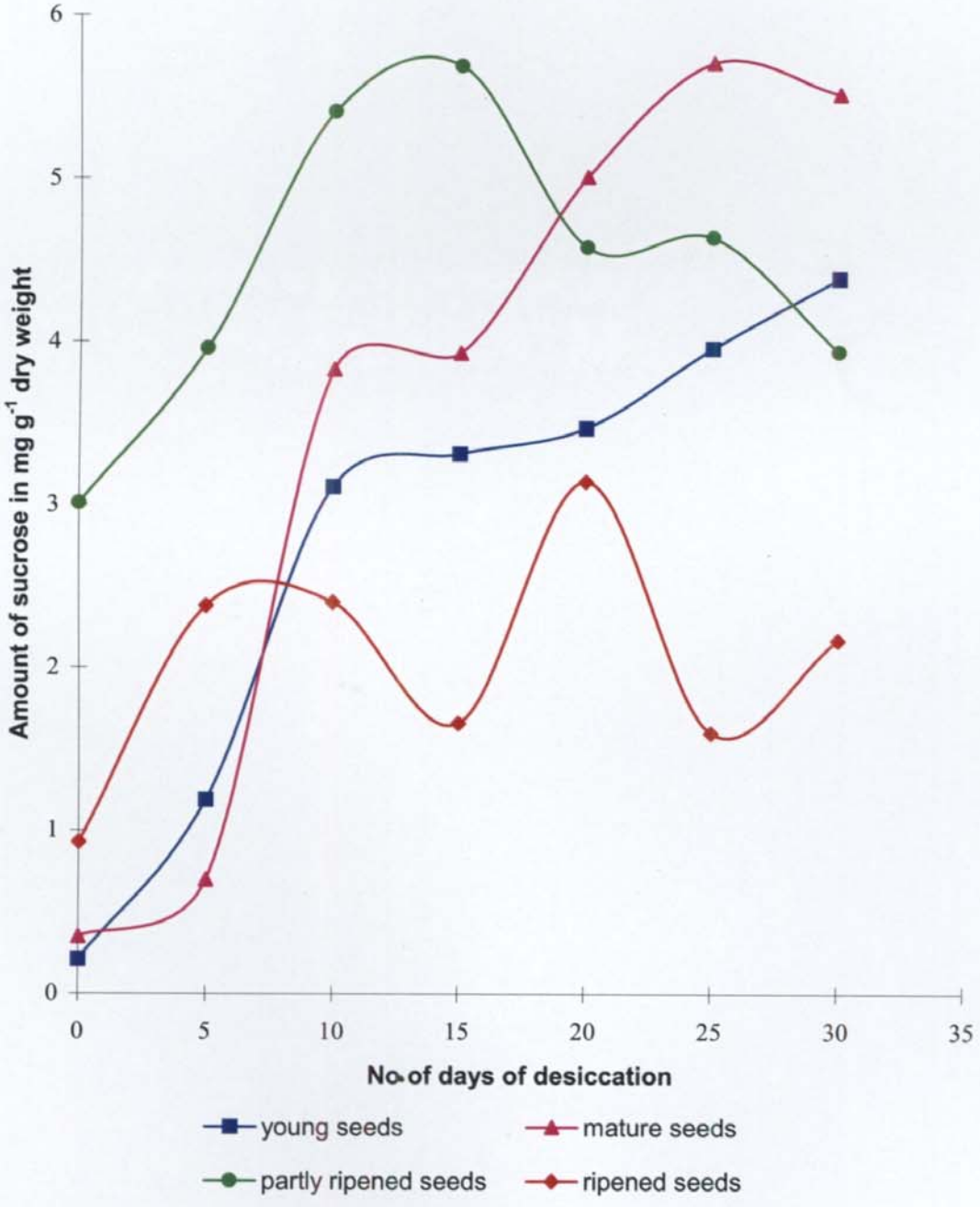


Figure 8: Effect of desiccation on raffinose content in *Mangifera indica* seeds (mg g^{-1} dry weight)

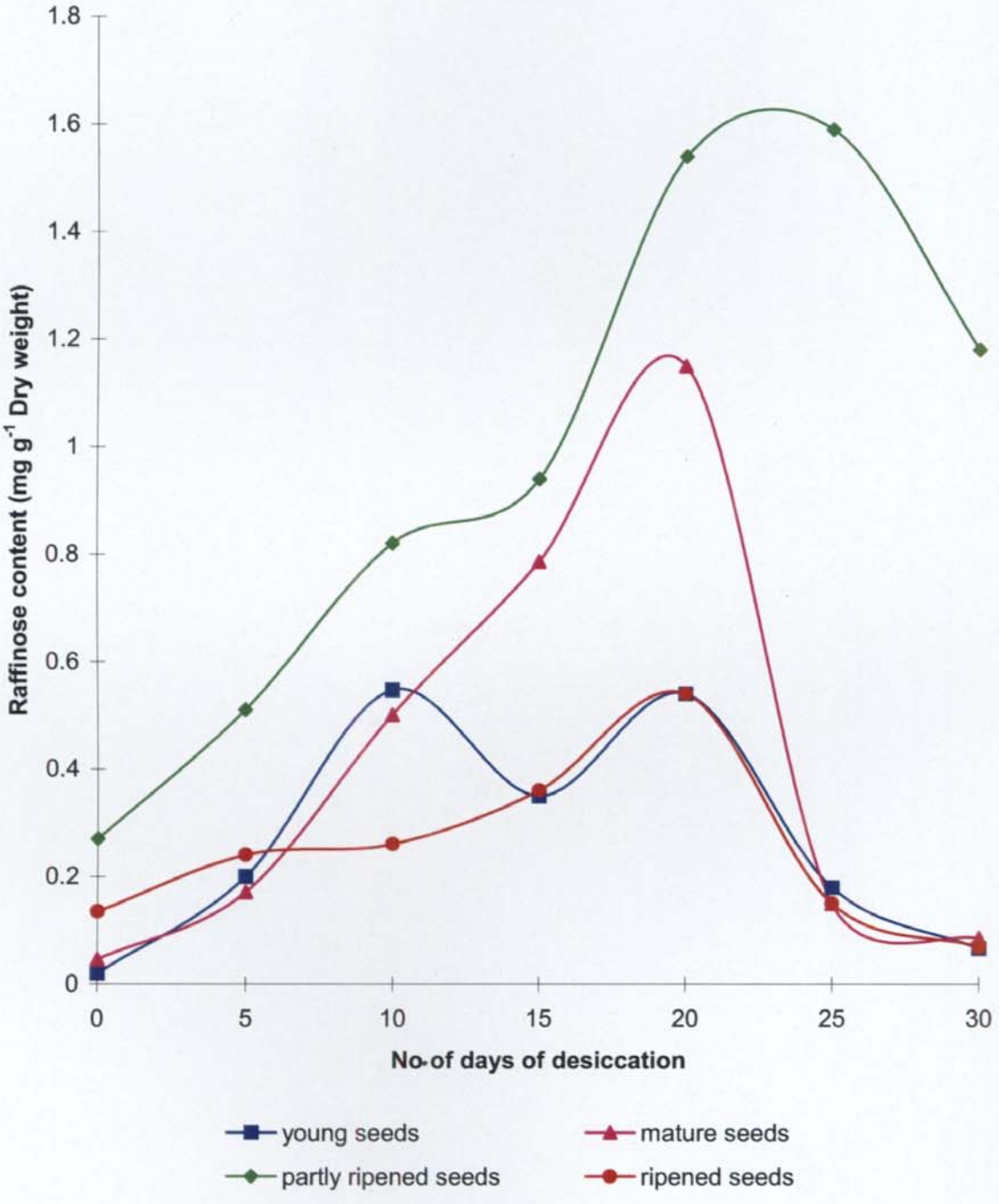
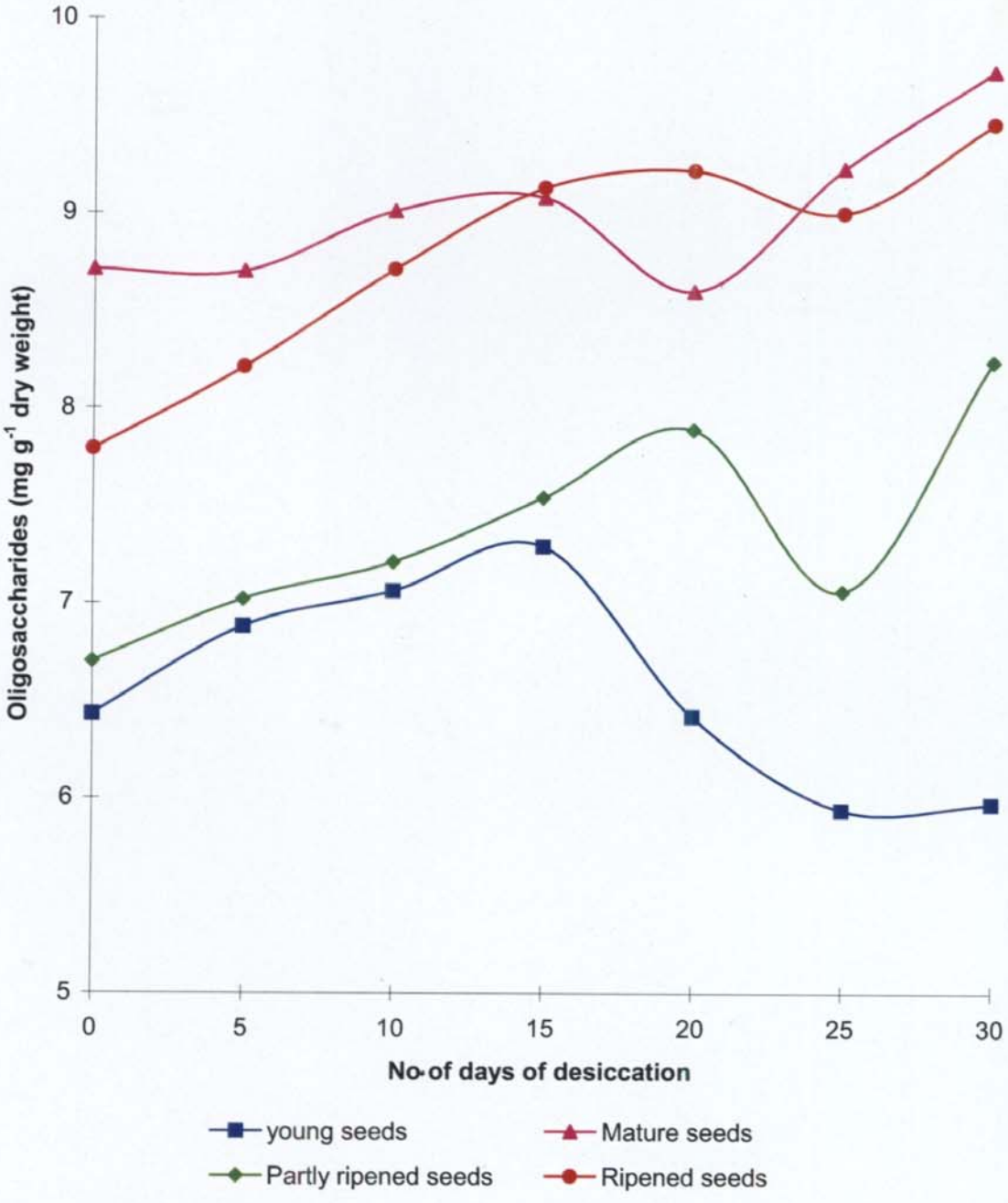


Figure 9: Effect of desiccation on oligosaccharides in *Mangifera indica* seeds (mg g^{-1} dry weight)



disappeared in 30 days desiccated seeds. Sucrose content was reduced in seeds after 15 days of desiccation.

Fructose increased gradually in ripened seeds throughout the period of desiccation. Glucose and maltose content exhibited the same pattern of changes as in partly ripened seeds. Raffinose content was found to increase up to 20 days of desiccation and beyond that a sharp reduction was observed in seeds of 25 and 30 days of desiccation. Rhamnose content was decreased during desiccation and disappeared after 15 days. Stachyose was increased upto 15 days and then declined. No regular pattern of reduction was observed in sucrose content.

2.4. Distribution of protein content

In young control seeds the protein content only 6% (Table 12, Fig.10). During the entire period of desiccation, the protein content was increased gradually but not significantly upto about 12%.

Mature control seeds showed the maximum protein content (i.e., 10%) compared to control seeds of other developmental stages. During desiccation the protein content of mature seeds registered a regular increase up to a maximum of 15% on 30^{th} day.

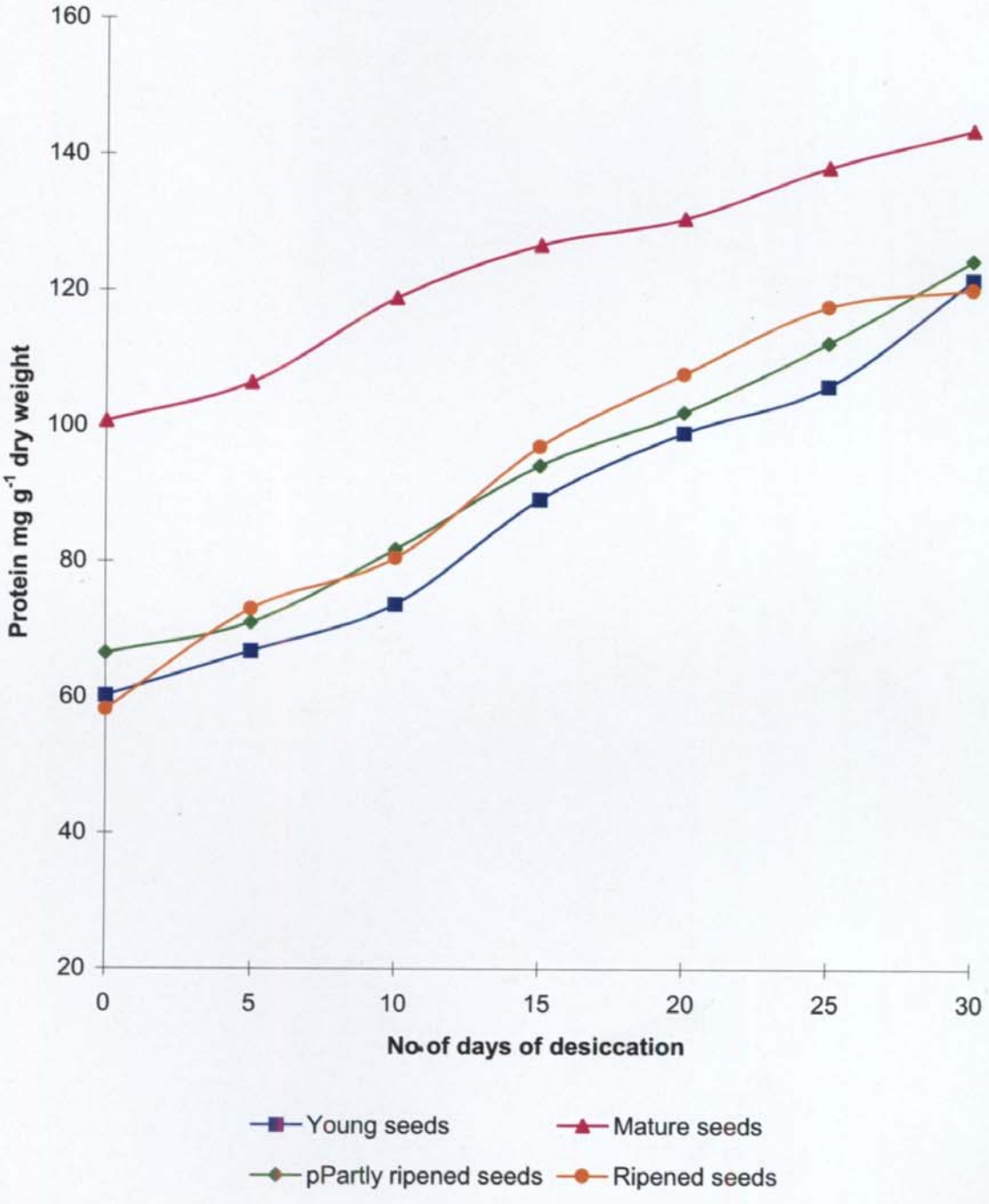
Partly ripened seeds composed of only 6-7% of protein which was found to increase during the entire period of desiccation. Seeds desiccated for 30 days showed 12% of protein content.

Like, young control seeds, ripened control seeds contained only 6% of protein. On 30^{th} day of desiccation, the protein content was increased to 12%. Seeds of all treatments showed a gradual increase in protein content during the desiccation period.

Table 12: Effect of desiccation on protein content in *Mangifera indica* seeds (mg g $^{-1}$ dry weight)

Samples (Seed)	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Protein in mg g $^{-1}$ dry weight						
Young	60.18 ± 4.15	66.76 ± 4.47	73.68 ± 5.68	89.11 ± 6.85	99.01 ± 5.58	105.92 ± 5.65	121.58 ± 6.54
Mature	100.63 ± 6.14	106.32 ± 4.15	118.82 + 3.23	126.64 ± 3.45	130.56 ± 4.15	138.16 ± 3.11	143.68 ± 3.48
Partly Ripened	66.45 ± 5.16	70.96 ± 4.82	81.74 ± 5.12	94.16 ± 4.41	102.04 ± 5.18	112.29 + 4.25	124.42 ± 6.49
Ripened	58.14 ± 6.17	72.97 ± 6.84	80.47 ± 5.36	96.97 ± 5.14	107.67 ± 5.41	117.65 ± 3.71	120.14 ± 4.29

Figure 10: Effect of desiccation on protein content in *Mangifera indica* seeds (mg g^{-1} dry weight)



2.5. Distribution of free amino acids

In young mango seeds amino acids like alanine, arginine, glycine and valine showed regular reduction of their amounts during the entire desiccation period (Table 13). Very slight reduction in aspartic acid was observed during desiccation. Cysteine was detected only upto 15 days. Metabolically important glutamic acid showed a steady state upto 15 days of desiccation and there was a slight reduction thereafter. Histidine content of 20 days desiccated seeds was reduced to half compared to the control. During further days of desiccation it was not detected. Leucine content remained unchanged upto 15 days of desiccation. Thereafter its content was slightly reduced. Lysine content was reduced to half in 30 days desiccated seeds.

Phenylalanine and tyrosine contents were not detected in seeds desiccated after 15 days, while phenylalanine was reduced to half. A slight reduction was observed in the tyrosine content when the seeds were desiccated to 15 days. However the threonine content remained almost same upto 10 days of desiccation. There was a slight increase of proline content during 5 and 10 days of desiccation and afterwards a slight reduction was observed during the entire desiccation period.

Amino acid distribution in mature mango seeds showed that control seed contained double the amount of alanine, arginine and cysteine compared to the young control seeds (Table 13 and 14). Aspartic acid, histidine and threonine were slightly higher than the young control seeds. But the glutamic acid, glycine, proline and tyrosine were slightly lower than that of young control seeds. In mature mango seeds, leucine content remained unchanged during the desiccation period. Aspartic acid, glutamic acid and lysine showed a very slight reduction in their contents during desiccation. Alanine, arginine, glycine, histidine, phenylalanine, threonine, tyrosine and valine contents were reduced during desiccation. The tyrosine content was not detected after 20 days of

Table 13: Effect of desiccation on amino acid content in young *Mangifera indica* seeds ($\mu g\ g^{-1}$ dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in $\mu g\ g^{-1}$ dry weight						
Alanine	16 ± 2	14 ± 1	14 ± 1	13 ± 1	12 ± 2	10 ± 1	9 ± 1
Arginine	12 ± 1	11 ± 1	10 ± 1	10 ± 1	9 ± 1	8 ± 2	8 ± 1
Aspartic acid	38 ± 1	38 ± 1	37 ± 2	36 ± 1	36 ± 1	35 ± 1	34 ± 2
Cysteine	9 ± 1	8 ± 1	6 ± 1	5 ± 2	ND	ND	ND
Glutamic acid	63 ± 1	60 ± 1	60 ± 1	58 ± 2	57 ± 1	56 ±1	56 ± 1
Glycine	26 ± 2	24 ± 1	21 ± 1	19 ± 2	18 ± 2	16 ±1	15 ± 1
Histidine	11 ± 1	10 ± 1	8 ± 1	7 ± 2	5 ± 2	ND	ND
Leucine	34 ± 2	33 ± 1	33 ± 1	31 ± 1	29 ± 2	28 ±1	26 ± 2
Lysine	18 ± 2	17 ±1	15 ± 1	13 ± 1	12 ± 2	10 ±1	8 ± 2
Phenylalanine	12 ± 1	9 ± 2	7 ± 2	5 ± 1	ND	ND	ND
Proline	18 ± 1	19 ± 1	20 ± 2	18 ± 2	17 ± 1	16 ±1	14 ±1
Threonine	8 ± 2	9 ± 1	6 ± 2	ND	ND	ND	ND
Tyrosine	9 ± 1	8 ± 1	6 ± 1	6 ± 1	ND	ND	ND
Valine	22 ± 1	21± 1	20 ± 2	17 ± 1	17 ± 1	15 ±2	14 ± 1
Total	**296**	**281**	**263**	**238**	**212**	**194**	**184**

ND –Not Detected

Table 14: Effect of desiccation on amino acid content in mature *Mangifera indica* seeds (µg g $^{-1}$ dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g $^{-1}$ dry weight						
Alanine	31± 1	29 ± 4	27 ± 1	25 ± 1	24 ± 2	22 ± 2	20 ± 1
Arginine	24 ± 2	23 ± 1	21 ± 2	20 ± 2	19 ± 3	18 ± 1	17 ± 2
Aspartic acid	42 ± 2	41 ± 2	39 ± 1	38 ± 3	38 ± 1	36 ± 1	36 ± 2
Cysteine	14 ± 2	13 ± 3	13 ± 1	12 ± 1	11 ± 2	9 ± 2	7 ± 3
Glutamic acid	41 ± 1	40 ± 1	38 ± 1	36 ± 1	36 ± 1	35 ± 1	34 ± 1
Glycine	20 ± 2	19 ± 3	18 ± 2	16 ± 1	15 ± 3	14 ± 2	13 ± 3
Histidine	14 ± 2	13 ± 2	13 ± 2	12 ± 2	10 ± 4	9 ± 1	7 ± 2
Leucine	32 ± 4	31 ± 1	30 ± 1	29 ± 1	29 ± 3	28 ± 2	28 ± 1
Lysine	20 ± 3	20 ± 1	19 ± 1	17 ± 3	17 ± 2	16 ± 2	15 ± 2
Phenylalanine	10 ± 1	9 ± 2	8 ± 2	8 ± 2	7 ± 3	7 ± 3	5 ± 1
Proline	14 ± 2	12 ± 1	11 ± 2	10 ± 4	9 ± 1	9 ± 1	7 ± 1
Threonine	13 ± 1	12 ± 3	10 ± 2	8 ± 2	7 ± 1	5 ± 1	ND
Tyrosine	10 ± 3	8 ± 1	6 ± 1	6 ± 1	5 ± 2	ND	ND
Valine	20 ± 1	18 ± 2	17 ± 2	16 ± 2	16 ± 2	15 ± 1	14 ± 2
Total	**305**	**280**	**270**	**250**	**227**	**223**	**203**

ND –Not Detected

desiccation. Cysteine and proline contents also showed a similar pattern of reduction.

The distribution pattern of amino acids in partly ripened mango seeds was different from that of young and mature seeds (Table 15). Amino acid content of partly ripened control seeds was almost similar to mature seeds in the case of arginine and glutamic acid. On 30^{th} day the arginine content was reduced to half. But the glutamic acid content remained the same upto 15 days of desiccation and thereafter a slight reduction was noted. Partly ripened control seeds contained lesser amount of alanine and aspartic acid compared to mature control seeds.

The cysteine, proline, threonine and tyrosine contents were nearly double to the amount that present in mature control seeds. Proline content increased slightly upto 15 days of desiccation and thereafter a slight reduction was noted. Threoine and tyrosine contents were found to decrease during desiccation period and they were finally reduced to half the amount compared to the control.

Other amino acids like glycine, histidine, leucine, lysine, phenylalanine and valine were slightly higher in partly ripened control seeds than mature control seeds. During desiccation these amino acid contents other than valine and leucine were found to be reduced to half compared to those in control seeds. A slight reduction in valine and leucine contents was noticed in desiccated seeds. Phenylalanine was not detected after 25 days of desiccation.

The pattern of distribution of amino acids in ripened seeds during desiccation was different from that of other treatments (Table 16). The control ripened seeds showed increase only in alanine content compared to the control partly ripened seeds. These seeds contained the same amount of aspartic acid, glutamic acid and valine. Ripened control seeds showed a decrease in the

Table 15: Effect of desiccation on amino acid content in partly ripened *Mangifera indica* seeds (µg g $^{-1}$ dry weight)

Amino acids	Number of days of desiccation of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g $^{-1}$ dry weight						
Alanine	22 ± 1	21 ± 1	20 ± 1	19± 1	19± 1	17± 2	15 ± 2
Arginine	23 ± 2	21 ± 2	19 ± 1	18 ±1	16 ±2	15 ± 1	14 ± 1
Aspartic acid	32 ± 2	35 ± 2	39 ± 2	34± 2	31± 1	30 ± 1	28 ± 1
Cysteine	23 ± 1	21 ± 1	20 ± 2	18 ± 1	18± 2	16 ± 1	14 ± 1
Glutamic acid	40 ± 1	42 ± 1	40 ± 1	38 ± 2	36 ± 2	36 ± 1	33 ± 2
Glycine	25 ± 1	23 ± 1	21 ± 2	20 ± 1	18 ± 2	17 ± 1	17 ± 1
Histidine	18 ± 1	17 ± 2	15 ± 1	14 ± 1	12 ± 1	10 ± 2	8 ± 1
Leucine	41 ± 1	42 ± 1	44 ± 1	40 ± 2	38 ± 2	37 ± 2	35 ± 2
Lysine	25 ± 2	23 ± 2	21 ± 1	20 ±1	18 ± 2	16 ± 1	14 ± 1
Phenylalanine	14 ± 1	13 ± 1	11 ± 1	9 ± 2	7 ± 1	7 ± 1	ND
Proline	23 ± 1	25 ± 2	27 ± 1	25 ±1	23 ± 2	20 ± 2	18 ± 2
Threonine	24 ± 2	23 ± 1	21 ± 2	19 ±1	17 ± 2	15 ± 2	12 ± 2
Tyrosine	20 ± 1	20 ± 1	18 ± 2	16 ±1	14 ± 2	13 ± 1	11 ± 2
Valine	28 ± 2	26 ± 1	25 ± 1	23 ±2	21 ±1	19 ± 2	17 ± 1
Total	**358**	**352**	**341**	**313**	**288**	**268**	**236**

ND –Not Detected

Table 16: Effect of desiccation on amino acid content in ripened *Mangifera indica* seeds (µg g $^{-1}$ dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g $^{-1}$ dry weight						
Alanine	30 ± 2	28 ±1	27 ±2	27 ±1	26 ±2	24 ±1	20 ±1
Arginine	15 ± 1	14 ±1	12 ±1	10 ±2	8 ± 1	8 ± 1	7 ± 1
Aspartic acid	30 ± 1	28 ±1	27 ±1	25 ±1	23 ±2	22 ±1	20 ±1
Cysteine	10 ± 1	9 ± 1	7 ± 1	7 ± 1	ND	ND	ND
Glutamic acid	42± 1	40 ±2	38 ±1	37 ±1	35± 2	35 ±1	31 ±1
Glycine	20 ± 1	19 ±1	17 ±1	16 ±1	15 ±1	13 ± 1	11 ±1
Histidine	11 ± 1	10 ±1	8 ± 2	7 ± 1	ND	ND	ND
Leucine	30 ± 2	31 ±1	31 ±2	29 ±1	28 ±1	26 ± 2	25 ±1
Lysine	19 ± 2	17 ±1	15 ±1	15 ±1	14 ±1	12 ± 1	11 ±1
Phenylalanine	10 ± 1	9 ± 1	8 ± 1	6 ± 2	ND	ND	ND
Proline	20 ± 1	21 ±1	22 ±1	22 ±1	20 ±2	18 ± 2	17 ±1
Threonine	18 ± 2	17 ±1	17 ±1	15 ±1	14 ±1	12 ± 1	11 ±1
Tyrosine	9 ± 1	8 ± 1	8 ± 1	6 ± 1	ND	ND	ND
Valine	24 ± 1	23 ±1	23 ±1	21 ±2	20 ±1	18 ± 1	16 ±1
Total	**288**	**265**	**260**	**243**	**203**	**188**	**177**

Not Deteceted

amount of arginine, cysteine, glycine, histidine, leucine, lysine, phenylalanine, proline, threonine and tyrosine than the partly ripened control seeds. Of these, the cysteine and tyrosine content was reduced to half. Invariably all amino acids were found to decrease during desiccation of ripened seeds. Among all amino acids, glutamic acid was found maximum in control seeds and a gradual reduction was noticed during desiccation. The only amino acid content slightly increased during desiccation was proline that increased slightly during 15 days of desiccation and thereafter a decline was occurred. Tyrosine, histidine and cysteine were not detected in ripened seeds after 15 days of desiccation.

2.6. Distribution of phenolic content

Phenolic content in mango seeds during the desiccation was given Table 17 (Fig. 11). Young seeds showed a regular increase in phenolic content during desiccation up to 30 days. The phenolic content ranges from 15% and 21% in control and 30 days desiccated seeds respectively.

The mature seeds contained maximum amount (23%) of phenolic content when compared to seeds of other developmental stages. A further and gradual increase was noticed during desiccation. Seeds desiccated for 30 days registered maximum phenolic content i.e., 34%.

The partly ripened and ripened seeds consisted of almost the same quantity of phenolic content. More or less same pattern of phenolic content distribution was observed throughout the period of desiccation.

3. Histochemical studies of desiccated seeds.

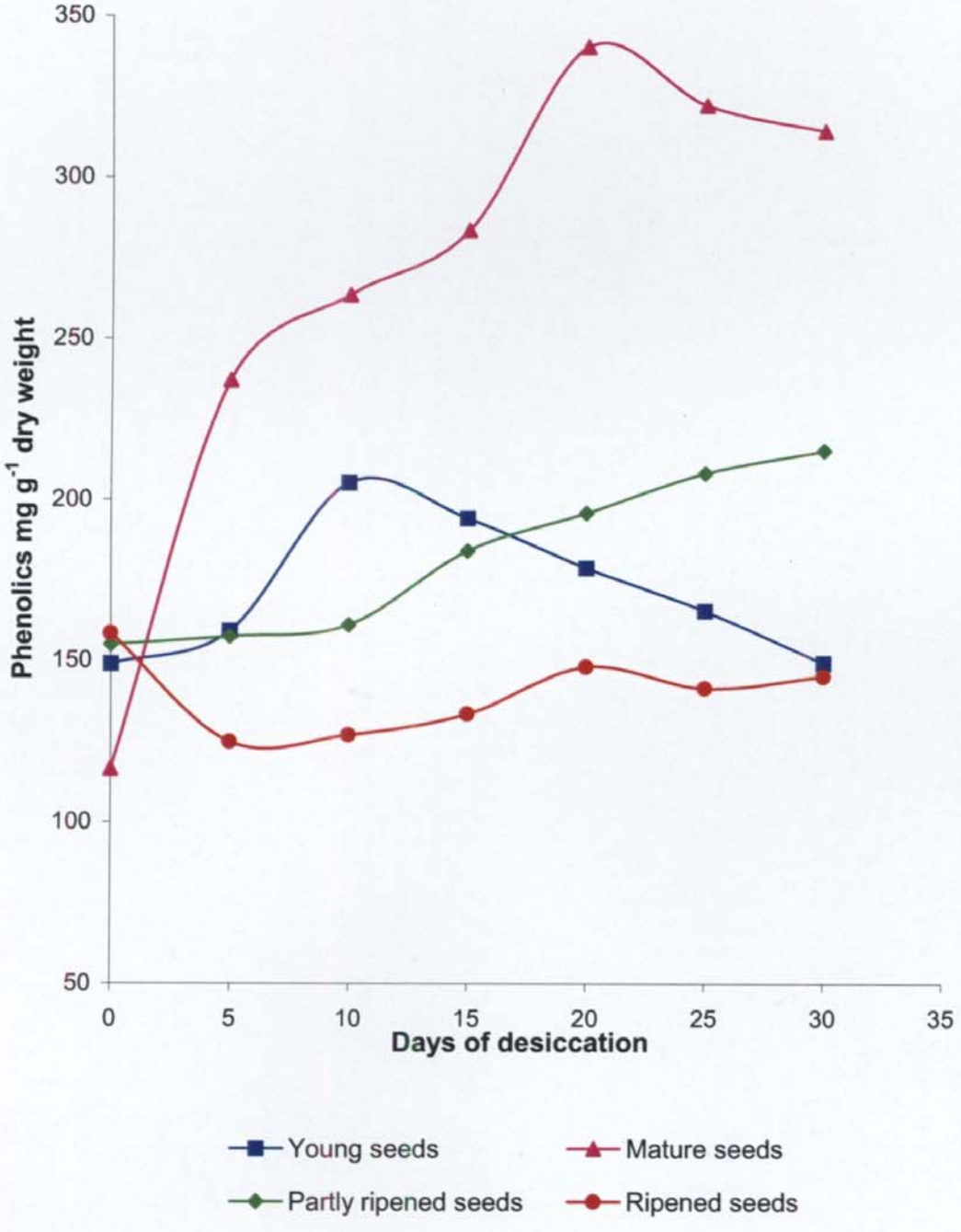
3.1. Localization of starch

In the cross section of cotyledons of young control seeds, the epidermal cells were small without any starch grains (Plate 1, Fig. A). Cortical cells were of different sizes containing 2 to 6 starch grains per cell, which were small in

Table: 17 Effect of desiccation on phenolic content in *Mangifera indica* seeds (mg g^{-1} dry weight)

Samples (Seed)	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Phenolics in mg g^{-1} dry weight						
Young	149.02 ± 5.12	159.27 ± 5.23	165.50 ± 7.18	178.78 ± 4.18	194.18 ± 5.23	204.11 ± 7.31	214.29 ± 8.18
Mature	226.50 ± 7.22	237.06 ± 5.12	263.45 ± 7.31	283.45 ± 8.32	320.45 ± 5.41	332.28 ± 5.08	344.34 ± 6.31
Partly Ripened	155.00 ± 7.22	157.51 ± 5.11	161.03 ± 5.14	184.09 ± 4.08	195.84 ± 7.31	208.18 ± 4.00	215.17 ± 3.16
Ripened	158.45 ± 2.44	164.86 ± 3.21	177.00 ± 3.11	188.50 ± 4.08	198.17 ± 4.66	207.50 ± 2.61	215.17 ± 4376

Figure 11: Effect of desiccation on phenolic content in *Mangifera indica* seeds (mg g^{-1} dry weight)



size. The starch grains were unevenly distributed towards the peripheral region of the cell. The shapes of grains vary from round, oval and /or elliptical. The procambial cells, which are small in size and were devoid of starch grains.

The epidermal cells of cotyledons of young seeds desiccated for five days were larger in size than control seeds, and starch grains were absent as in control seeds (Plate:1, Fig. B). There was an increase in number of starch grains in the cortical cells that ranges from 3 to 8 per cell. The size of the starch grains was also increased as compared to that of the control seeds. Some of the grains were found adhered to the cell wall. The shape of starch grains was similar to that of control seeds. No starch grains were observed in procambial cells.

The size of the epidermal cells of cotyledon of young seeds desiccated for 10 days was similar to that of 5 days desiccated seeds and a few grains were observed in the epidermis (Plate 1, Fig. C). Starch grains were found distributed in the cells of cortical region. The number and size of the grains in cortical cells were increased, as compared to the previous two stages. Each cell contained 6 to 14 small grains having the same shape as in the control and 5 days desiccated seeds. In the procambial cells starch grains were not seen.

The epidermal cells of cotyledons of 15 days desiccated seeds showed no starch grains (Plate 1, Fig. D). A brown stained substance was seen in epidermal and cortical cells. More number of grains was observed in the cortical cells than in the epidermal cells. Almost all the cells of the cortex were filled with starch grains. The size and shape of grains varied as observed in 10 days desiccated seeds. The number of grains in the cortical cells ranges from 8 to 14 and there was an increase in size than the previous stages. Procambial strands seen without any starch grain.

After 20 days of desiccation the epidermal cells of the seeds were devoid of starch grains, and composed of deeply stained substances (Plate 1, Fig. E). The cells in the cortical region were also completely filled with starch grains and exhibited variation in size and shape. The cell wall breakage was observed in some cells. The number of grains ranges from 6 – 14 per cell and size was increased than that of previous stages. Starch grains were absent in the procambial strands with elongated cells.

The epidermal cells of cotyledons of 25 days desiccated seeds possesed no starch grains (Plate 1, Fig. F). The cortical cells contained starch grains which were unevenly distributed and the size was found increased compared to the previous stages of desiccation and control. The cells in the cortical region were not intact and the grains were found intermingled. The number of grains in each cell ranges from 8 – 14 per cell and was small round to large oval shaped. The procambial cells, which are seen as elongated cells and devoid of starch grains.

The cotyledons of seeds desiccated for 30 days showed epidermal cells with large grains as compared to other stages of desiccation and control (Plate 1, Fig. G). The walls of cortical cells were not intact. The grains in the cells were found mixed together. The size of the grains was larger as compared to previous stages and control and shape varied from round, oval and/or elliptical. The number of grains ranges from 10 - 14 per cell. The procambial strands devoid of starch grains.

The epidermal cells of mature control seeds were small in size and consisted of no starch grains (Plate 2, Fig. A). The cortical cells were intact and the distribution of grains was uneven. Most of the grains were attached towards the peripheral region of the cell. The number of grains in each cell varied considerably and ranges from 2 to 8 per cell. The grains were small in size and

oval to elliptical in shape. Procambial cells were small and thin walled which were devoid of starch grains.

In cotyledons of seeds desiccated for 5 days, the epidermal cells were small in size with no starch grains (Plate 2, Fig. B). The cells in the cortical region were intact and starch grains were distributed unevenly. The number of grains ranges form 4 – 10 per cell and were larger in size compared to control seeds. The size and shape of grains showed considerable variation and most of them were elliptical and few of the grains were oval and round. Some grains were found distributed towards the peripheral region of the cells. Starch grains were absent in procambial cells which were smaller in size.

The epidermal cells of cotyledons of 10 days desiccated seeds were small in size and devoid of starch grains (Plate 2, Fig. C). In the cells of the cortex, starch grains were distributed unevenly, the size of the cells was increased as compared to control. The size of grains varied considerably, the number of grains per cell ranges from 4 - 12 and shape range from oval, round and/or elliptical. Procambial strands were seen as patches consisting of thin walled elongated cells with no starch grains.

No starch grains were observed in the epidermal cells of cotyledons of seeds desiccated for 15 days (Plate 2, Fig. D). The cortical cells remained intact and the distribution of starch grains was uneven. The number of grains ranges from 6 – 12 per cell, the size was found increased than the control seeds and was round, oval and elliptical in shape. Procambial cells were elongated and thin walled and starch grains were absent.

The epidermal and cortical cells of cotyledons of 20 days desiccated seeds were completely filled with starch grains (Plate 2, Fig. E). The wall of some of the cells of the cortex found degenerated and the grains were mixed together. The sizes of the grains were increased as compared to that of previous

stages. The number of grains ranges from 9 – 13 per cell. The shape of the grains was similar to that of previous stages. Starch grains were not present in procambial strands.

In the cotyledons of seeds desiccated for 25 days, the epidermal cells were found filled with starch grains (Plate 2, Fig. F). The grains were also distributed throughout the cortical cell and varied in size and shape. The wall of cortical cells was found ruptured and grains were mixed. The number of starch grains ranged from 9 – 14 per cell, size was larger and shape ranges from round to elliptical. The starch grains were absent in the procmabial cell.

The cells of the epidermis and cortex of cotyledon of seeds desiccated for 30 days were completely filled with starch grains (Plate 2, Fig. G). The size of the grains was found maximum at this stage of desiccation. The cortical cells were not intact and most of the grains were found mixed. The grains were almost oval to elliptical in shape and the number ranges from 9 - 14 per cell. No starch grains were observed in the procambial strands.

The cotyledons of partly ripened control seed showed the same structural features as that of young and mature seeds (Plate 3, Fig. A). The epidermal cells were devoid of starch grains. The cortical cells were intact and consisted of starch grains ranged from 4 – 8 per cell. The grains were unevenly distributed towards the peripheral region of the cell. The grains were of differ in shape as in the previous stages. Procambial strands with small thin walled cells, and starch grains were absent.

Similar structure as in the case of control seeds was observed in the cotyledons of 5 days desiccated seeds (Plate 3, Fig. B). Cortical cells were intact and contained unevenly distributed starch grains towards peripheral region of the cell as in the control seeds. The number of grains ranges from 8 - 12 per cell and showed variation in size. The shape of grains showed

variation from that of control seeds. No starch grains observed in thin walled elongated cells of procambial strands.

The epidermis of cotyledons of 10 days desiccated seeds contained starch grains (Plate 3, Fig. C). The cortical cells were intact and contained starch grains. The number of grains ranged from 8 – 14 per cell and the shape was identical to that observed in seeds desiccated for 5 days. The size of the grains was found increased. Starch grains were absent in procambial cells.

The epidermis of cotyledons of the seeds desiccated for 15 days exhibited starch grains (Plate 3, Fig. D). The cortical cells were intact and fully filled with starch grains. The sizes of the grains were increased compared to seeds desiccated for 10 days and control. The shape of grains varied considerably and was similar to that of 10 days desiccated seeds and control. The number of grains in each cell ranges from 8 – 14. Starch grains were not observed in the cells of procambial strands.

Structural changes were not observed in the cotyledons of seeds desiccated for 20 days, starch grains were observed in the epidermal cells (Plate 3, Fig. E). The wall of cortical cells was found broken at certain regions and the grains were mixed up. The size and shape of grains were varied considerably. The sizes of the grains were found increased compared to that of 15 days desiccated seeds. The shape of the grains was identical to that observed in the previous stages. The number of grains ranged from 10 – 16 per cell. Procambial strands were observed with elongated cells and without starch grains.

The cells of epidermis and cortex of cotyledons of 25 days desiccated seeds consisted of starch grains (Plate 3, Fig. F). The size of the grains in the epidermal cells and cortical cells were larger as compared to seeds desiccated for 20 days and control. The cells in the cortical region were not intact and the

grains were intermingled. The sizes of grains were found increased as compared to other stages of desiccation and control. Shape of the grains was similar to all the other stages and the number per cell ranged from 10 – 16. No starch grains were observed in the cells of procambial strands.

As in the previous stage of desiccation, the epidermal cells of cotyledons of 30 days desiccated seeds also contained of well defined starch grains (Plate 3, Fig. G). The cortical cells were ruptured and the grains were distributed throughout the cortical region. The sizes of the grains were larger than that of 25 days desiccated seeds and control. The shape was similar to that observed in previous stages and the number ranges from 10 – 16 per cell. The procambial cells were elongated and devoid of starch grains.

The epidermal cells of cotyledons of ripened control seeds contained starch grains (Plate 4, Fig. A). The cortical cells were intact and starch grains were distributed unevenly towards the peripheral portion of the cells. The grains were small in size and varied in shape from round to oval. The number of grains ranged from 4 to 9 per cell. Procambial cells were thin walled and without starch grains.

In the cotyledon of seeds desiccated for 5 days, the epidermal cells were small and without starch grains (Plate 4, Fig. B). The cortical cells were intact and starch grains were not uniformly distributed. The size and shape of grains varied considerably and the number of grains in each cell ranges from 6 – 10. Starch grains were absent in procambial cells.

The epidermis of cotyledon of 10 days desiccated seeds showed no starch grains (Plate 4, Fig. C). The cortex comprised of starch grains and the size and shape varied considerably. The size were found increased as compared to that of 5 days desiccated and control seeds. The shape of grains was round, oval and elliptical. The number of grains in cortical cells ranged from 8 – 12

per cell. Procambial cells were seen as elongated cells and devoid of starch grains.

The starch grains were absent in the epidermal cells of cotyledons of seeds desiccated for 15 days (Plate 4, Fig. D). The cortical cells were intact and were completely filled with starch grains. The grain size was increased as compared to that of 10 days desiccated and control seeds. The shape of grains varied considerably as in the former cases. The number of grains ranges from 12 to 18 per cortical cell. The procambial cells were elongated and starch grains were absent.

The epidermis of cotyledons of seeds desiccated for 20 days contained starch grains (Plate 4, Fig. E). The cortical cells were not intact, the grains were mixed up and varied in size and shape. The number of grains in the cortical cell ranges from 12 – 20 per cell and was round, oval, and elliptical in shape. Procambial strands consisted of thin walled cells and starch grains were absent.

In the cotyledon of seeds desiccated for 25 days, the epidemical cells were filled with starch grains (Plate 4, Fig. F). The cortical cells were not intact and the grains were mixed together. The size of the grains was increased than that of 20 days desiccated and control seeds and shape was round, oval, and/or elliptical. The number of grains per cortical cell ranges from 12 - 20. Starch grains were absent in the thin walled procambial strands.

The epidermal cells of cotyledon of 30 days desiccated seeds showed starch grains (Plate 4, Fig. G). The cells in the cortical region were not intact. The starch grains in each cell in the cortex were fully mixed up. The size of the grains was almost same in the 25 days desiccated seeds. The shape varied from round, oval and/or elliptical. The number of grains ranged from 12–28 per cortical cell. No starch grains were observed in the cells of procambial strands.

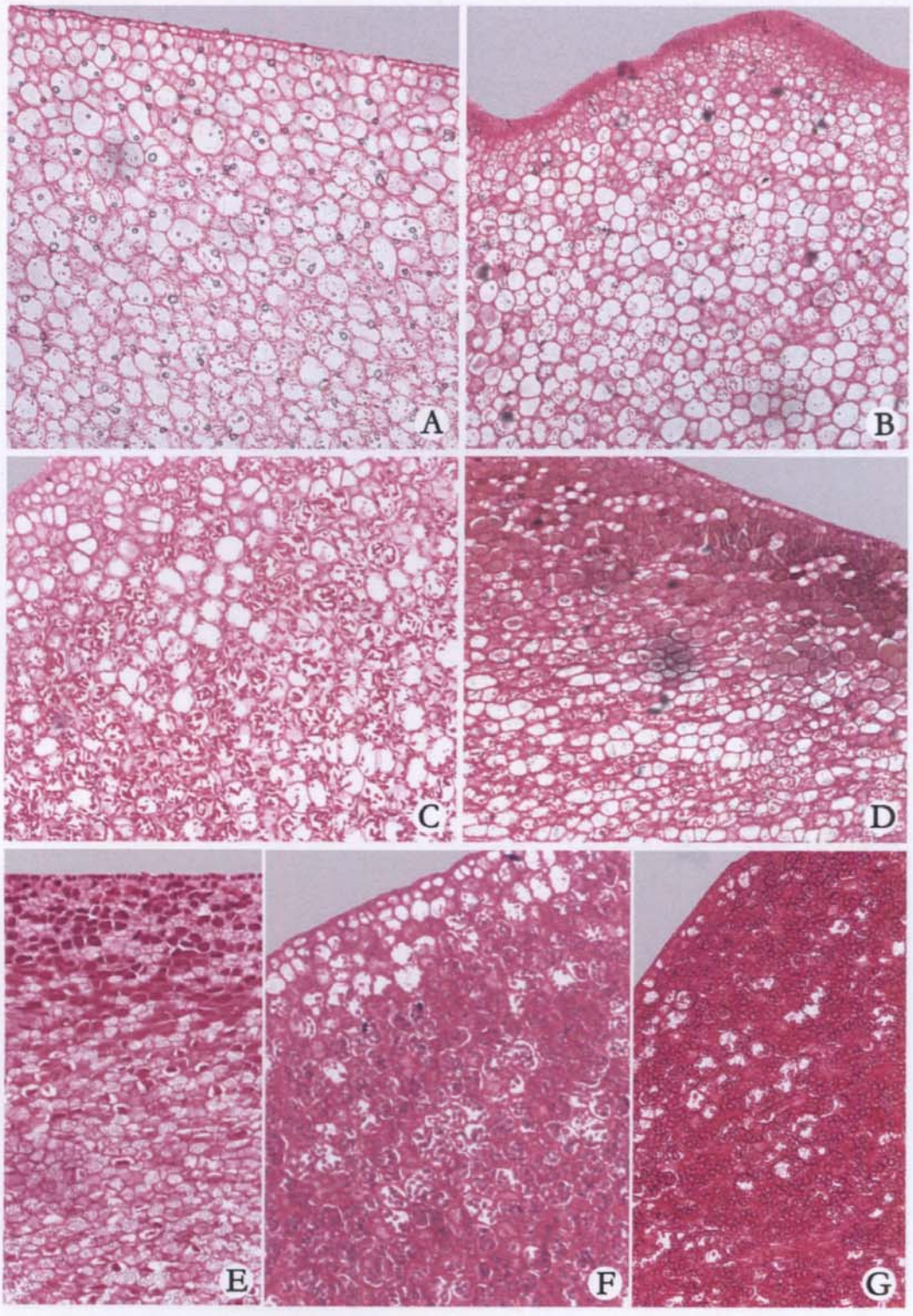


Plate 1 : Effect of desiccation on localization of starch in young Mango (*Mangifera indica*) seeds.
Fig. A: Control seeds; **B, C, D, E, F & G**: Seeds desiccated for 5, 10, 15, 20, 25, & 30 days respectively.

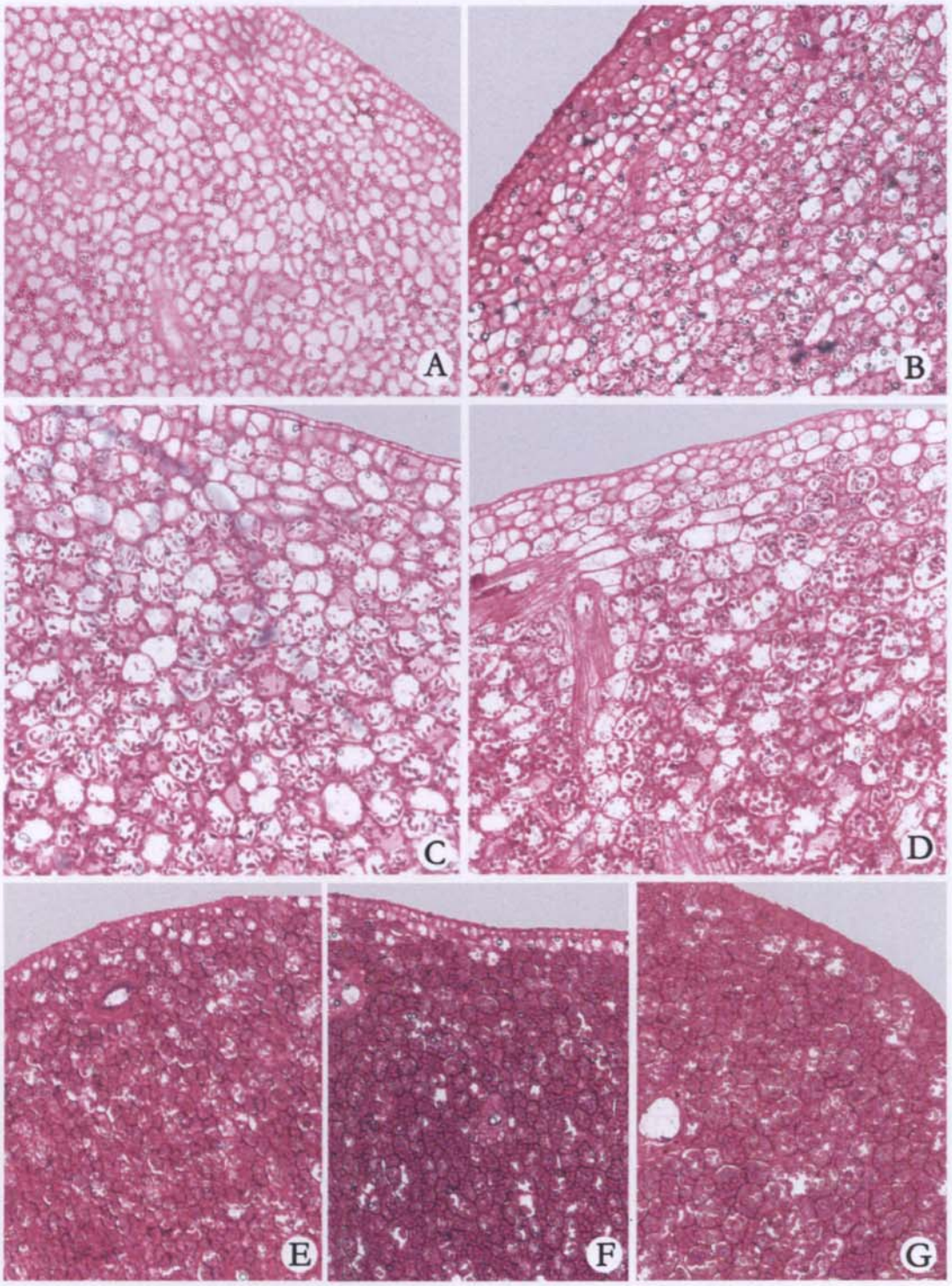


Plate 2 : Effect of desiccation on localization of starch in mature Mango (*Mangifera indica*) seeds.
Fig. **A**: Control Seeds; **B**, **C**, **D**, **E**, **F** & **G** : Seeds desiccated for 5, 10, 15, 20, 25, 30 days respectively.

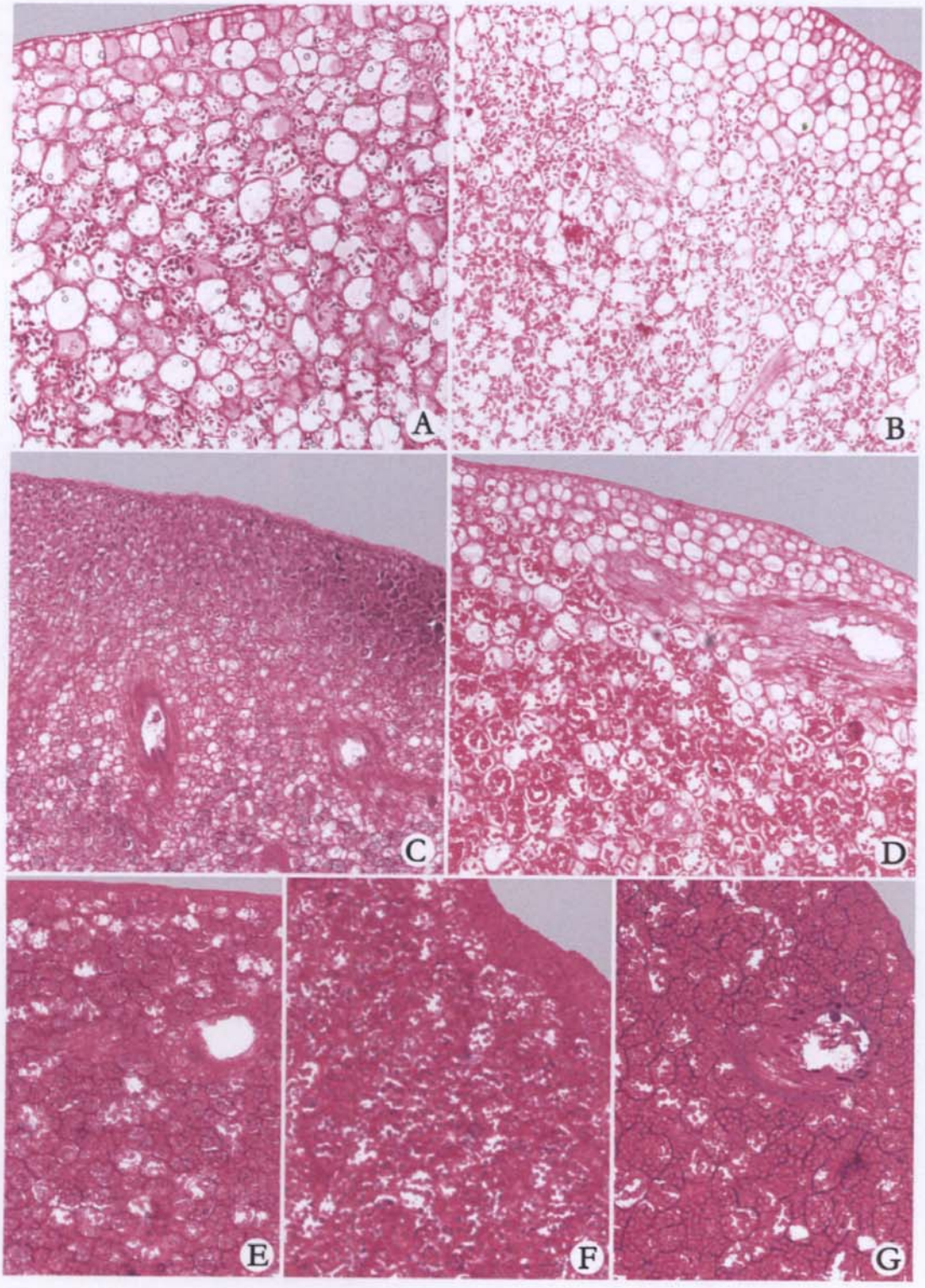


Plate 3 : Effect of desiccation on localization of starch in partly ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

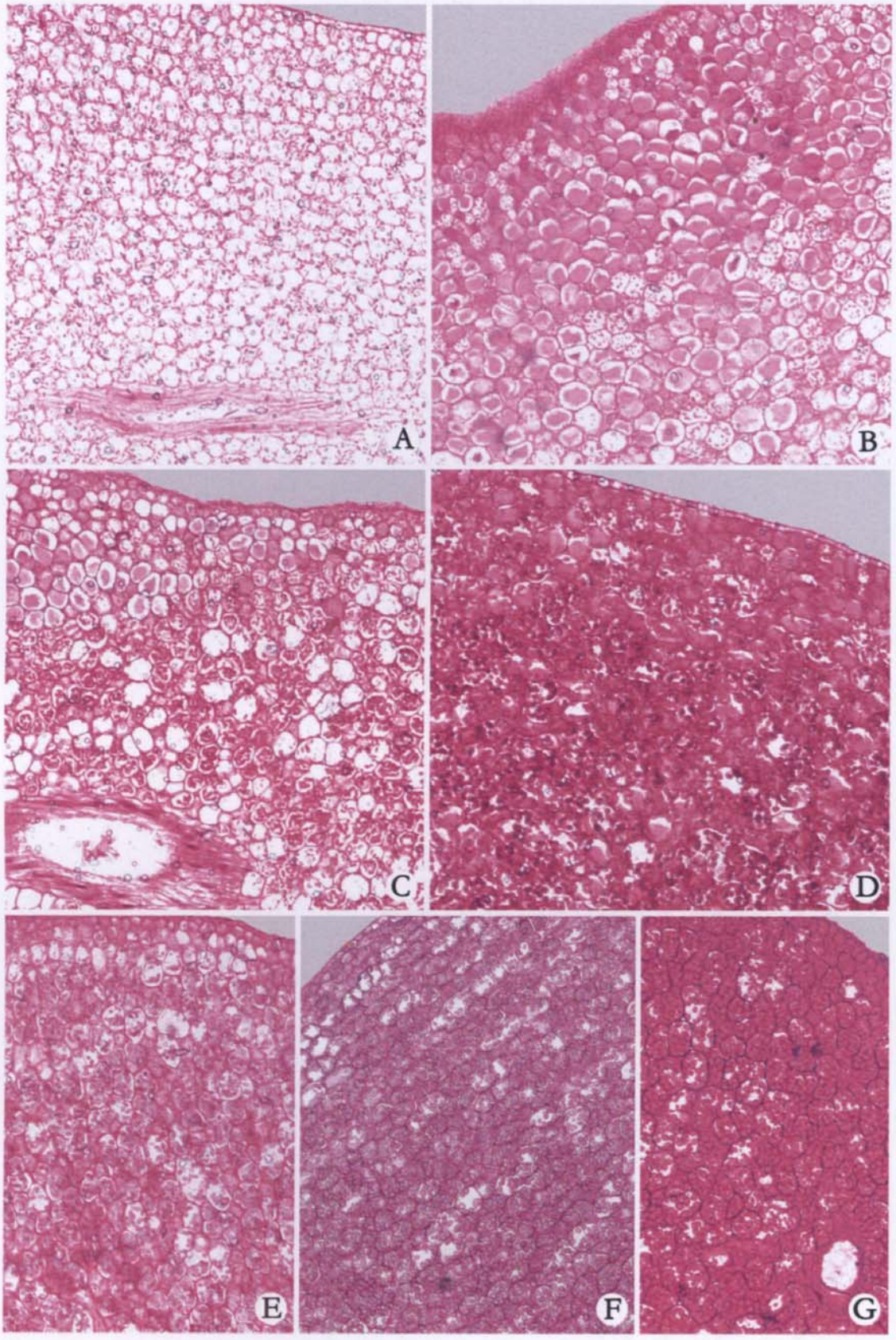


Plate 4 : Effect of desiccation on localization of starch in ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

When a comparison is made between control and desiccated seeds cell wall thickening and staining intensity for starch was more in desiccated seeds than compared to the control (Plate 1-7).

3.2. Localization of protein

The cross section of cotyledon of young control seeds showed feebly stained blue coloured patches in all the cells (Plate 5, Fig. A). The epidermal cells were small and deeply stained than the cortical cells. The staining intensity of cortical cells varied considerably from cell to cell. The procambial strands were seen as elongated cells with feebly stained regions.

The staining intensity of 5 days desiccated seeds was deeper than control seeds (Plate 5, Fig. B). The epidermal cells were small in size and deeply stained than control seeds. Below the epidermis one layer of cells was also found deeply stained than cortical cells. The cortical cells were feebly stained and the staining intensity varied considerably in individual cells. The stain was concentrated around the starch grains. The procambial strands consist of blue stained cells.

The epidermal cells of 10 days desiccated seeds showed greater staining intensity than that of the control (Plate 5, Fig. C). The staining intensity of the cortical cells varied considerably, which was more than that of the previous stages and only the inner zone of cells was blue in colour. The cell wall was not seen clearly. Blue stained elongated cells were seen as procambial strands.

The seeds desiccated for 15 days showed slight increase in staining intensity for proteins than the 10 days desiccated seeds (Plate 5, Fig. D). The staining intensity of the epidermal and cortical cells was almost similar. The protein mass were unevenly distributed in the cortex and a few cells contained more amount of protein than other cells. The staining intensity of procambial strands was deeper than the previous stages and other tissues in the section.

The epidermis of 20 days desiccated seeds consisted of protein masses which were more deeply stained than the seeds desiccated for 15 days and control (Plate 5, Fig. E). The protein masses in the cortical cells were unevenly distributed, and the staining intensity was more than that of the previous stages. The procambial strands were also composed of protein masses which were deeply stained than that of the previous stages of desiccation.

The seeds desiccated for 25 days showed protein masses in the epidermis and cortex (Plate 5, Fig. F). The epidermal cells were deeply stained than that of the previous stages. The protein masses in cortical cells were unevenly distributed, some of the cells composed of very less protein masses than the remaining cells. Majority of cells were almost completely filled with protein masses. The staining intensity for protein in procambial cells was found lesser than that of the cortical cells; but greater than that observed in the previous stages.

The staining intensity for protein content in seeds desiccated for 30 days was higher as compared to that of the previous stages of desiccation and control (Plate 5, Fig. G). The epidermal cells were found completely filled with protein masses. The cortical cells were also composed of maximum amount of protein masses than other stages of desiccation but were lesser than that observed in the epidermis. A few of the cortical cells exhibited very less amount of protein. The staining intensity of procambial strands showed a slight increase than the cortical cells and of the previous stages.

Mature control seeds showed protein masses in both epidermis and cortex (Plate 6, Fig. A). Almost same intensity for protein staining was observed in the epidermal and cortical cells and the staining intensity was not uniform. Procambial strands were found as blue stained cells which were darkly stained than cortical cells.

After 5 days of desiccation the seeds showed high staining intensity for proteins than control seeds (Plate 6, Fig. B). The epidermal cells were evenly stained with protein. The cortical cells consisted of protein masses with high staining intensity and which was unevenly distributed throughout the section. The procambial strands were deeply stained than cortex.

The epidermal cells and cortical cells of 10 days desiccated seeds exhibited deeper staining intensity compared to that of previous stages of desiccation and control (Plate 6, Fig. C). The protein mass in the cortical cells was almost evenly distributed. The staining intensity of procambial strands was more as compared to the previous stages.

The seeds desiccated for 15 days showed a slight increase in protein content as compared to the previous stages (Plate 6, Fig. D). The epidermal cells were stained almost similar to that of cortical cells. The protein mass was observed in all cells of cortex but the staining intensity varied considerably and was greater than the previous stages and control. The procambial strands consisted of elongated cells showing feebly stained protein mass.

The protein content was found in the epidermal cells of seeds desiccated for 20 days (Plate 6, Fig. E). In cortex the protein masses were distributed in all the cells and a slight increase was noted as compared to the previous stages of desiccation and control. The procambial strands also showed the presence of proteins.

The seeds desiccated for 25 days showed much increase in the protein mass compared to that of the previous stages (Plate 6, Fig. F). The epidermis was found deeply stained for proteins and was evenly distributed. Cortical cells composed of protein and were more than that of 20 days desiccated seeds and control. The procambial strands consisted of elongated cells and were deeply stained for proteins.

The protein content in the cotyledon of seeds desiccated for 30 days was observed maximum at this stage than the previous stages of desiccation and control (Plate 6, Fig. G). The epidermal cells were completely filled with protein mass. The cortical cells were also found deeply stained for proteins and the staining intensity was more than that of the previous stages. Deeply stained protein mass was also seen in the elongated procambial strands.

The cotyledon of partly ripened control seeds showed the presence of proteins in the tissues (Plate7, Fig. A). The epidermal cells were feebly stained for proteins. The staining intensity of cortical cells varied considerably and protein masses were found in a few cells, but the staining intensity was feeble. The procambial strands with elongated cells composed of blue stand protein masses.

The seeds desiccated for 5 days showed a slight increase in the staining intensity, compared to the control seeds (Plate 7, Fig. B). The epidermis was found deeply stained than that of the control seeds. The protein mass in the cortical cells was observed unevenly distributed. The procambial strands also showed the presence of high protein content than control seeds.

The protein content in the epidermis of seeds desiccated for 10 days showed slight increase than that of previous stages (Plate 7, Fig. C). In cortical cells the distribution of protein was not uniform and the protein masses were located at the peripheral region of the cell and the intensity of stain was greater than that of the seeds desiccated for 5 days. The procambial strands were also filled with protein masses.

An increase in the protein content was observed in the cotyledon of 15 days desiccated seeds as compared to that of the previous stages of desiccation and control (Plate 7, Fig. D). The epidermal cells were uniformly stained for proteins which were more than that of the previous stages. The distribution of

protein in the cortical cells was restricted to a few cells but the staining intensity was more than that of previous stages. The procambial strands also showed an increase in protein mass.

The seeds desiccated for 20 days showed an increase in the protein mass than the previous stage (Plate 7, Fig. E). The epidermal and cortical cells exhibited an increased straining intensity than the previous stages of desiccation and control. The cortical cells were found completely occupied with the protein mass. The procambial strands were observed with elongated cells with fully stained protein masses which were more than that of the previous stages and control.

The epidermal cells of seeds desiccated for 25 days showed slight increase in staining intensity than the previous stage of desiccation (Plate 7, Fig. F). The cortical cells were deeply stained for proteins. The procambial strands were also stained deeply for proteins.

The protein content, in the cotyledon of seeds desiccated for 30 days was found greater than that of the previous stages of desiccation and control (Plate 7, Fig. G). The epidermis and cortex were found with deeply stained proteins. The protein masses were found completely filled in the cortical cells and were evenly distributed than all other stages of desiccation and control. Procambial strands were also found deeply stained for proteins and filled with the protein masses.

The lightly stained protein masses were observed in the cotyledon of ripened control seeds (Plate 8, Fig. A). The epidermal cells and cortical cells were found feebly stained for proteins. In the epidermal cells the protein masses distributed almost evenly and in the cortical cells the distribution was unevenly. The procambial strands were almost uniformly stained with protein than that of cortical cells.

The cotyledon of seeds desiccated for 5 days showed a slight increase in the intensity of staining for proteins (Plate 8, Fig. B). The epidermal cells consisted of protein masses and were distributed evenly. The protein mass was evenly distributed in the cells of cortex also. The staining intensity for proteins of the cells of procambial strands was similar to that of the cortical cells.

The epidermal and cortical cells of 10 days desiccated seeds showed a slight increase in protein content than that of the 5 days desiccated and control seeds (Plate 8, Fig. C). The protein masses were found uniformly distributed in the entire cortical cell. The elongated cells of procambial strands were observed with more deeply stained masses as compared to that of 5 days desiccated seeds.

After 15 days desiccation the seeds exhibited an increase in protein content in the epidermis and cortex than the previous two stages of desiccation (Plate 8, Fig. D). The cortical cells were found completely filled with protein masses. The procambial strands were seen as elongated cells with deeply stained protein masses.

The epidermal and cortical cells of seeds desiccated for 20 days showed higher staining intensity for proteins as compared to that of the earlier stages of desiccation and control (Plate 8, Fig. E). The protein masses in the cortical cells were deeply stained. The elongated celled procambial strands were observed deeply stained for protein, which was more as compared to previous stages of desiccation.

The seeds desiccated for 25 days showed an increase in the protein content over the control and previous stages of desiccation (Plate 8, Fig. F). The epidermal and cortical cells were found filled with the protein masses. The protein mass in the cells were found evenly distributed throughout the section.

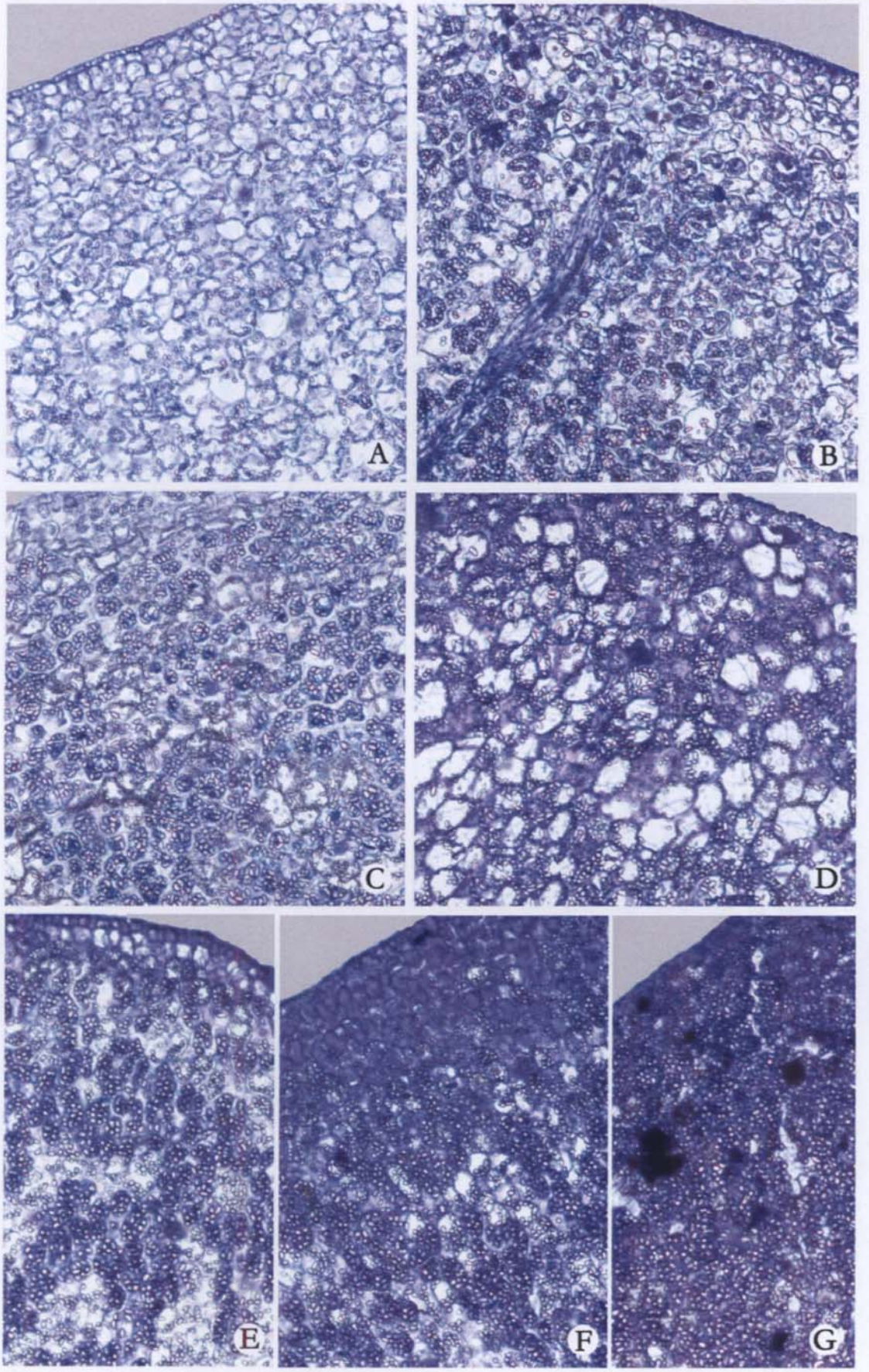


Plate 5 : Effect of desiccation on localization of protein in young Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

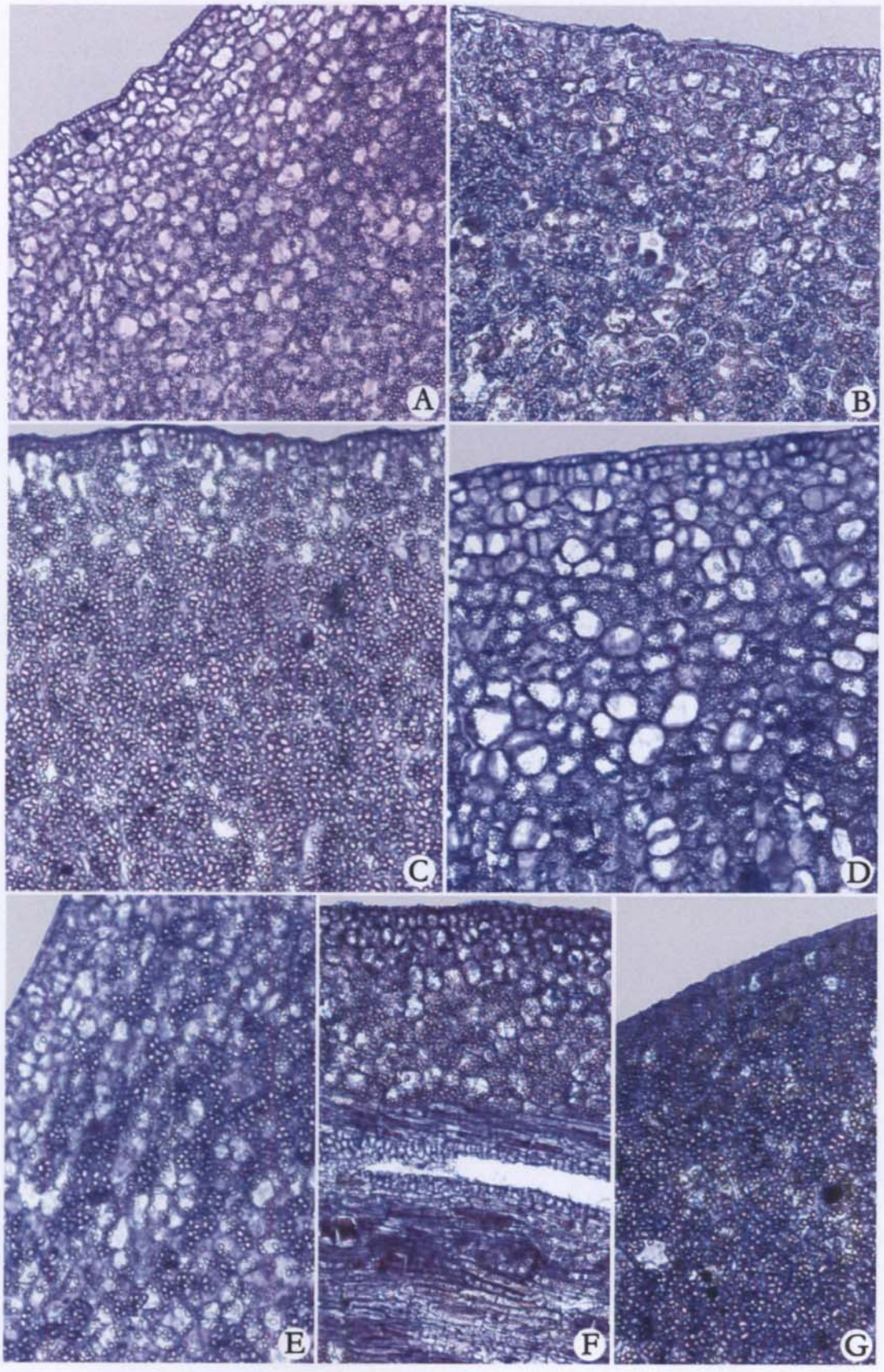


Plate 6 : Effect of desiccation on localization of protein in mature Mango (*Mangifera indica*) seeds.
Fig. A: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

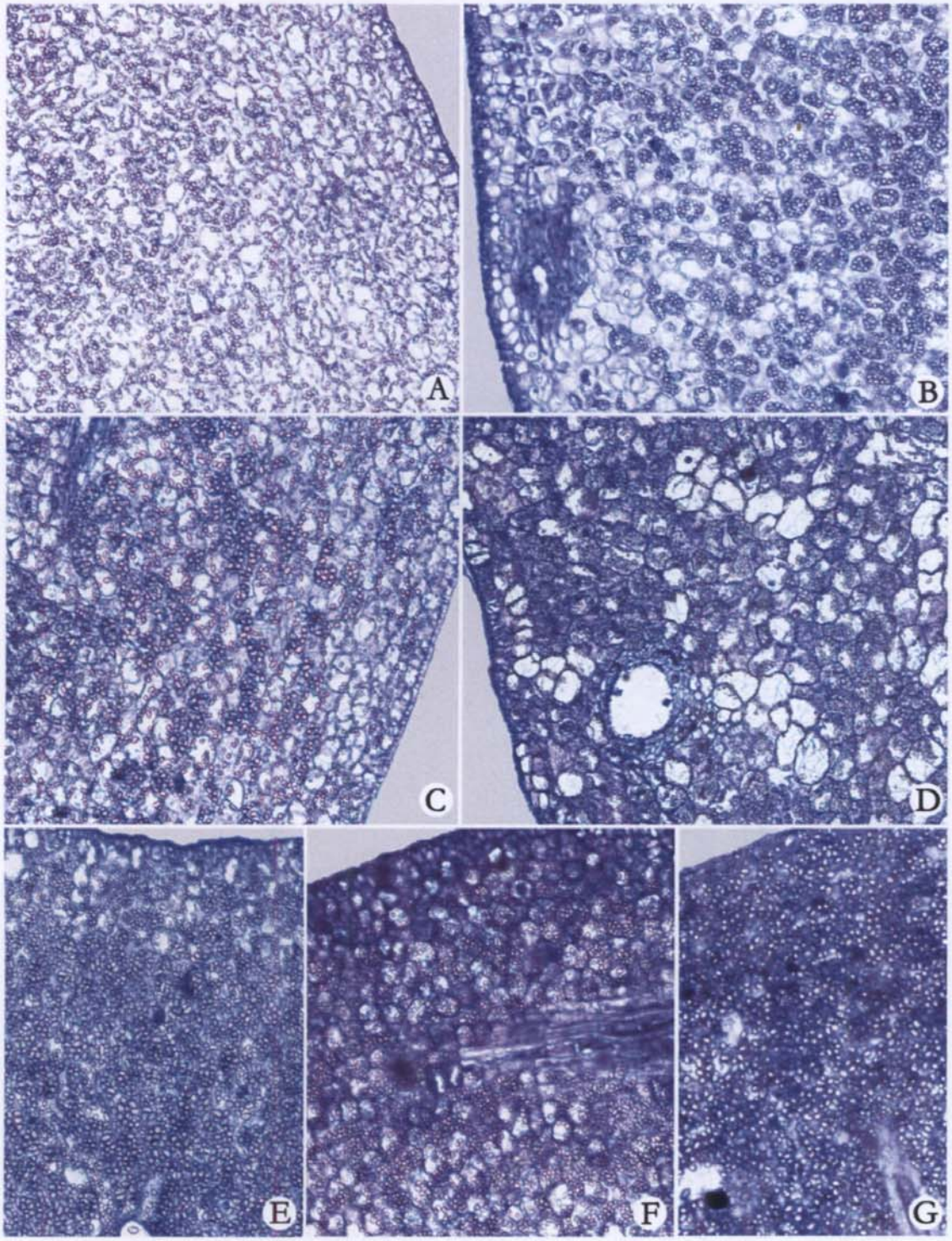


Plate 7 : Effect of desiccation on localization of protein in partly ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

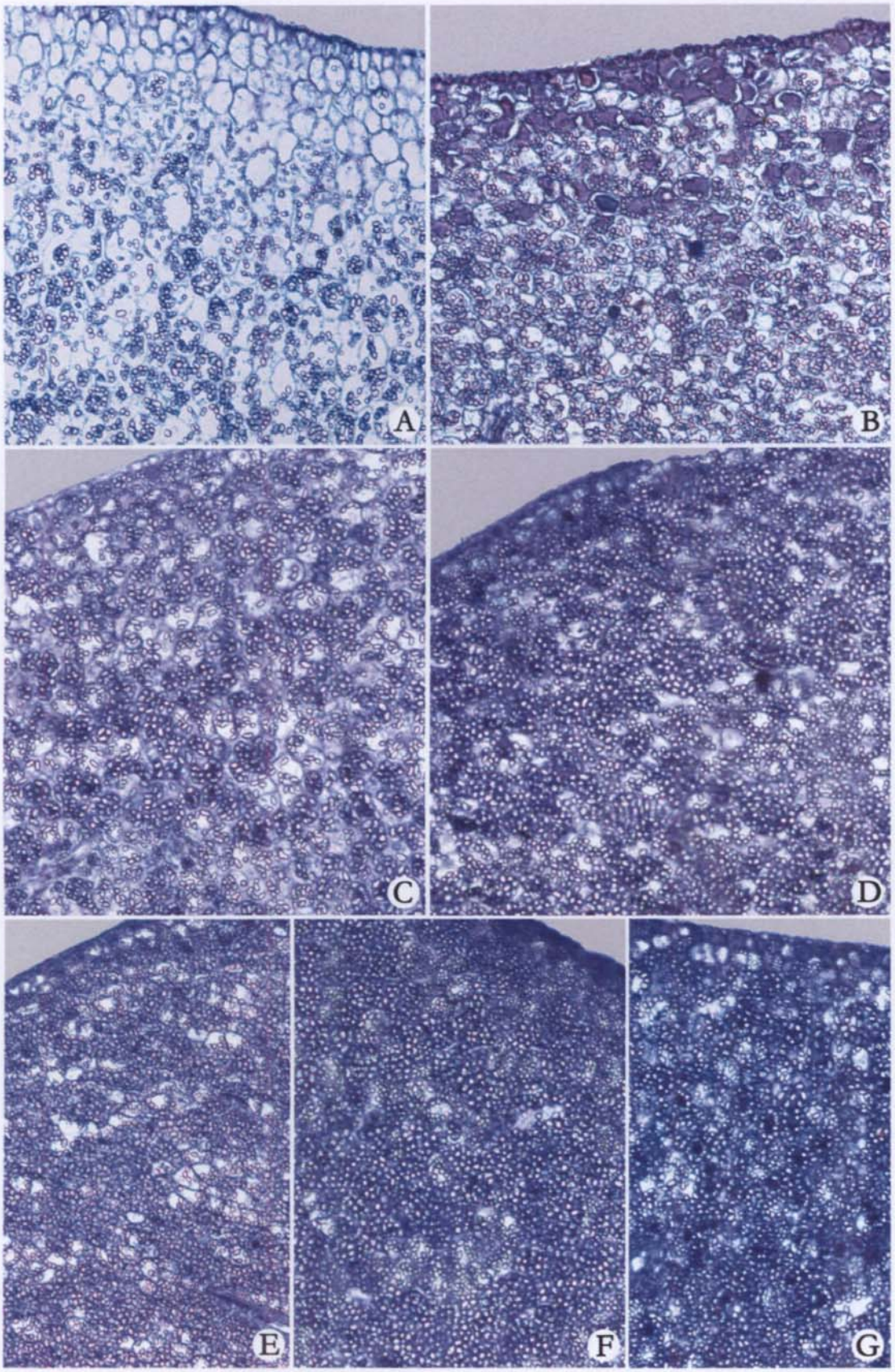


Plate 8 : Effect of desiccation on localization of protein in ripened Mango (*Mangifera indica*) seeds.
Fig. A: Control seeds; B, C, D, E, F & G : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

The procambial strands were observed with deeply stained masses and the staining intensity of which was greater than that of the previous stages.

The protein content in the epidermal and cortical cells of 30 days desiccated seeds was greater than that of the previous stages of desiccation and control (Plate 8, Fig. G). The epidermal and cortical cells were composed of deeply stained protein masses. In the cortical cells the protein masses were uniformly distributed throughout the section of the cotyledon. The elongated procambial strands were also composed deeply stained masses, the staining intensity of which was greater than that of the previous stages of desiccation and control.

4. Biochemical studies of germinated seeds

4.1. Distribution of starch content

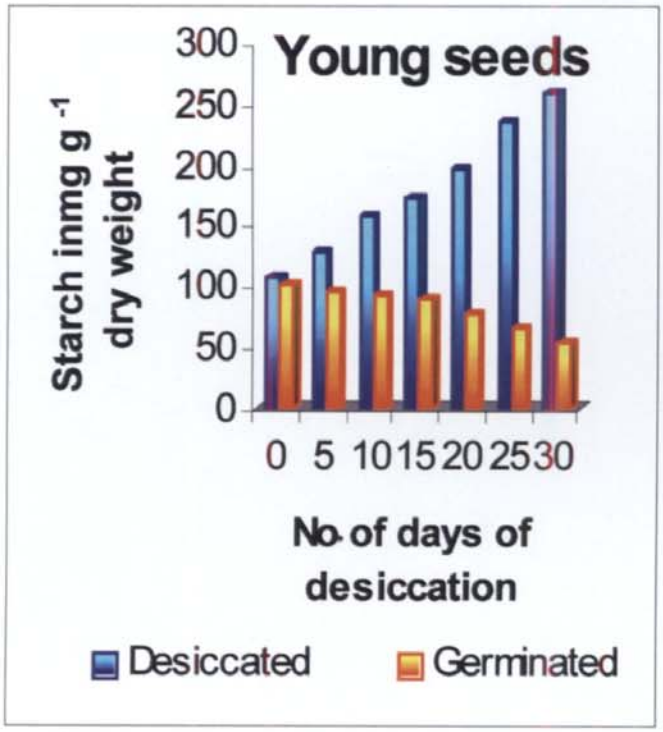
Changes in starch content of mango seeds during germination of desiccated seeds were given in Table 18 (Fig. 12). During germination, seeds of all treatments showed a regular decrease in starch content. Maximum reduction in starch content was observed in young and mature seeds of 30 days of desiccation. In these seeds the starch content was reduced to half when compared to that of control germinated seeds of same type. Partly ripened and ripened seeds showed similarity in the pattern of reduction of starch content during germination upto 30 days desiccated seeds on germination.

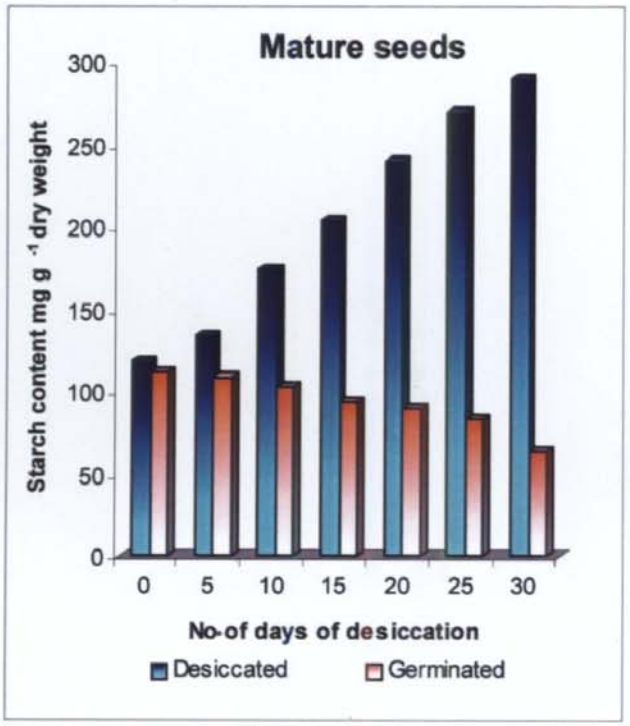
A comparison of starch content of seeds during desiccation and germination showed that the starch content of young seeds was increased 2.5 times compared to the control seeds during desiccation and during germination the starch content was reduced to half (Table 18, Fig. 12). Similar pattern of changes in starch content was observed in mature seeds also. In partly ripened seeds the starch content was found to increase 3 times than that of the control seeds during desiccation but during germination, the starch content was

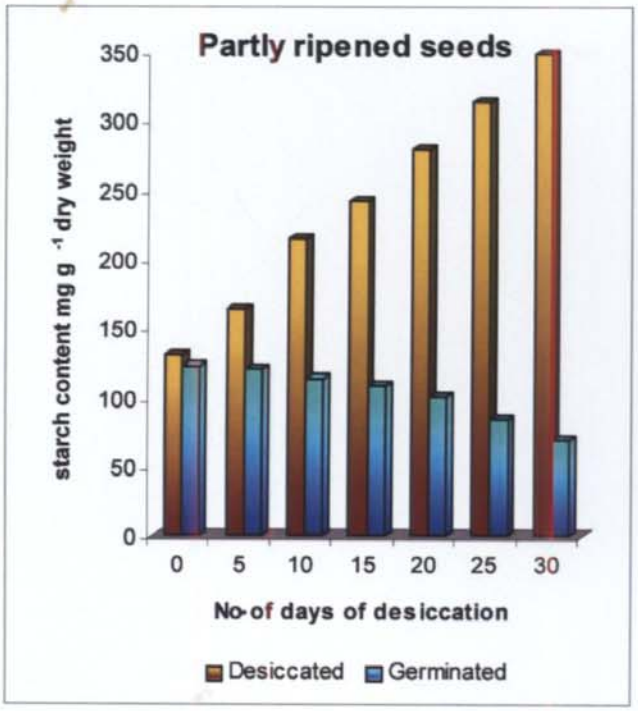
Table 18: Effect of desiccation on mobilization of starch content in *Mangifera indica* seeds during germination (mg g $^{-1}$ dry weight)

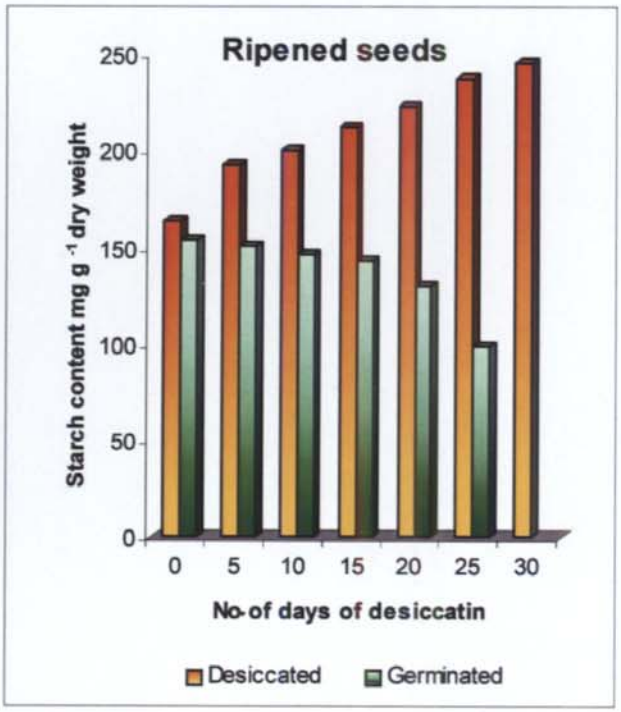
Samples (Seed)	Number of days of desiccation													
	0		5		10		15		20		25		30	
	Control	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated
	Starch in mg g $^{-1}$ dry weight													
Young	107.69 ± 6.47	100.45 ± 8.45	126.15 ± 7.25	95.42 ± 3.18	156.05 ± 8.47	92.35 ± 2.44	171.41 ± 8.36	87.52 ± 4.12	196.34 ± 7.87	77.71 ± 6.47	235.44 ± 6.58	64.14 ± 5.42	248.28 ± 5.48	34.17 ± 5.47
Mature	118.54 ± 6.58	112.12 ± 2.77	134.46 ± 8.56	109.13 ± 3.01	174.99 ± 7.58	103.45 ± 4.25	204.15 ± 7.78	94.17 ± 4.21	240.53 ± 6.36	90.17 ± 3.12	270.34 ± 8.25	84.15 ± 3.52	290.34 ± 8.45	64.18 ± 7.56
Partly ripened	130.90 ± 5.64	123.20 ± 2.45	164.51 ± 6.42	120.81 ± 2.44	215.38 ± 8.32	114.15 ± 4.36	242.31 ± 7.56	109.14 ± 4.21	280.21 ± 7.62	101.81 ± 4.74	314.15 ± 8.48	84.23 ± 6.455	350.00 ± 6.47	70.25 ± 7.45
Ripened	164.34 ± 6.23	154.16 ± 3.16	193.85 ± 5.25	150.81 ± 3.11	200.55 ± 4.58	147.17 ± 2.18	213.17 ± 5.45	143.14 ± 3.55	224.15 ± 4.35	130.13 ± 6.48	238.45 ± 5.57	99.15 ± 8.47	246.15 ± 4.41	–

Figure 12: Effect of desiccation on mobilization of starch in *Mangifera indica* seeds during germination (mg g^{-1} dry weight)









reduced to 65% of control seeds. Ripened seeds showed only negligible increase in starch content during 30 days of desiccation. These seeds showed a gradual decrease of starch content to 65% of the control seeds during germination.

4.2. Distribution of sugar content

Sugar content of germinated seeds of different treatments and desiccation of 30 days was showed (Table 19). Except ripened seeds, all other treatments contained the six sugars; fructose, glucose, maltose, rhamnose, stachyose and sucrose. The control and desiccated ripened seeds showed the presence of sucrose only. Maltose was absent in partly ripened seeds but it appeared during germination of control and desiccated seeds.

Young germinated seeds showed a gradual and significant reduction in glucose, maltose and fructose content during germination. The rhamnose content was reduced to half in seeds germinated after 10 days of desiccation. But during germination of 15 days and 20 days desiccated seeds the rhamnose content was increased significantly but again decreased in 25 days desiccated seeds ($p<0.01$). During germination of 30 days desiccated seeds, the maltose and rhamnose contents were not detected.

Fructose content of mature germinated seeds showed a rapid reduction in seeds germinated for 10 days desiccated seeds. Seeds germinated after 15 days of desiccation showed an increase in fructose content which decreased during further days of desiccation. Glucose and rhamnose contents in germinated mature seeds showed a gradual increase upto 10 days of desiccation. After 15 days, the glucose and rhamnose contents were reduced to half during germination of 30 days desiccated seeds. Maltose content showed a significant but gradual reduction during germination of mature seeds desiccated for 20 days ($p<0.02$). Stachyose was detected only in seeds germinated after 5

Table 19: Effect of desiccation on sugar content in *Mangifera indica* seeds (mg g^{-1} dry weight)

Samples (seeds)	Sugar	Number of days of desiccation						
		0 (control)	5	10	15	20	25	30
		Sugar content in mgg^{-1} dry weight						
Young	Fructose	4.29 ± 0.015 (5.44 ± 0.05)	4.63 ± 0.02 (5.26 ± 0.05)	4.76 ± 0.06 (4.38 ± 0.03)	4.88 ± 0.04 (3.51 ± 0.01)	3.89 ± 0.05 (2.43 ± 0.007)	3.32 ± 0.02 (1.53 ± 0.041)	3.25 ± 0.05 (1.176 ± 0.005)
	Glucose	2.14 ± 0.06 (2.021 ± 0.020)	2.25 ±0.01 (2.11 ± 0.08)	2.30 ± 0.03 (3.181± 0.008)	2.41± 0.05 (2.516 ± 0.021)	2.53 ± 0.02 (2.312 ±0.002)	2.62 ± 0.02 (1.048 ± 0.009)	2.72 ± 0.01 (0.415 ± 0.004)
	Maltose	1.34 ± 0.015 (3.12 ± 0.005)	1.46 ± 0.05 (2.93 ± 0.006)	1.75 ±0.04 (1.83 ± 0.005)	1.85 ± 0.02 (1.51±0.012)	2.10 ± 0.02 (0.81±0.03)	2.17 ± 0.03 (0.35±0.007)	ND (ND)
	Raffinose	0.02 ± 0.001 (ND)	0.2 ± 0.03 (ND)	0.54 ± 0.02 (ND)	0.35 ± 0.03 (ND)	0.54 ± 0.04 (ND)	0.18 ± 0.04 (ND)	0.067±0.02 (ND)
	Rhamnose	1.57 ± 0.02 (3.917 ± 0.039)	1.18 ± 0.03 (4.175 ± 0.035)	1.23 ± 0.04 (2.737 ± 0.036)	1.14 ±0.04 (5.564 ± 0.007)	0.92 ± 0.03 (5.999 ±0.051)	0.52 ± 0.03 (3.412 ± 0.006)	0.34 ± 0.02 (ND)
	Stachyose	0.01 ± 0.001 (0.33±0.008)	0.10 ± 0.01 (0.68±0.035)	0.27 ± 0.05 (0.078 ± 0.006)	0.35 ± 0.03 (0.116±0.005)	0.41 ±0.02 (0.05±0.004)	ND (1.34±0.03)	ND (0.128 ± 0.034)
	Sucrose	0.214 ± 0.04 (3.008 ± 0.007)	1.19 ± 0.03 (3.136 ± 0.004)	3.11 ± 0.03 (3.166 ± 0.005)	3.31 ± 0.02 (3.22 ± 0.010)	3.47 ± 0 .03 (3.24 ± 0.007)	3.96 ± 0.02 (3.316 ± 0.034)	4.39 ±0.01 (3.12 ± 0.031)
Mature	Fructose	4.03 ± 0.03 (5.542 ± 0.050)	4.16 ± 0.04 (5.241 ± 0.009)	4.80 ± 0.04 (0.426 ± 0. 005)	5.05 ± 0.03 (3.506 ± 0.004)	5.17 ± 0.03 (2.432 ±0.002)	5.27 ± 0.05 (1.534 ± 0.02)	5.34 ± 0.03 (1.241 ± 0.007)
	Glucose	4.68 ± 0.04 (1.428 ± 0.041)	4.54 ± 0.04 (1.568±0.007)	4.21 ± 0.02 (1.912 ± 0.012)	4.03 ± 0.03 (1.616 ± 0.017)	3.43 ± 0.03 (1.423 ±0.070)	3.23 ± 0.004 (1.041 ± 0.008)	2.40 ± 0.02 (0.842 ± 0.071)
	Maltose	1.62 ± 0.05 (3.101 ± 0.004)	1.81 ± 0.03 (2.939 ± 0.005)	1.90 ±0.02 (1.837 ± 0.004)	2.17 ±0.03 (1.511 ± 0.012)	2.30 ±0.06 (0.817 ±0.031)	2.24 ±0.06 (0.357 ± 0.008)	2.17 ± 0.05 (0.241 ± 0.004)
	Raffinose	0.046 ± 0.02 (ND)	0.171 ± 0.03 (ND)	0.501 ± 0.02 (ND)	0.786 ± 0.04 (ND)	1.15± 0.03 (ND)	0.15 ±0.03 (ND)	0.086 ± 0.04 (ND)
	Rhamnose	2.74 ±0.02 (2.612 ± 0.014)	2.88 ± 0.25 (3.141 ± 0.002)	2.97 ± 0.35 (4.002 ± 0.003)	3.17 ± 0.08 (2.831 ± 0.006)	3.26 ± 0.12 (1.885 ±0.078)	3.41 ± 0.50 (1.525 ± 0.002)	4.77 ± 0.07 (1.371 ± 0.007)
	Stachyose	0.025 ± 0.005 (0.412 ± 0.012)	0.085 ± 0.005 (0.213 ± 0.021)	0.25 ± 0.04 (ND)	0.39 ± 0.03 (ND)	0.57 ± 0.04 (ND0	0.07 ± 0.002 (ND)	0.12 ± 0.01 (ND)
	Sucrose	0.35 ± 0.05 (3.505 ± 0.004)	0.70 ± 0.05 (3.438 ± 0.042)	3.83 ± 0.03 (2 116 ± 0.004)	3.93 ± 0.02 (2.279 ± 0.012)	5.01 ± 0.04 (4.907 ±0.046)	5.71 ± 0.05 (5.171 ± 0.027)	5.52 ± 0.02 (5.341 ± 0.010)
Partly Ripened	Fructose	4.62 ± 0.05 (2.456 ± 0.006)	4.84 ± 0.04 (2.212 ± 0.014)	4.97 ± 0.07 (2.018 ± 0.043)	5.15 ± 0.04 (1.904 ± 0.007)	5.42 ± 0.06 (1.604 ± 0.014)	5.51 ± 0.01 (1.321 ± 0.012)	5.57 ± 0.02 (0.741 ± 0.008)
	Glucose	2.08 ± 0.05 (3.712 ± 0.014)	2.18 ± 0.06 (3.521 ± 0.023)	2.24 ± 0.07 (3.146 ± 0.07)	2.39 ± 0.03 (2.012 ± 0.080)	2.47 ± 0.04 (1.567 ±0.048)	2.55 ± 0.0.06 (1.218 ± 0.006)	2.67 ± 0.05 (1.009 ± 0.007)
	Maltose	ND (0.510 ± 0.003)	ND (0.415 ± 0.004)	ND (0.388 ± 0.003)	ND (0.271 ± 0.005)	ND (0.165 ±0.012)	ND (0.107 ± 0.003)	ND (0.080 ± 0.004)
	Raffinose	0.27 ± 0.02 ND	0.51 ± 0.03 ND	0.82 ± 0.02 ND	0.94 ± 0.03 ND	1.54 ± 0.04 ND	1.59 ± 0.03 ND	1.18 ± 0.04 ND
	Rhamnose	0.68 ± 0.06 (4.016 ± 0.070)	4.58 ± 0.02 (2.739 ± 0.002)	3.55 ±0.02 (1.142 ± 0.012)	0.89 ± 0.04 (0.942 ± 0.078)	2.38 ± 2.55 (0.457 ±0.071)	2.55 ± 0.01 (ND)	2.82 ± 0.04 (ND)
	Stachyose	0.13 ± 0.03 (2.016 ± 0.002)	0.25 ± 0.04 (2.001 ± 0.012)	0.41 ± 0.05 (1.816 ± 0.024)	0.47 ± 0.04 (1.423 ± 0.041)	0.77 ± 0.04 (1.012 ±0.008)	0.29 ± 0.02 (0.412 ± 0.110)	ND (ND)
	Sucrose	3.01 ± 0.04 (3.093 ± 0.014)	3.96 ± 0.02 (4.645 ± 0.029	5.41 ± 0.03 (4.815 ± 0.078)	5.69 ± 0.05 (5.966 ± 0.016)	4.58 ± 0.06 (5.847 ±0.014)	4.64± 0.03 (4.672 ± 0.028)	3.94± 0.05 (4.541 ± 0.007)
Ripened	Fructose	4.67 ± 0.04	5.03 ± 0.02	5.47 ± 0.03	5.84 ± 0.02	5.88 ± 0.04	5.96 ± 0.02	6.01 ± 0.01
	Glucose	3.21± 0.09	3.18 ± 0.08	3.24 ± 0.03	3.29 ± 0.01	3.34 ± 0.02	3.04 ± 0.06	3.45 ± 0.06
	Maltose	ND	ND	ND	ND	ND	ND	ND
	Raffinose	0.135 ± 0.03	0.24 ± 0.01	0.26 ± 0.01	0.36 ±0.02	0.54±0.03	0.15 ± 0.03	0.07 ± 0.02
	Rhamnose	2.79 ± 0.04	1.58 ± 0.03	3.79 ± 0.04	0.59 ± 0.03	ND	ND	ND
	Stachyose	0.067 ± 0.07	0.12 ± 0.04	0.13 ± 0.06	0.10 ± 0.04	0.027 ±0.02	0.075 ± 0.003	0.07 ± 0.03
	Sucrose	2.93 ± 0.03 (5.707 ± .045)	2.38 ± 0.03 (4.908 ± 0.033)	2.40 ± 0.08 (3.547 ± 0.030)	1.66 ± 0.03 (2.195 ± 0.046)	3.14 ± 0.04 (1.562 ±0.061)	1.60 ± 0.02 (1.232 ± 0.013)	2.17 ± 0.01 (1.014 ± 0.056)

Values in parenthesis indicates sugar content in germinated seeds

ND – Not Detected

days of desiccation. These seeds possessed only half the amount than that of the mature control seeds. Sucrose content of seeds during germination showed a gradual decrease upto 10 days of desiccation and after that the content was increased sharply during the entire period of germination.

During germination of partly ripened control seeds the maximum content of rhamnose was observed. Fructose, glucose, maltose, rhamnose and stachyose contents of partly ripened seeds showed similar pattern of significant decrease during germination of desiccated seeds. Rhamnose was absent in 25 and 30 days desiccated seeds during germination. Stachyose was not detected in 30 days desiccated seeds. Raffinose content was not detected in partly ripened germinated seeds.

During germination of control and desiccated ripened seeds, only sucrose was present which was found to decrease gradually during germination of desiccated seeds.

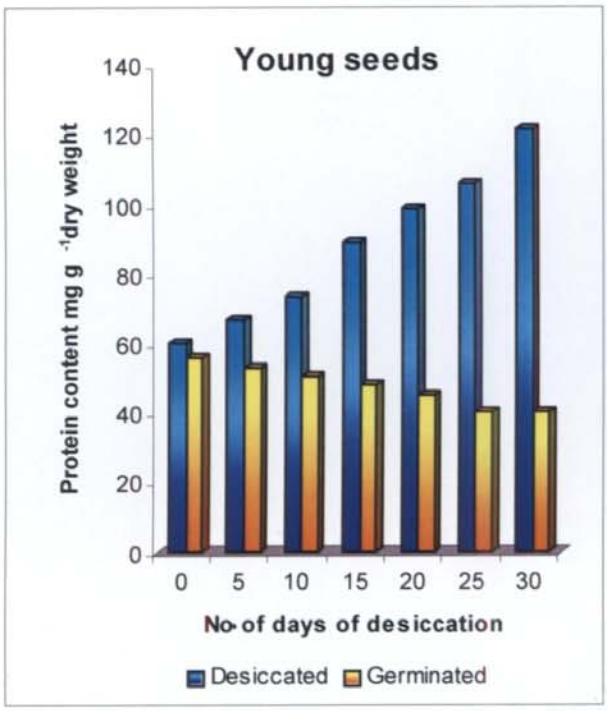
4.3. Distribution of protein

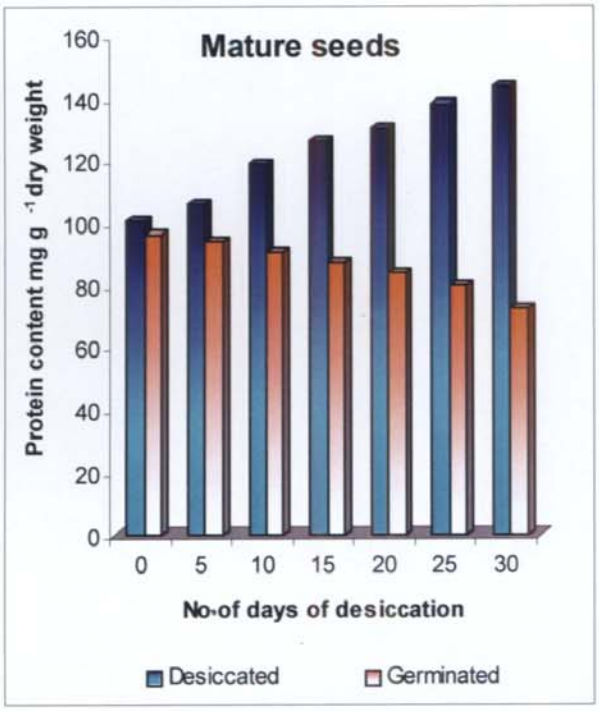
The changes in protein content during seed germination of all treatments are given (Table 20, Fig. 13). In all treatments, the protein content was found to decrease gradually during germination. In young seeds desiccated for 30 days the protein content was reduced to 60% of the control seeds. In the case of mature seeds the reduction in protein content during germination was slow. In 30 days desiccated seeds protein content was about 75% of the control seeds. Partly ripened control seeds showed about 6% of protein. Seeds desiccated for 30 days retained about 63% of protein compared to that of the control seeds. Ripened seeds also showed the same pattern during germination but the protein reduction was maximum. Seeds desiccated for 25 days showed that protein content was only 55% of control. Mature seeds exhibited minimum loss of protein content.

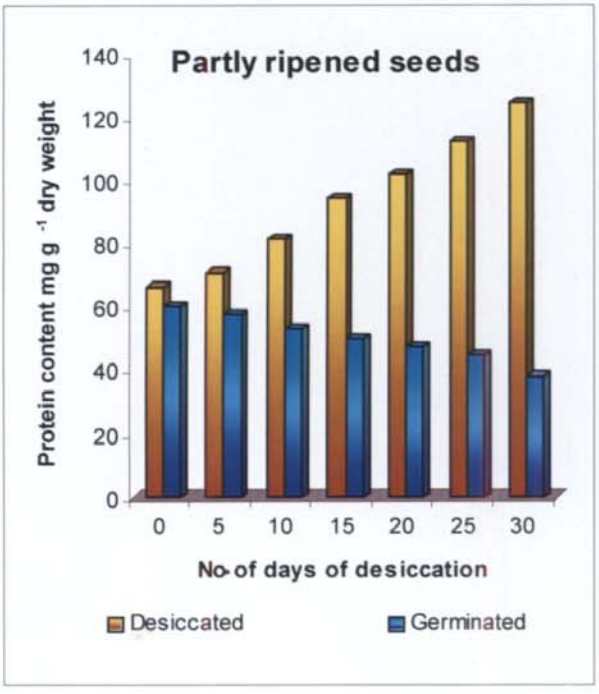
Table 20: Effect of desiccation on mobilization of protein content in *Mangifera indica* seeds during germination (mg g $^{-1}$ dry weight)

Samples (Seed)	Number of days of desiccation													
	0		5		10		15		20		25		30	
	control	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated
	Protein in mg g $^{-1}$ dry weight													
Young	60.18 ± 4.15	56.32 ± 1.25	66.76 ± 4.47	53.25 ± 2.12	73.68 ± 5.68	5 0.45 ± 1.44	89.11 ± 6.85	48.56 ± 1.54	99.01 ± 5.58	45.47 ± 5.14	105.92 ± 5.65	40.25 ± 4.14	121.58 ± 6.54	34.17 ± 3.81
Mature	100.63 ± 6.14	96.45 ± 1.21	106.32 ± 4.15	94.25 ± 2.10	118.82 ± 3.23	90.54 ± 2.14	126.64 ± 3.45	87.54 ± 2.45	130.56 ± 4.15	84.23 ± 2.74	138.16 ± 3.11	80.27 ± 3.45	143.68 ± 3.48	72.56 ± 5.45
Partly ripened	66.45 ± 5.16	60.23 ± 2.12	70.96 ± 4.82	57.54 ± 2.12	81.74 ± 5.12	53.12 ± 3.55	94.16 ± 4.41	50.12 ± 2.12	102.04 ± 5.18	47.52 ± 2.14	112.29 ± 4.25	45.23 ± 2.14	124.42 ± 6.49	38.23 ± 3.21
Ripened	58.14 ± 6.17	54.23 ± 4.25	72.97 ± 6.84	50.12 ± 3.45	80.47 ± 5.36	46.28 ± 2.47	96.97 ± 5.14	40.23 ± 4.25	107.67 ± 5.41	35.23 ± 3.24	117.65 ± 3.71	29.23 ± 2.14	120.14 ± 4.29	—

Figure 13: Effect of desiccation on mobilization of protein in *Mangifera indica* seeds during germination (mg g^{-1} dry weight)







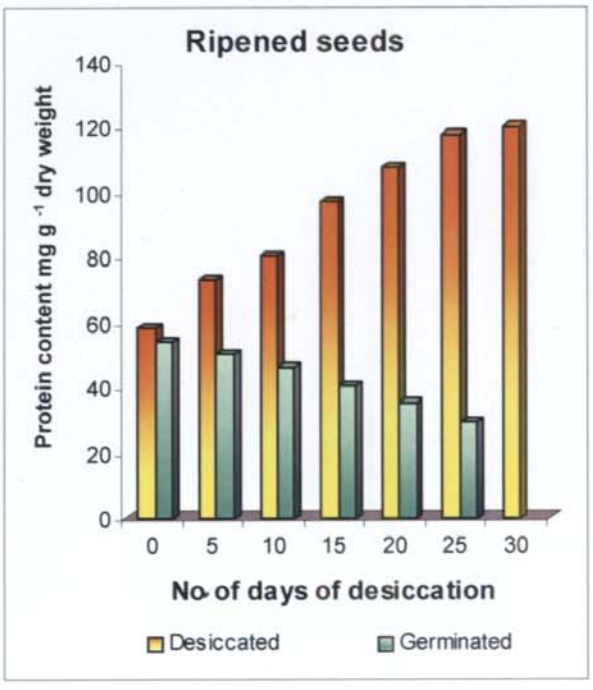
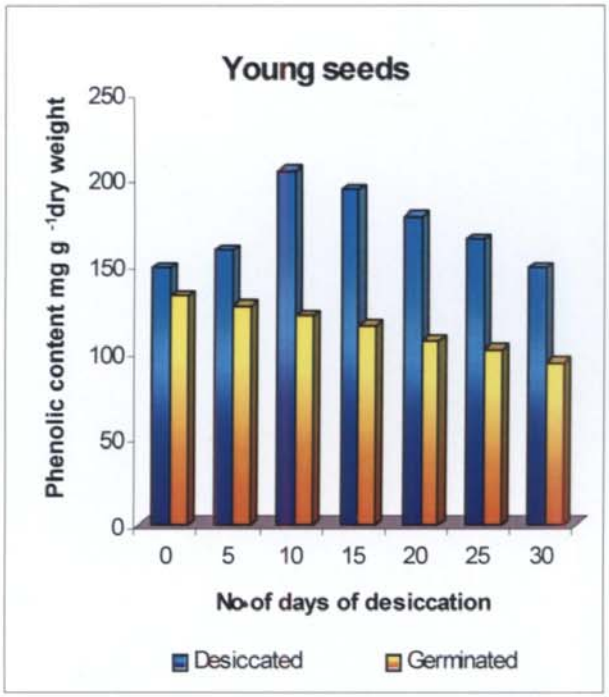
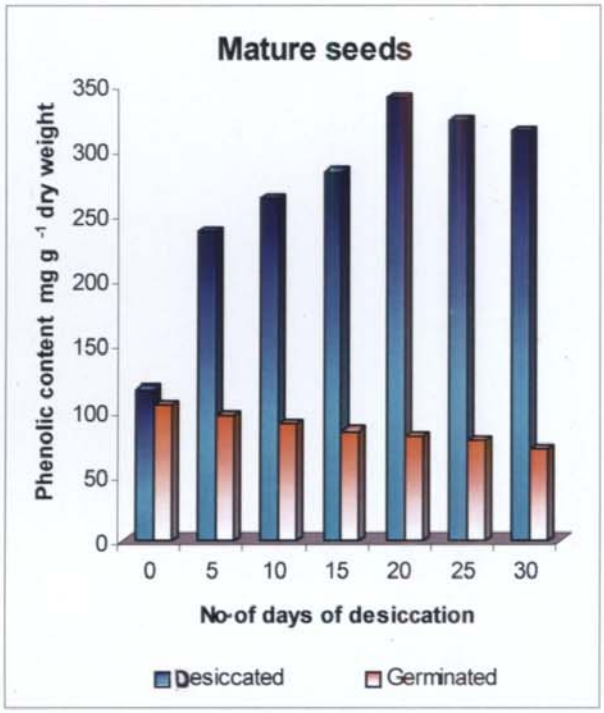
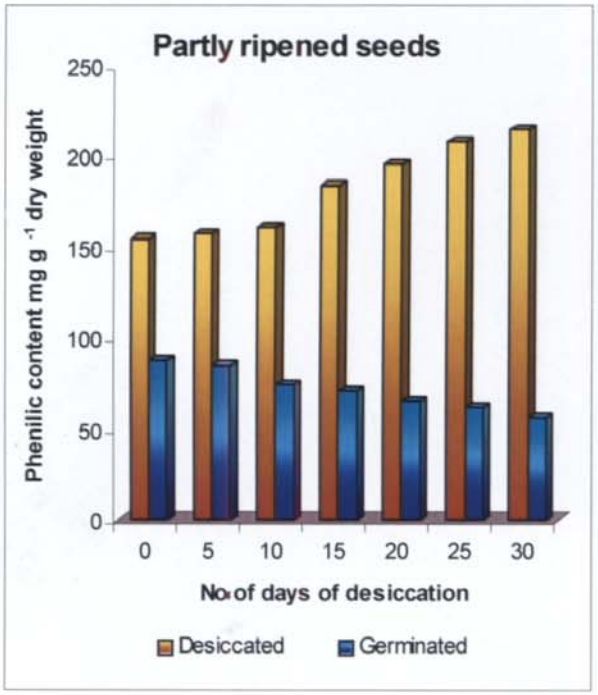
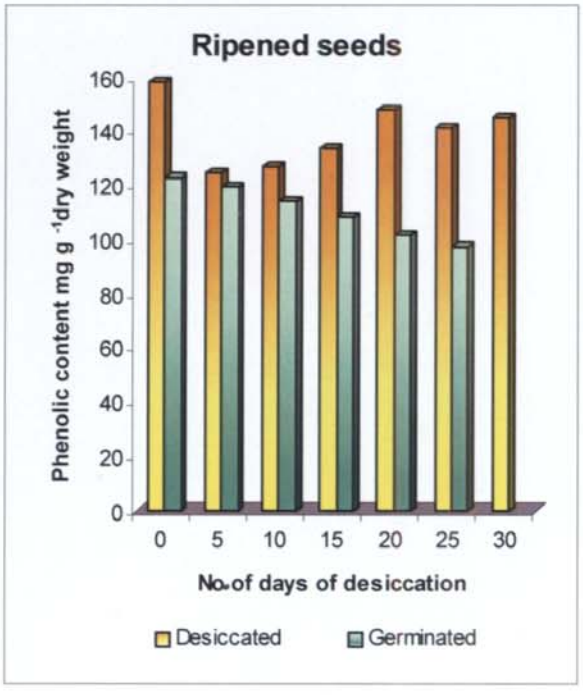


Figure 14: Effect of desiccation on mobilization of phenolics in *Mangifera indica* seeds during germination (mg g^{-1} dry weight)









69C

A comparison of changes in protein content during desiccation and germination showed a regular increase in content during desiccation and a regular reduction during germination (Table 20, Fig. 13). In young seeds during desiccation protein content was increased 2 times, but during germination, it was reduced to 60% of control seeds. Mature seeds showed maximum protein content during desiccation and germination. The mature seeds desiccated for 30 days maintained about 75% of the protein of control seeds during germination. Seeds of other treatments could not retain as much protein of their respective controls compared to the mature seeds. Partly ripened and ripened seeds showed similar pattern of increase of protein content during desiccation. During germination the retention of their protein content was 63% and 55% of their respective controls.

4.4. Distribution of free amino acid contents

The amino acid contents of young control and desiccated seeds during germination were given in Table 21. The maximum amount of amino acid in control and desiccated young seeds was that of glutamic acid, which was found to increase significantly in desiccated seeds.

During germination of desiccated seeds, all amino acids were increased except proline. There was a gradual increase in proline content during germination of 10 days desiccated seeds and thereafter declined gradually. The arginine, cysteine, histidine, phenylalanine, threonie and tyrosine content in germinating desiccated seeds were doubled compared to that of the control seeds. The seeds desiccated for 25 days and 30 days maintained almost same amino acid content level during germination.

The mature control seeds showed a different amino acid distribution pattern (Table 22). The control seeds during germination showed an increased level of amino acids like alanine, arginine, aspartic acid, cysteine, glycine and

Table: 21 Effect of desiccation on amino acid content in young *Mangifera indica* seeds during germination (µg g^{-1} dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g^{-1} dry weight						
Alanine	20 ± 2	22 ± 1	23 ± 2	26 ± 1	29 ± 2	31 ± 1	31 ± 1
Arginine	15 ± 1	17 ± 1	20 ± 2	24 ± 2	26 ± 1	28 ± 1	29 ± 1
Aspartic acid	40 ± 2	43 ± 1	44 ± 1	46 ± 1	49 ± 2	52 ± 1	53 ± 1
Cysteine	13 ± 1	14 ± 1	17 ± 2	19 ± 1	22 ± 2	25 ± 1	26 ± 1
Glutamic acid	66 ± 1	69 ± 2	71 ± 1	74 ± 1	77 ± 2	79 ± 1	80 ± 1
Glycine	27 ± 1	29 ± 3	33 ± 2	36 ± 1	37 ± 1	39 ± 1	41 ± 1
Histidine	14 ± 1	16 ± 1	17 ± 1	21 ± 1	24 ± 1	26 ± 1	27 ± 1
Leucine	37 ± 2	39 ± 1	42 ± 1	46 ± 1	49 ± 2	53 ± 3	55 ± 1
Lysine	20 ± 2	21 ± 1	21 ± 2	23 ± 1	25 ± 1	28 ± 1	29 ± 1
Phenylalanine	14 ± 1	16 ± 1	19 ± 1	21 ± 2	22 ± 1	24 ± 1	26 ± 1
Proline	19 ± 1	21 ± 1	22 ± 1	21 ± 1	18 ± 2	17 ± 2	15 ± 1
Threonine	10 ± 2	13 ± 1	17 ± 1	16 ± 1	18 ± 1	20 ± 1	20 ± 1
Tyrosine	11 ± 1	12 ± 1	14 ± 1	14 ± 1	17 ± 2	19 ± 1	19 ± 1
Valine	24 ± 1	25 ± 1	28 ± 1	31 ± 2	34 ± 1	36 ± 1	36 ± 1
Total	**330**	**357**	**381**	**418**	**447**	**477**	**487**

Table: 22 Effect of desiccation on amino acid content in mature *Mangifera indica* seeds during germination (µg g $^{-1}$ dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g $^{-1}$ dry weight						
Alanine	33 ± 2	35 ± 2	38 ± 2	40 ± 1	43 ± 2	44± 1	46 ± 1
Arginine	26 ± 1	28 ± 1	31 ± 1	34 ± 2	36 ± 1	39 ± 1	41 ± 1
Aspartic acid	44 ± 1	46 ± 1	49 ± 1	52 ± 2	55 ± 3	57 ± 1	58 ± 1
Cysteine	16 ± 1	18 ± 1	22 ± 1	20 ± 1	17 ± 2	15 ± 1	13 ± 1
Glutamic acid	43 ± 1	46 ± 2	49 ± 1	52 ± 1	54 ± 2	56 ± 1	58 ± 1
Glycine	24 ± 1	26 ± 1	29 ± 1	31 ± 2	34 ± 2	36 ± 1	38 ± 1
Histidine	16 ± 1	19 ± 1	22 ± 2	24 ± 1	20 ± 2	17 ± 2	15 ± 1
Leucine	34 ± 2	36 ± 1	39 ± 2	43 ± 3	45 ± 1	47 ± 2	49 ± 1
Lysine	23 ± 2	25 ± 1	29 ± 2	32 ± 1	28 ± 1	25 ± 1	22 ± 1
Phenylalanine	14 ± 1	16 ± 1	19 ± 1	21 ± 2	23 ± 1	24 ± 2	25 ± 1
Proline	15 ± 1	16 ± 1	14 ± 2	12 ± 1	10 ± 2	8 ± 2	6 ± 1
Threonine	15 ± 2	18 ± 1	21 ± 1	23 ± 1	25 ± 1	26 ± 1	11 ± 1
Tyrosine	14 ± 1	16 ± 1	18 ± 1	22 ± 1	19 ± 3	15 ± 2	12 ± 2
Valine	23 ± 1	25 ± 1	29 ± 1	31 ± 2	28 ± 2	25 ± 2	23 ± 1
Total	**340**	**370**	**410**	**437**	**437**	**434**	**417**

tyrosine than the young control seeds. Comparatively lesser amount of glutamic acid and proline was noted in mature control seeds. Contents of histidine, leucine, lysine, phenylalanine and valine were almost same in control of young and mature mango seeds. During germination of desiccated seeds, there was a gradual increase in alanine, arginine, aspartic acid, glutamic acid, glycine, leucine and phenylalanine. Seeds germinated after 15 days desiccation showed the maximum contents of cysteine, histidine, lysine, tyrosine and valine. After this the contents of these amino acids showed a gradual increase and thereafter a gradual reduction. Seeds desiccated for 30 days showed a maximum content of alanine, arginine, aspartic acid, glutamic acid, glycine, leucine and phenylalanine during germination. These amino acids were found to increase with the increase in desiccation period. Threonine content was maximum in 25 days desiccated seeds and thereafter showed a sharp decline.

Germinated partly ripened desiccated mango seeds showed a specific pattern of distribution of amino acids (Table 23). During germination of partly ripened control seeds, contents of arginine, glutamic acid, lysine and phenyl alanine were same as that of mature control seeds. In these seeds like alanine, aspartic acid and leucine are slightly lower than those of mature control seeds. These seeds showed a higher content of cysteine, glycine, hystidine, proline, threonine, tyrosine and valine than the mature control seeds. During germination of 30 days desiccated seeds, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, phenylalanine and threonine contents were maximum. These amino acids were showed a gradual increase during germination after different desiccation period. A regular pattern of initial increase and followed by decrease in amino acid contents like alanine, arginine, lysine, tyrosine and valine was also exhibited by partly ripened seeds during germination after desiccation. Alanine and arginine contents were maximum during germination of 25 days desiccated seeds. Maximum lysine content was shown by germinated seeds after 15 days of desiccation, tyrosine content was

Table: 23 Effect of desiccation on amino acid content in partly ripened *Mangifera indica* seeds during germination (µg g $^{-1}$ dry weight)

Amino acids	Number of days of desiccation						
	0 (control)	5	10	15	20	25	30
	Amino acids in µg g $^{-1}$ dry weight						
Alanine	25 ± 2	28 ± 1	29 ± 1	32 ± 2	34 ± 2	33 ± 1	30 ± 1
Arginine	26 ± 1	27 ± 1	31 ± 1	33 ± 2	35 ± 1	37 ± 1	35 ± 1
Aspartic acid	35 ± 1	36 ± 1	38 ± 1	40 ± 1	43 ± 2	45 ± 1	46 ± 1
Cysteine	25 ± 1	27 ± 1	30 ± 2	32 ± 1	33 ± 1	35 ± 2	37 ± 1
Glutamic acid	44 ±1	46 ± 2	49 ± 1	53 ±1	55 ±2	57 ± 1	59 ± 1
Glycine	27 ± 1	29 ± 1	32 ± 1	35 ± 1	37 ± 1	38 ± 1	40 ± 1
Histidine	21 ± 1	23 ± 1	26 ± 2	29 ± 2	31 ± 1	33 ± 2	36 ± 1
Leucine	21 ± 2	23 ± 1	26 ± 2	29 ± 1	32 ± 1	35 ± 2	37 ± 1
Lysine	26 ± 2	28 ± 2	31 ± 1	33 ± 1	32 ± 1	30 ± 1	28 ± 1
Phenylalanine	16 ± 1	18 ± 1	21 ± 1	23 ± 2	26 ± 2	27 ± 1	29 ± 2
Proline	25 ± 1	26 ± 1	22 ± 2	20 ± 1	18 ± 2	16 ± 2	13 ± 1
Threonine	26 ± 2	28 ± 1	32 ± 1	34 ± 1	37 ± 1	39 ± 1	41 ± 1
Tyrosine	25 ± 1	26 ± 1	29 ± 1	27 ± 1	24 ± 2	22 ± 1	20 ± 1
Valine	30 ± 1	32 ± 1	35 ± 1	37 ± 2	39 ± 2	36 ± 1	33 ± 1
Total	**349**	**388**	**431**	**457**	**476**	**483**	**484**

ND –Not Detected

maximum in seeds germinated after 10 days of desiccation and that of valine in 20 days desiccated seeds. The only amino acid, which showed regular and significant reduction during germination of desiccated seeds, was proline. This was found to be decreased gradually. It was reduced to half compared to that of control seeds in seeds desiccated for 30 days on germination.

The pattern of amino acid content distribution during germination of ripened seeds is given in Table 24. Compared to control seeds of partly ripened and ripened seeds, both contained almost same contents of aspartic acid and glutamic acid. Except alanine all other amino acid contents were lesser in control-ripened seeds. Cysteine and tyrosine contents were lowered to half that of partly ripened seeds. Only alanine was found in a slightly increased level in ripened control seeds during germination. All amino acid contents remained almost same in germinating 25 days and 30 days desiccated seeds. Except proline all other amino acid contents were found to increase during germination of progressively desiccated seeds as that of partly ripened seeds. Proline content was increased slightly in 10 days desiccated seeds during germination and thereafter sharp decline were occurred. Glutamic acid was found maximum in control and desiccated ripened seeds during germination.

4.5. Distribution of phenolic content

During germination young control seeds showed maximum phenolic content compared to the control and desiccated seeds of all other treatments (Table 25, Fig. 14). Phenolic content decreased regularly during germination of all desiccated seeds. In young seeds, germination of 30 days desiccated seeds showed only 28% reduction in phenolic content. The germination of 30 days desiccated mature seeds resulted in 33% reduction of phenolic content. But 39% of reduction of phenolic content was shown by partly ripened seeds during germination of final days of desiccation period. Germinated ripened

Table: 24 Effect of desiccation on amino acid content in ripened *Mangifera indica* seeds during germination (µg g^{-1} dry weight)

	Number of days of desiccation						
Amino acids	0 (control)	5	10	15	20	25	30
	Amino acids in µg g^{-1} dry weight						
Alanine	32 ± 2	34 ± 1	35 ± 2	37 ± 1	40 ± 2	42 ± 1	42 ± 1
Arginine	17 ± 1	21 ± 1	24 ± 2	30 ± 2	33 ± 1	36 ± 1	38 ± 1
Aspartic acid	33 ± 1	36 ± 1	40 ± 1	44 ± 1	49 ± 2	54 ± 1	56 ± 1
Cysteine	13 ± 1	14 ± 1	14 ± 1	16 ± 1	17 ± 1	19 ± 1	20 ± 1
Glutamic acid	44 ± 2	48 ± 2	53 ± 1	55 ± 2	58 ± 2	62 ± 1	64 ±1
Glycine	21 ± 1	23 ± 1	27 ± 2	31 ± 1	34 ± 1	38 ± 2	40 ± 1
Histidine	13 ± 1	15 ± 1	15 ± 2	17 ± 1	17 ± 1	18 ± 1	20 ± 2
Leucine	33 ± 2	36 ± 1	39 ± 2	43 ± 3	47 ± 3	50 ± 2	52 ± 1
Lysine	21 ± 2	23 ± 1	26 ± 1	13 ± 1	33 ± 1	35 ± 1	37 ± 1
Phenylalanine	11 ± 1	12 ± 1	12 ± 1	14 ± 2	15 ± 2	17 ± 1	17 ± 1
Proline	22 ± 1	22 ± 1	24 ± 1	18 ± 1	16 ± 1	15 ± 1	15 ± 1
Threonine	19 ± 2	21 ± 1	22 ± 1	24 ± 2	27 ± 1	29 ± 1	30 ± 1
Tyrosine	11 ± 1	12 ± 1	12 ± 1	14 ± 1	15 ± 1	15 ± 1	16 ± 1
Valine	26 ± 1	27 ± 1	30 ± 2	33 ± 2	37 ± 1	39 ± 1	41 ± 1
Total	**316**	**344**	**373**	**389**	**438**	**469**	**488**

ND – Not detected

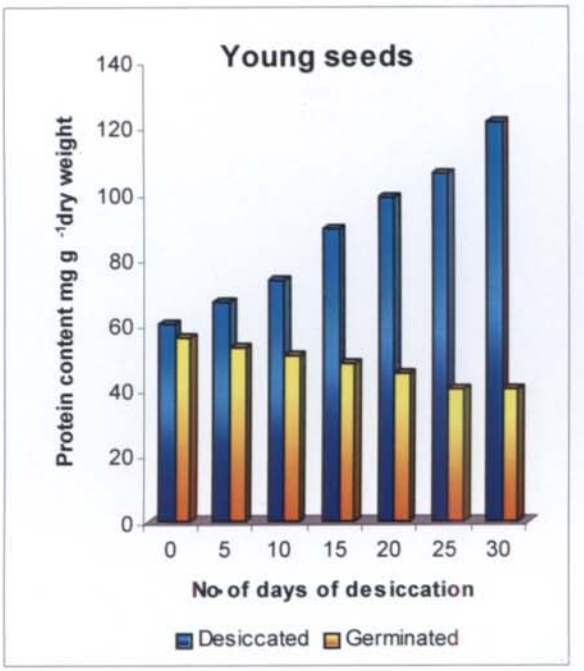
613

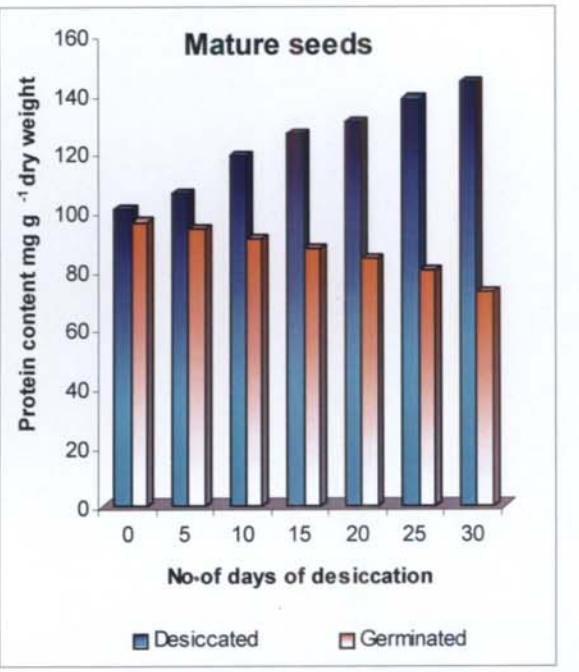
Table 25: Effect of desiccation on mobilization of phenolic content in *Mangifera indica* seeds during germination (mg g $^{-1}$ dry weight)

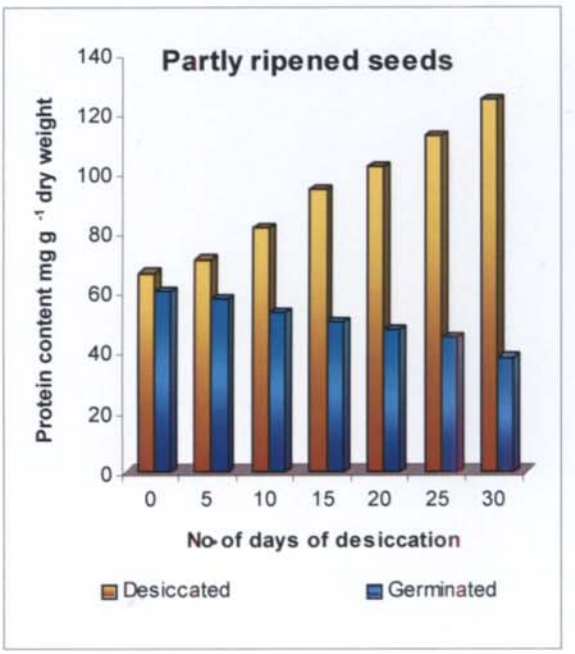
Samples (Seed)	Number of days of desiccation													
	0		5		10		15		20		25		30	
	Control	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated	Desiccated	Germinated
	Phenolic in mg g $^{-1}$ dry weight													
Young	149.02 ± 5.12	132.18 ± 4.07	159.27 ± 8.23	126.82 ± 2.71	165.50 ± 7.18	121.03 ± 3.18	178.78 ± 4.18	115.62 ± 5.78	194.18 ± 5.23	106.81 ± 3.14	204.11 ± 7.31	101.50 ± 3.13	214.11 ± 8.18	94.21 ± 4.01
Mature	226.50 ± 8.11	103.93 ± 4.01	237.06 ± 9.18	96.15 ± 3.11	263.45 ± 7.31	90.47 ± 3.01	283.45 ± 8.32	84.18 ± 3.28	320.45 ± 5.41	80.18 ± 2.58	332.28 ± 5.08	76.81 ± 2.41	344.34 ± 6.31	70.5 ± 3.23
Partly ripened	155.00 ± 7.22	88.00 ± 5.13	157.51 ± 5.11	84.90 ± 4.27	161.03 ± 5.14	74..52 ± 4.08	184.09 ± 4.08	70.68 ± 3.18	195.84 ± 7.31	65.14 ± 2.18	208.18 ± 4.00	61.83 ± 3.19	215.17 ± 3.16	56.59 ± 3.41
Ripened	155.45 ± 2.44	122.99 ± 5.45	164.86 ± 3.11	119.43 ± 3.18	177.00 ± 3.11	114.17 ± 4.48	188.50 ± 4 .08	108.18 ± 3.11	198.17 ± 4.66	101.79 ± 4.56	207.50 ± 2.61	97.58 ± 3.66	–	–

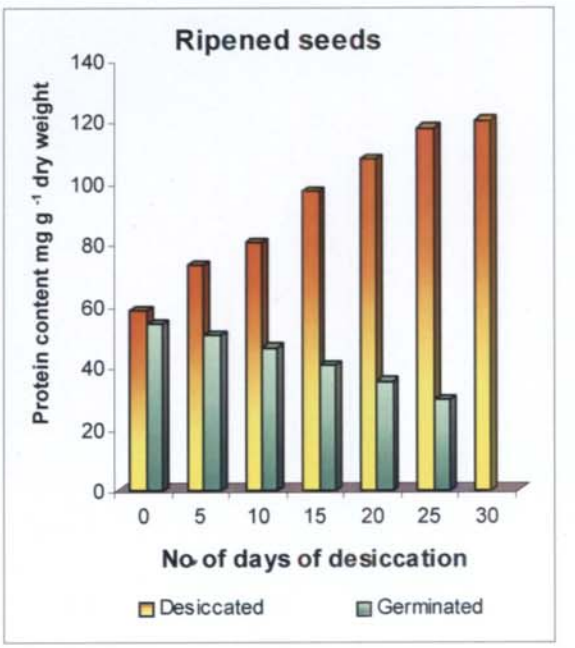
57

Figure 13: Effect of desiccation on mobilization of protein in *Mangifera indica* seeds during germination (mg g^{-1} dry weight)









seeds showed the minimum reduction in phenolic content. These seeds showed only 22% of reduction in phenolic content than its control seeds.

Comparison of phenolic content change during desiccation and germination showed that there is regularity in increase during desiccation treatments and a gradual reduction during germination of all treatments. The changes in phenolic content during desiccation are rapid but those of germination are gradual. Mature seeds showed the maximum increase and maximum content of phenolics during desiccation. Partly ripened and mature seeds showed the maximum reduction in phenolic content during germination. The ripened seeds showed minimum reduction in phenolic content during germination of desiccated seeds

5. Histochemical studies of germinated seeds

5.1. Localization of starch

The cross section of cotyledons of germinated control young seeds consisted of starch grains in the epidermal and cortical cells (Plate 9, Fig. A). The number of starch grains ranged from 10 – 15 per cortical cell. The grains varied in size and shape. The shape of grains was round or ovoid. Procambial strands with a group of thin walled elongated cells were seen without starch grains.

The germinated seeds after 5 days desiccation showed less number of starch grains than control germinated seeds. The epidermal cells were devoid of starch grains and the cortical cells contained starch grains (Plate 9, Fig. B). The number of grains in cortical cells varied from 10 – 15 per cell and were round or oval in shape. The grains were of different size and some are smaller. Starch grains were absent in thin walled elongated procambial cells seen in the cortical region.

The number of starch grains in the cotyledon of 10 days desiccated germinated seeds was found decreased than previous stages (Plate 9, Fig. C). No starch grains were observed in the epidermal cells and cortical cells. The size, shape and number of grains showed variation. The number of grains varied from 8 – 12 per cortical cell and the shape was oval, round and /or elliptical. The procambial strands with narrow elongated cells were seen without any grains.

The cross section of cotyledons of seeds germinated after 15 days desiccation showed less starch grains than that of the previous stages (Plate 9, Fig. D). The starch grains were seen only in the cortical cells and no grains were seen in the epidermal cells. The number of grains varied from 8 – 12 per cell and round, oval and/or elliptical in shape. The grains were variously sized. Procambial stands were seen without any starch grains.

The germinated seeds after 20 days of desiccation showed less number of starch grains compared to the earlier stages of desiccation and control (Plate 9, Fig. E). The epidermal cells were without any grains but the cortical cells contained starch grains. The size of the grains was found reduced than that of the previous stages. The number of grains was decreased as compared to that of previous stages and varied from 6 – 10 per cell. The shapes of grains were oval, elliptical and/or round. No starch grains were observed in the procambial strands.

The seeds germinated after 25 days of desiccation showed starch grains, which was very less than that of the previous stages (Plate 9, Fig. F). No starch grains were observed in the epidermal cells. The number of grains in the cortical cells varied from 6 – 10 per cell. The size of grains was smaller than the previous stages. The grains were variously shaped such as oval, elliptical and round. The procambial strands were with thin walled elongated cells, lacking starch grains.

The seeds desiccated for 30 days of germination showed no starch grains in epidermis but only a few grains were found in cortex (Plate 9, Fig. G). The number of grains was less compared to those of previous stages of germination and it was ranged from 6-8 per cell. The size of grains was found reduced as compared to that of the previous stages. The procambial strands were seen with thin walled cells and devoid of starch grains.

The cross section of cotyledons of germinated mature control seeds consisted of starch grains completely filled in each cell (Plate 10, Fig. A). The epidermal cells are intact and contained starch grains, ranging from 3 – 4 per cell. The cortical cells were intact with 12 – 16 starch grains per cell. The grains were large in size and showed variation in its shape such as oval, elliptical and/or round. Starch grains were not observed in the procambial strands.

The 5 days desiccated seeds on germination showed lesser number of starch grains than the cotyledons of germinated control seeds (Plate 10, Fig. B). The epidermis and cortical cells contained starch grains. The number of grains in epidermis was 3 – 4 per cell and in cortex it was 8 – 15 per cell. The shape of the grains was similar to that observed in germinated control seeds. Size of the grains in both epidermis and cortex showed variation. The procambial strands were seen without starch grains.

The number of starch grains in 10 days desiccated germinated seeds was fewer than that observed in the previous stages (Plate 10, Fig. C). Starch grains were observed in epidermis and cortex. The number of starch grains in the epidermis was 2 – 3 per cell and in the cortex it was 8 – 13 per cell. Shape of the grains was similar to that observed in the previous stage. Starch grains were not seen in the procambial strands.

The germinated seeds after 15 days of desiccation showed lesser number of starch grains than control seeds (Plate 10, Fig. D). Starch grains were absent in the epidermis and the cortical cells consisted of starch grains, 7 – 10 per cell. The size of the grains showed variation and shape was oval, elliptical and/or round. Starch grains were absent in procambial strands.

Seeds germinated after 20 days of desiccation showed the presence of starch grains which was lesser than that of the previous stages and control (Plate 10, Fig. E). The epidermal cells were devoid of starch grains and grains were present only in the cortical cells. The number of grains in cortical cells varied from 6 – 10 per cell and oval, elliptical and/or round in shape. The procambial stands were devoid of starch grains.

The starch grains in seeds germinated after 25 days desiccation exhibited a decrease, than that of the previous stages (Plate 10, Fig. F). No starch grains were observed in the epidermal cells and the cortical cells contained starch grains. The number of grains per cortical cell varied from 6 – 10 and the size was found reduced when compared to that of the previous stages. The shape of the grains was oval, round and/or elliptical. The procambial strands consisted of elongated thin walled cells and devoid of starch grains.

The cotyledons of seeds germinated after 30 days of desiccation showed more or less same pattern of distribution of starch grains (Plate 10, Fig. G). Epidermal cells were devoid of starch grains, but the cortical cells were composed of starch grains, which were varied in number, size and shape. The cortical cells contained 6 – 10 starch grains per cell. The sizes of the grains were decreased as compared to that of the previous stages and the shape was oval, elliptical and/or round. Starch grains were absent in the procambial strands.

The partly ripened control seeds on germination exhibited starch grains both in the epidermal and cortical cells (Plate 11, Fig. A). The number of starch grains in epidermal cells ranges from 3 – 5 per cell. The cortical cells contain more number of starch grains than epidermal cells and it was 7 – 17 per cells. The size and shape of grains also showed variation. The shape was identical as in the previous stages. Procambial strands consisted of thin walled elongated cells and starch grains were absent in these cells.

The seeds germinated after 5 days of desiccation showed a reduction in number of starch grains than control germinated seeds (Plate 11, Fig. B). The epidermal cells composed of 3-4 starch grains and the cortical cells with 7-15 starch grains per cell. The size and shape of grains showed variation and were almost similar to that of control seeds. The starch grains were absent in procambial strands.

The number of starch grains was lesser in 10 days desiccated germinated seeds than the pervious stages (Plate 11, Fig. C). Both epidermal and cortical cells contained starch grains and cells were remaining intact. The number of grains was 2 per epidermal cell and 7 – 14 per cortical cell. The size and shape of grains showed variation but was identical to that of the previous stages. No starch grains observed in procambial strands.

The seeds germinated after 15 days of desiccation showed starch grains only in the cortical cells and the cells were intact (Plate 11, Fig. D). No starch grains were observed in epidermal cells. There was a decrease in number of grains as compared to the previous stages and varied from 6 – 10 per cell. The shape and size of grains were almost similar to previous stages. Starch grains were absent in procambial stands.

The starch grains were absent in the epidermal cells of 20 days desiccated germinated seeds (Plate 11, Fig. E). The cells were remaining

intact. The number of starch grains in the cortical cells were more or less similar to that observed in the 15 days desiccated germinated seeds. The size was found reduced and the shape was oval, round and/or elliptical. The procambial strands were devoid of starch grains.

The seeds germinated after 25 days of desiccation showed no starch grains in the epidermal cells (Plate 11, Fig. F). But in cortical cells the number of grains was reduced to 5 – 8 per cell. The size and shape of the grains also showed variation. The shape of grains was oval, round and/or elliptical. Size variation was exhibited by the grains irrespective of their shape. Starch grains were absent in the procambial strands.

The starch content in seeds germinated after 30 days of desiccation was lesser than that of previous stages of germination (Plate 11, Fig. G). The epidermal cells were devoid of starch grains and cortical cells contained starch grains. The number of starch grains ranges from 5 – 8 per cells and varied in size, and shape. The shape of grains was oval, round and/or elliptical as in the previous stages. Irrespective of the shape some grains are small and others are large. Procambial strands composed of thin walled elongated cells and without starch grains.

The ripened control seeds on germination showed starch grains in the epidermal and cortical cells and the cells were intact (Plate 12, Fig. A). The epidermal cells contained 3 – 4 starch grains per cell. The number of starch grains per cortical cells was 10 – 22 and the starch grains exhibited variation in size and shape. Some of the grains were small and others large. The shape of the grains was oval, round and/or elliptical. The procambial strands consisted of elongated cells and were devoid of starch grains.

The starch grains in 5 days desiccated seeds on germination was lesser than the control germinated seeds and the cells were intact (Plate 12, Fig. B).

The number of starch grains in the epidermis ranged from 2 – 4 per cell and that of the cortical cells was 10 – 16 per cell. The size and shape of the starch grains was similar to that of the germinated control seeds. Starch grain were absent in the procambial strands.

A slight reduction in the number of starch grains was observed in the 10 days desiccated seeds on germination than the control (Plate 12, Fig. C). The number of starch grains was 2 – 3 per epidermal cell and 10 – 14 per cortical cell. Size and shape of these grains showed much variation. No starch grains were seen in procambial strands.

The seeds germinated after 15 days of desiccation showed a decrease in the starch content compared to the control (Plate 12, Fig. D). The epidermal cells were devoid of starch grains and cortical cells contained starch grains. The number, size and shape of starch grains vary considerably. The number of starch grains ranges from 6 – 12 per cell. The size of grains was reduced than that observed in the previous stages. The shape of grains was similar to that observed earlier. Procambial strands consisted of elongated cells and starch grains were absent.

The starch content in seeds germinated after 20 days of desiccation was limited in the cortical cells and the cells were remain intact (Plate 12, Fig. E). The number of starch grains in the cortical cells was decreased than that of the previous stages of desiccation and varied from 6 – 10 per cell and the size of grains was also found decreased as compared to that of the previous stages. The shape of grains was oval, round and/or elliptical. Starch grains were absent in the procambial strands.

In the epidermal cells of cotyledon of seeds germinated after 25 days of desiccation, starch grains were completely absent (Plate 12, Fig. F). The cortical cells consisted of 6 – 10 starch grains per cell. The size and shape

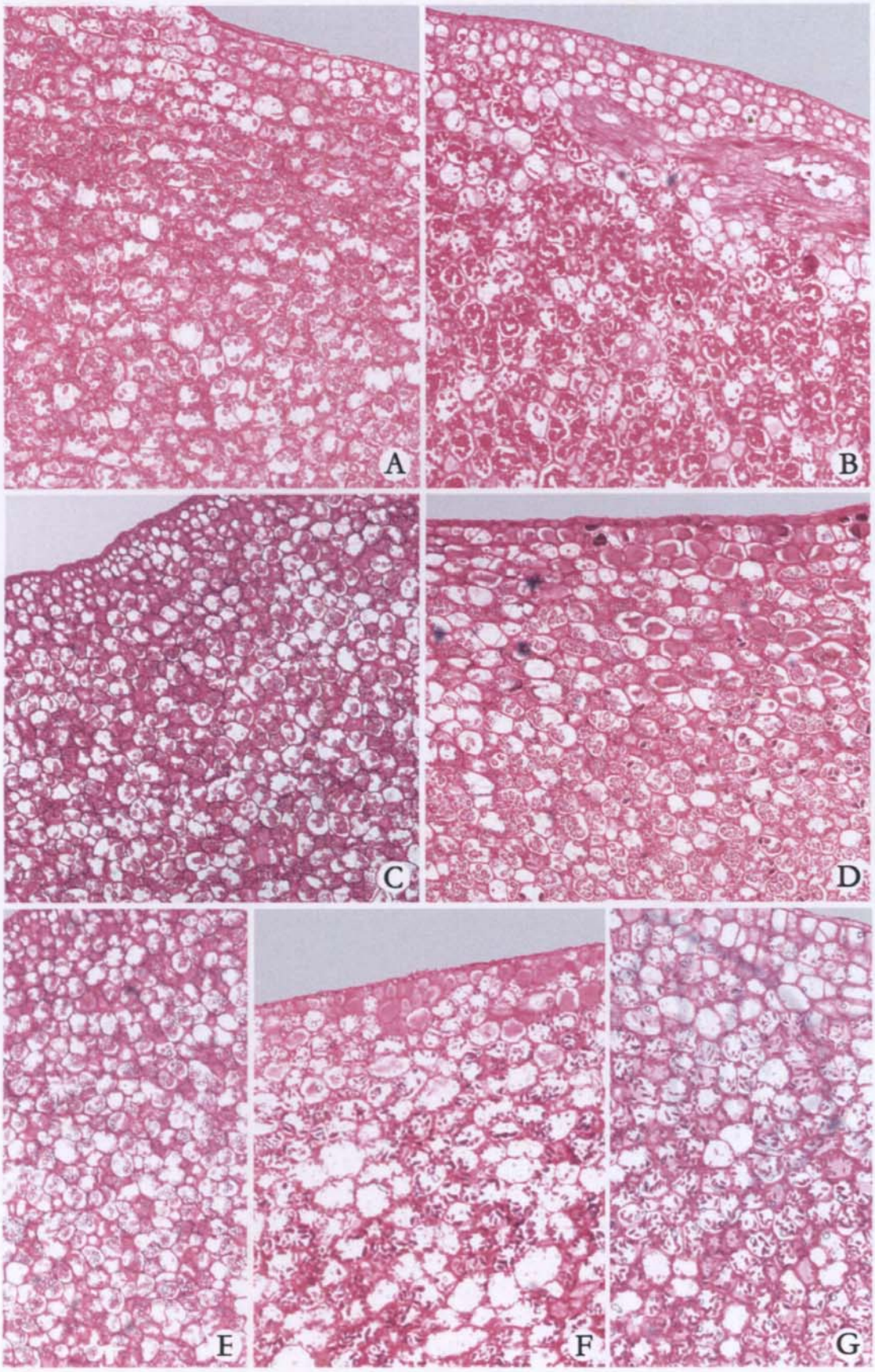


Plate 9 : Effect of desiccation on localization of starch during germination of young Mango (*Mangifera indica*) seeds.
Fig. A: Control seeds; **B, C, D, E, F & G**: Seeds desiccated for 5, 10, 15, 20, 25, & 30 days respectively.

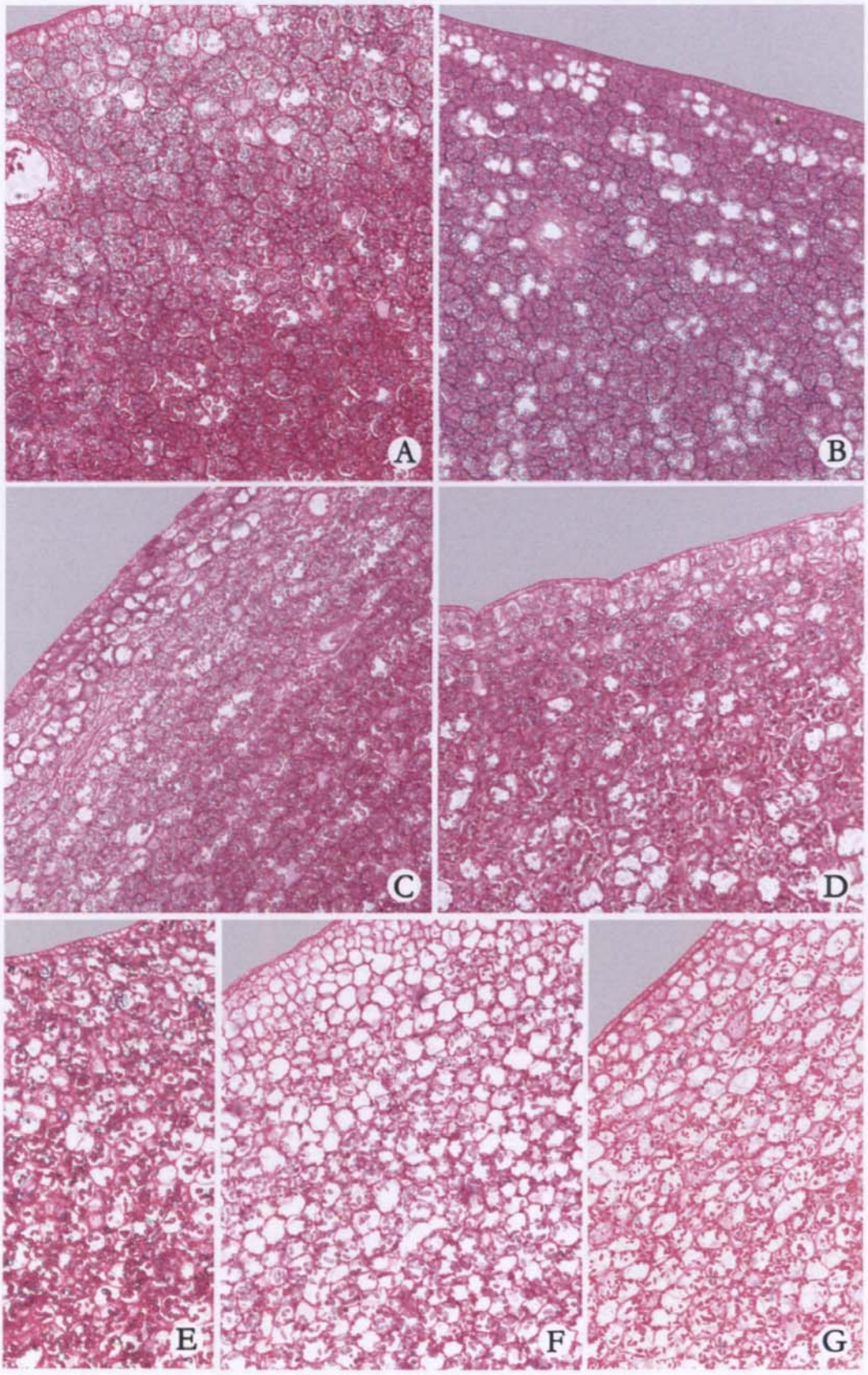


Plate 10: Effect of desiccation on localization of starch during germination of mature Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

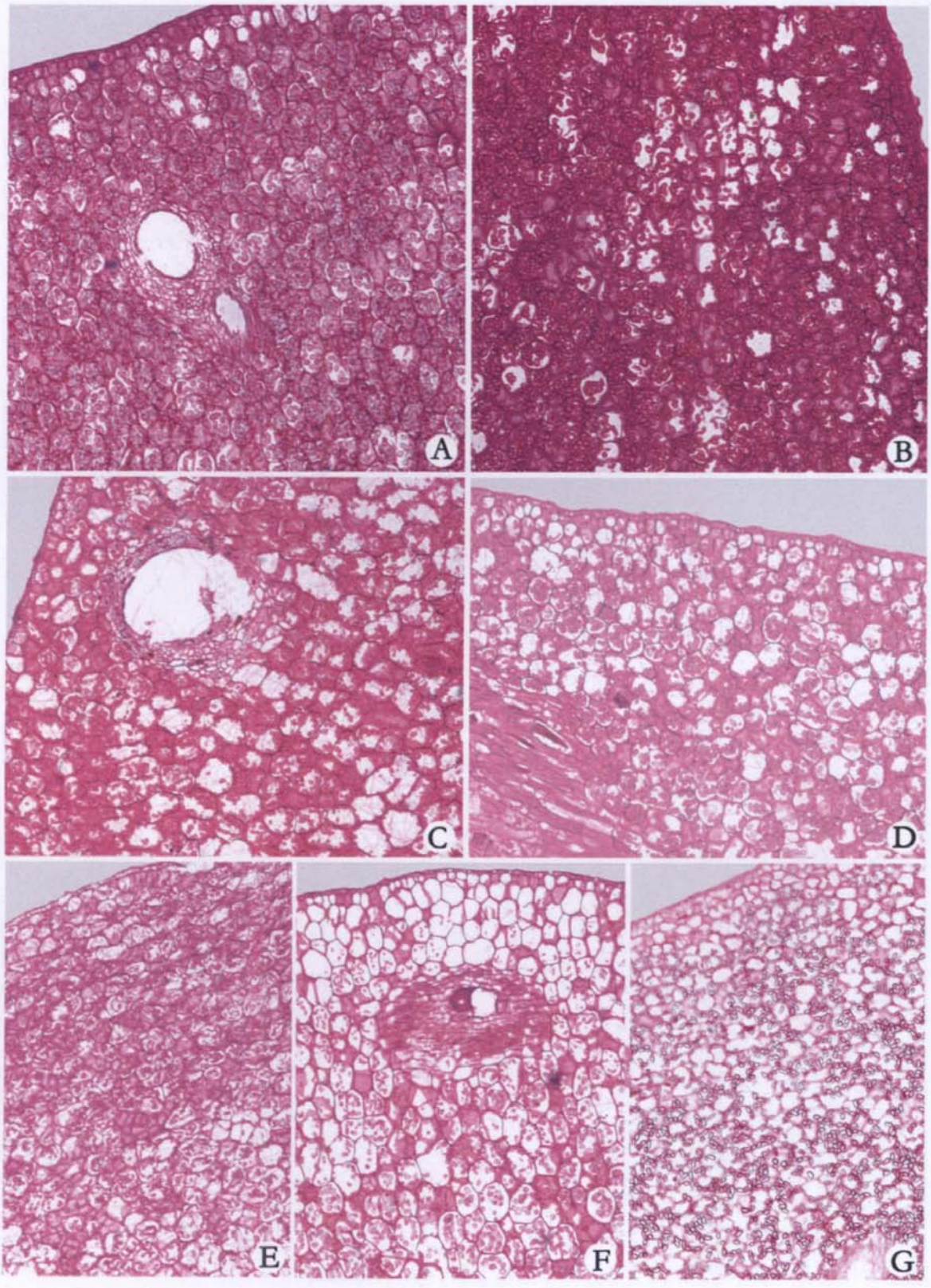


Plate 11 : Effect of desiccation on localization of starch during germination of partly ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

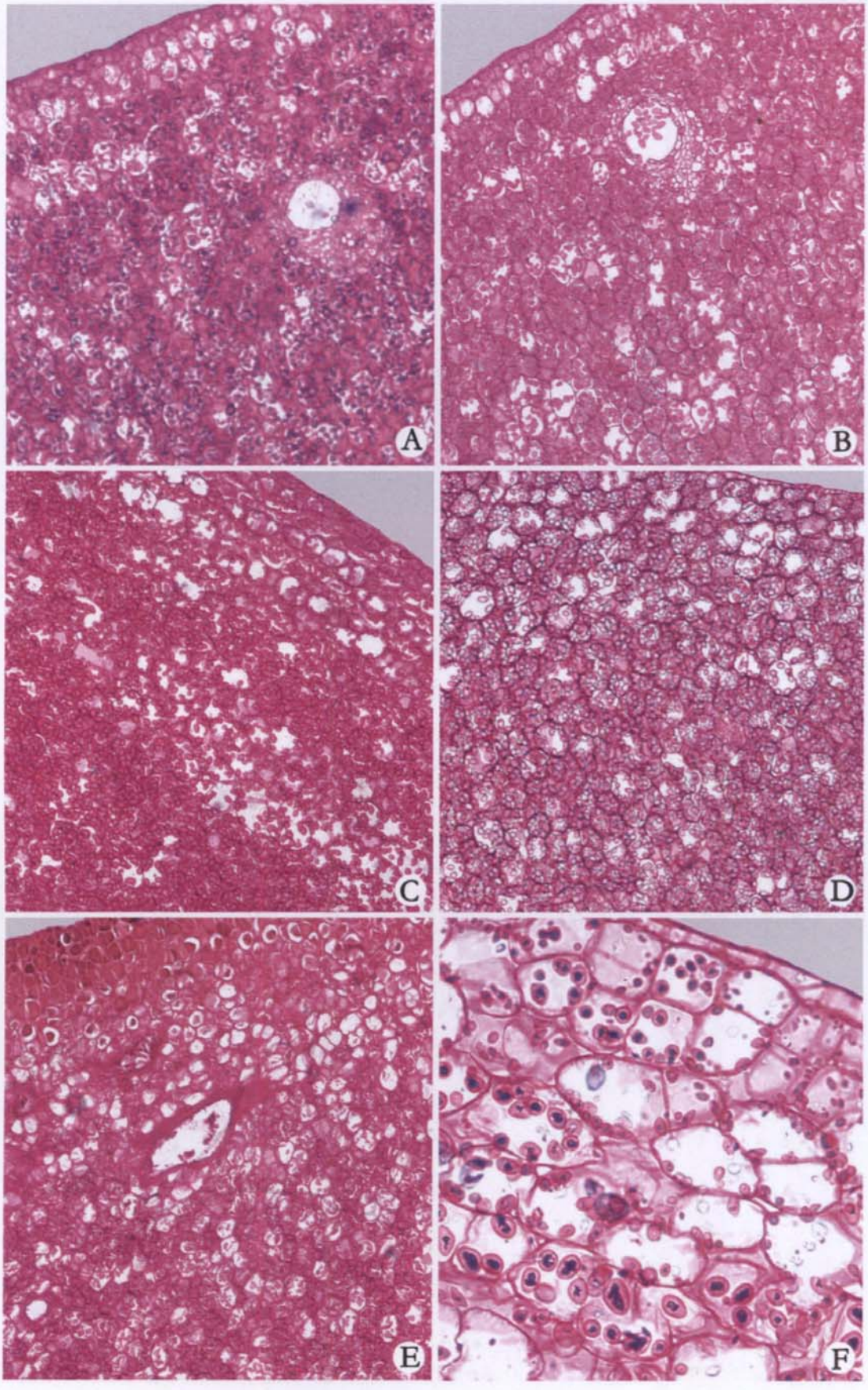


Plate 12 : Effect of desiccation on localization of starch during germination of ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E & F** : Seeds desiccated for 5, 10, 15, 20 & 25 days respectively.

showed variation which was similar to that observed in the previous stages. Starch grains were absent in procambial strands.

The starch grains in the seeds germinated after 30 days of desiccation were seen only in cortical cells (Plate 12, Fig. G). No starch grains were observed in the epidermal cells. The cortical cells consisted of 6 – 10 starch grains per cell. The size and shape of grains vary considerably. The shape of grains was round, oval and/or elliptical as in the previous stages. The size of grains was found decreased as compared to that of the previous stages. The starch grains were absent in procambial strands.

5.2. Localization of protein

The germinated young control seeds showed deep blue stained masses of protein in the epidermal and cortical cells (Plate 13, Fig. A). All the cells of epidermis were filled with protein masses. The procambial strands were seen with elongated cells filled with protein masses.

The staining intensity for protein in the cross section of cotyledon of 5 days desiccated germinated seeds was less as compared to that of control (Plate 13, Fig. B). The protein masses were uniformly distributed in the epidermal cells. The staining intensity for proteins was lesser than that of germinated control seeds and was unevenly distributed. The elongated cells of procambial strands also showed a slight decrease in the staining intensity for proteins.

The germinated seeds after 10 days of desiccation showed a decrease in the distribution of protein masses in both epidermis and cortex (Plate 13, Fig. C). As in the previous stages the distribution of protein content in the cortical cells was not uniform. The procambial strands with elongated cells were filled with protein masses and the staining intensity was lesser than that of the previous stages.

The epidermal cells of cotyledon of seeds germinated after 15 days of desiccation showed a decreased staining intensity for proteins than that of the previous stages of desiccation and control (Plate 13, Fig. D). The protein masses in the cortical cells were distributed unevenly throughout the section. The procambial strands were also showed a decrease in protein content than the previous stages.

The protein content of young seeds germinated after 20 days of desiccation was lesser than the previous stages (Plate 13, Fig. E). The epidermal and cortical cells were found feebly stained for proteins. The protein masses in the cortical cells were found unevenly distributed and the staining intensity was lesser as compared to that of previous stages of desiccation and control. A decreased staining intensity for proteins was also observed in the procambial strands.

The young seeds germinated after 25 days of desiccation showed a decrease in protein content both in the epidermis and cortex as compared to the previous stages (Plate 13, Fig. F). The distribution of protein masses in the cortical cells was not uniform and the staining intensity for protein was feeble than the earlier stages. The procambial strands were also composed of lightly stained masses of protein.

The protein masses in the epidermal and cortical cells of cotyledon of germinated seeds after 30 days of desiccation were very less as compared to that of the previous stages of desiccation and control (Plate 13, Fig. G). The staining intensity for proteins of procambial strands was lesser than that observed in the previous stages of desiccation and control.

The cross section of cotyledon of germinated mature control seeds showed blue stained protein masses in the epidermal and cortical cells (Plate 14, Fig. A). The epidermis was found feebly stained for protein and protein

masses were observed in all the cells. The protein masses in the cortical cells were deeply stained and evenly distributed throughout the section. The procambial strands consisted of elongated cells with feebly stained protein masses which were distributed evenly.

The seeds germinated after 5 days of desiccation showed a slight reduction in protein content compared to that of germinated control seeds (Plate 14, Fig. B). A reduction in the staining intensity was noticed in the epidermal cells than that of control and the protein mass was uniformly distributed. The distribution of protein masses in the cortical cells was not uniform but a slight reduction in the staining intensity was observed. The cells of procambial strands also showed a slight increase in staining intensity than control seeds.

The epidermal cells of seeds germinated after 10 days of desiccation showed almost same pattern of distribution of protein masses as that of seeds germinated after 5 days of desiccation (Plate 14, Fig. C). A decrease in protein content was observed in the cortical cells than the previous stages of desiccation and control and the protein masses were unevenly distributed. The elongated cells of procambial strands were also exhibited a reduction in the staining intensity for protein than the previous stages.

The protein content of the seeds germinated after 15 days of desiccation was reduced as compared to the previous stages (Plate 14, Fig. D). In the epidermal cells the protein masses were uniformly distributed, but in the cortical cells the protein masses were unevenly distributed. The staining intensity for proteins in the epidermal and cortical cells was lesser than that observed in the previous stages and control. The procambial strands consisted of elongated cells with less protein mass than that of the previous stages.

The seeds germinated after 20 days of desiccation, showed almost same staining intensity for proteins in the epidermal cells as observed in the seeds germinated after 15 days of desiccation (Plate 14, Fig. E). The protein masses in the cortical cells were decreased as compared to the previous stages of desiccation and control and were distributed unevenly. The cells of procambial strand also exhibited a decreased staining intensity than that of the previous stages.

The epidermal and cortical cells of cotyledon of seeds germinated after 25 days desiccation showed a decline in protein content as compared to the previous stages (plate 14, Fig. F). A few amount of protein was observed in the epidermis. The cortical cells were composed of lightly stained protein masses and it was distributed unevenly and was lesser than that occurred in previous stages of desiccation and control. The procambial cells also showed the same staining intensity of that of cortical cells and were lesser than that of the previous stages.

The protein content of seeds germinated after 30 days of desiccation was lesser than that observed in the previous stages (Plate 14, Fig. G). All the epidermal cells were found fully stained for proteins but a few cells in the cortex were composed of protein masses. A large number of cells were seen without protein masses. The cells in the procambial strands were also devoid of protein masses.

The cross section of cotyledon of germinated partly ripened control seeds showed higher staining intensity for proteins (Plate 15, Fig. A). The epidermal cells were found completely filled with protein masses and were deeply stained. Deeply stained protein masses were also observed in the cortical cells also and the staining intensity was uniform in the epidermis and cortex. The procambial strands consisted of elongated cells and feebly stained than the epidermal and cortical cells.

The seeds germinated after 5 days of desiccation showed a decrease in the staining intensity for protein than the control (Plate 15, Fig. B). The protein masses were evenly distributed in the epidermal and cortical cells as in the control seeds. The staining intensity, for protein of procambial strands was lesser than that observed in control.

The protein content in the seeds germinated after 10 days of desiccation showed a reduction as compared to that of seeds germinated after 5 days of desiccation and control (Plate 15, Fig. C). The protein mass in the epidermal cells was distributed evenly but unevenly in the cortical cells. A few cells of the cortex were seen without protein masses. The same staining pattern as that of cortical cells was observed in the procambial strands and was lesser than that of the previous stages.

The epidermal cells of cotyledon of seeds germinated after 15 days of desiccation exhibited a slight decrease in the staining intensity than the previous stages and the protein masses were found unevenly distributed (Plate 15, Fig. D). The cortical cells also exhibited a decreased staining intensity than the previous stages. The elongated cells of the procambial strand were also found feebly stained for protein masses which were lesser than that observed in the previous stages of desiccation and control.

The 20 days desiccated seeds on germination showed a slight decrease in the staining intensity for protein in the epidermal region (Plate 15, Fig. E). The cortical cells showed a less amount of protein masses than the previous stages. The staining intensity for proteins in the cells of procambial strands were also lesser than in the previous stages.

The staining intensity for protein in the epidermal and cortical cells of seeds germinated after 25 days of desiccation was found reduced as compared to that of the previous stages (Plate 15, Fig. F). The protein masses were

observed only in a few cells of the cortex and other cells remain vacant. The cells of the procambial strands consisted of lightly stained protein masses which were lesser than that of the previous stages.

The seeds germinated after 30 days of desiccation showed very less amount of protein in the epidermal and cortical cells than that of the previous stages (Plate 15, Fig. G). The protein masses were found unevenly distributed in the cortical cells. The number of cells, remaining vacant was more than that observed in the previous stages. The procambial strands composed of feebly stained protein masses which was lesser than that noticed in the earlier stages.

The ripened control mango seeds after germination exhibited a higher staining intensity for protein (Plate 16, Fig. A). The epidermal and cortical cells were observed with deeply stained protein masses. The protein masses were evenly distributed throughout the epidermis and cortex. The procambial strands consisted of elongated cells with slightly stained masses of protein.

A decrease in the protein content was observed in the epidermal and cortical cells of seeds germinated after 5 days of desiccation than the germinated control seeds (Plate 16, Fig. B). The protein masses were found evenly distributed in the cortical cells. The elongated procambial strands were also exhibited an increased staining intensity for protein than the control.

The seeds germinated after 10 days of desiccation showed a decrease in the protein content than the previous stages (Plate 16, Fig. C). The cells of the epidermis and cortex were observed feebly stained. The protein masses in the cortical cells were unevenly distributed throughout the section. The protein masses in the cells of procambial strands were lightly stained than the previous stages of desiccation.

The epidermis of seeds germinated after 15 days of desiccation showed almost same staining intensity as that of the 10 days desiccated seeds (Plate 16,

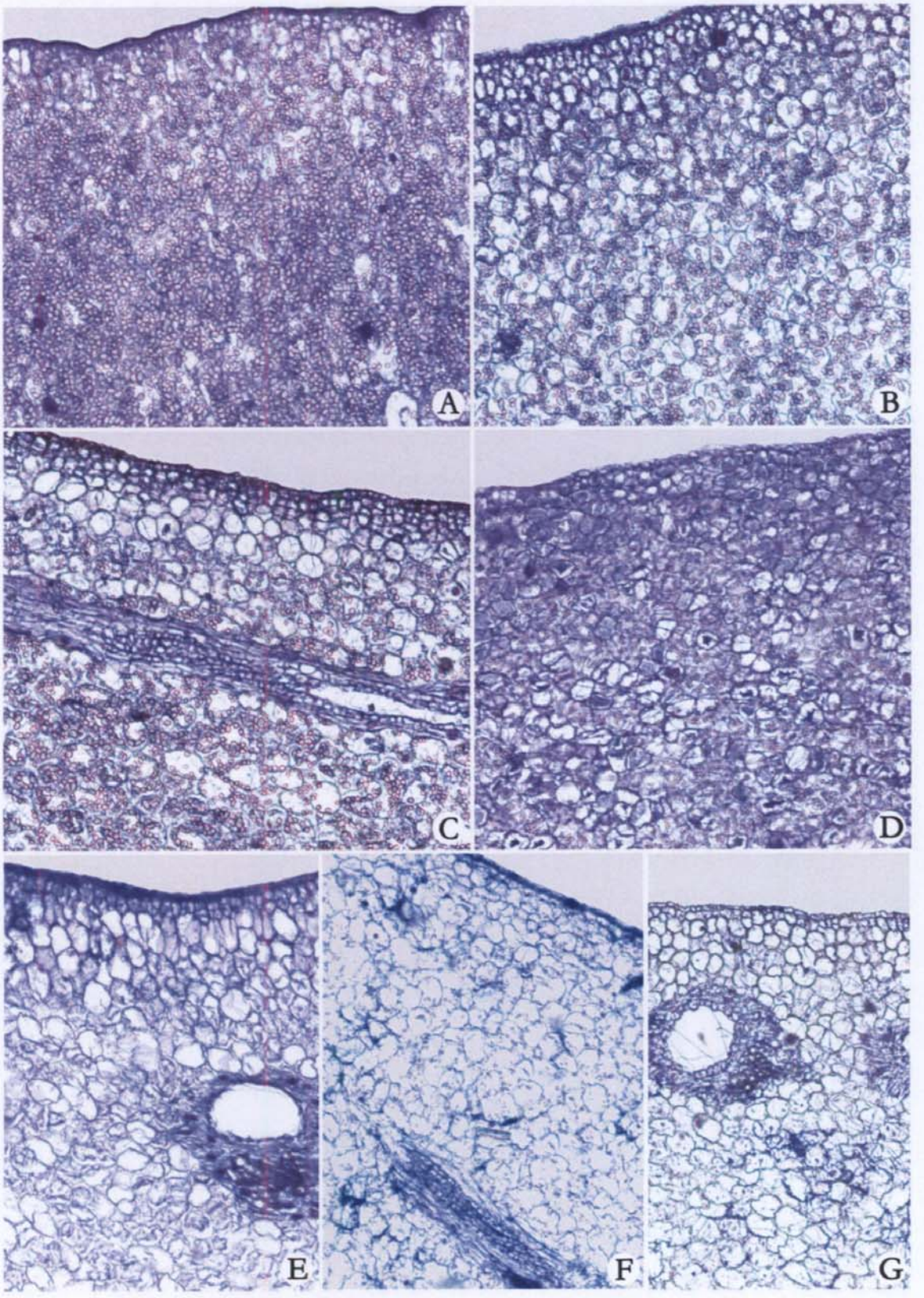


Plate 13 : Effect of desiccation on localization of protein during germination of young Mango (*Mangifera indica*) seeds. Fig. **A**: Control seeds; **B, C, D, E, F & G**: Seeds desiccated for 5, 10, 15, 20, 25, & 30 days respectively.

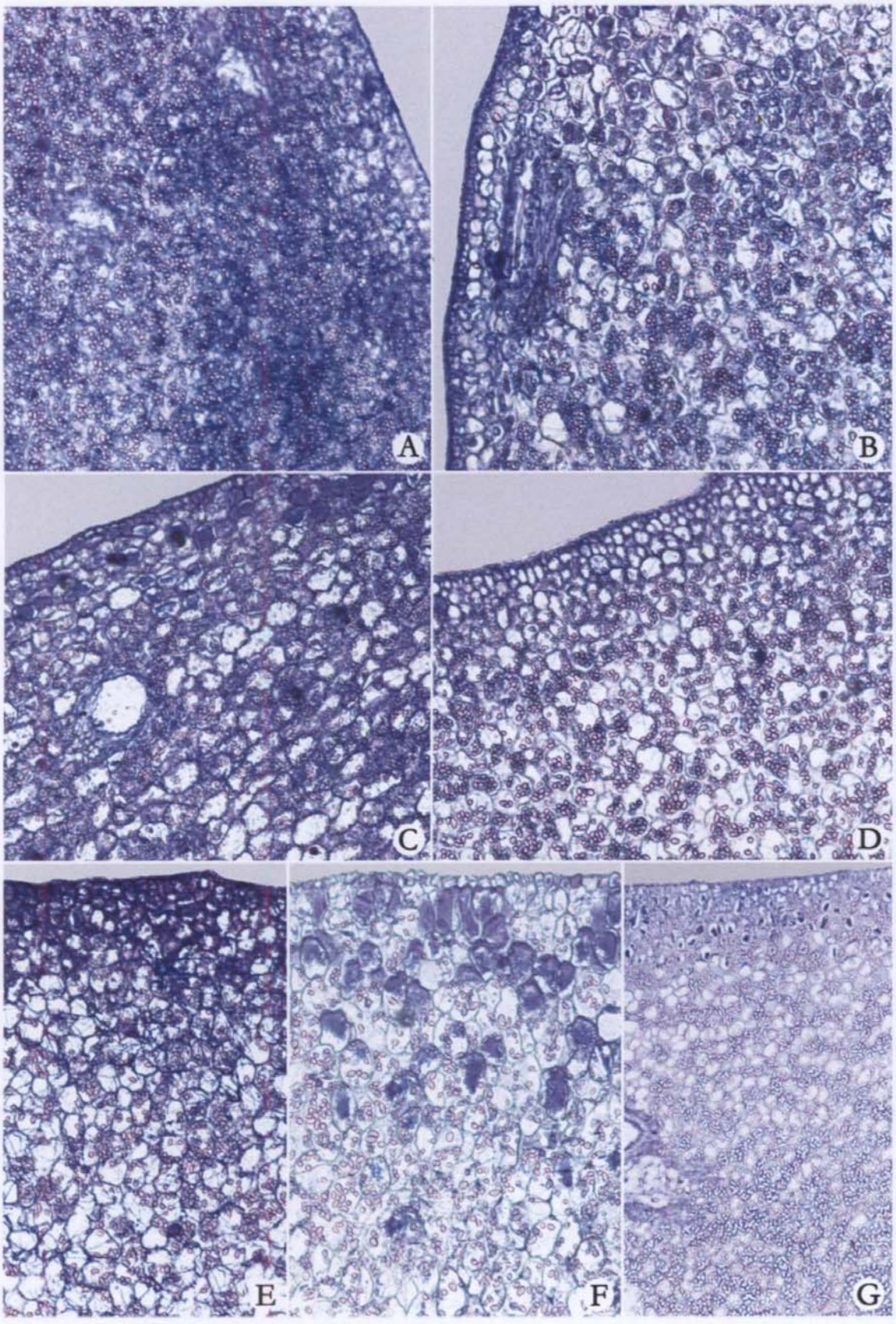


Plate 14 : Effect of desiccation on localization of protein during germination of mature Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E, F & G** : Seeds desiccated for 5, 10, 15, 20, 25 & 30 days respectively.

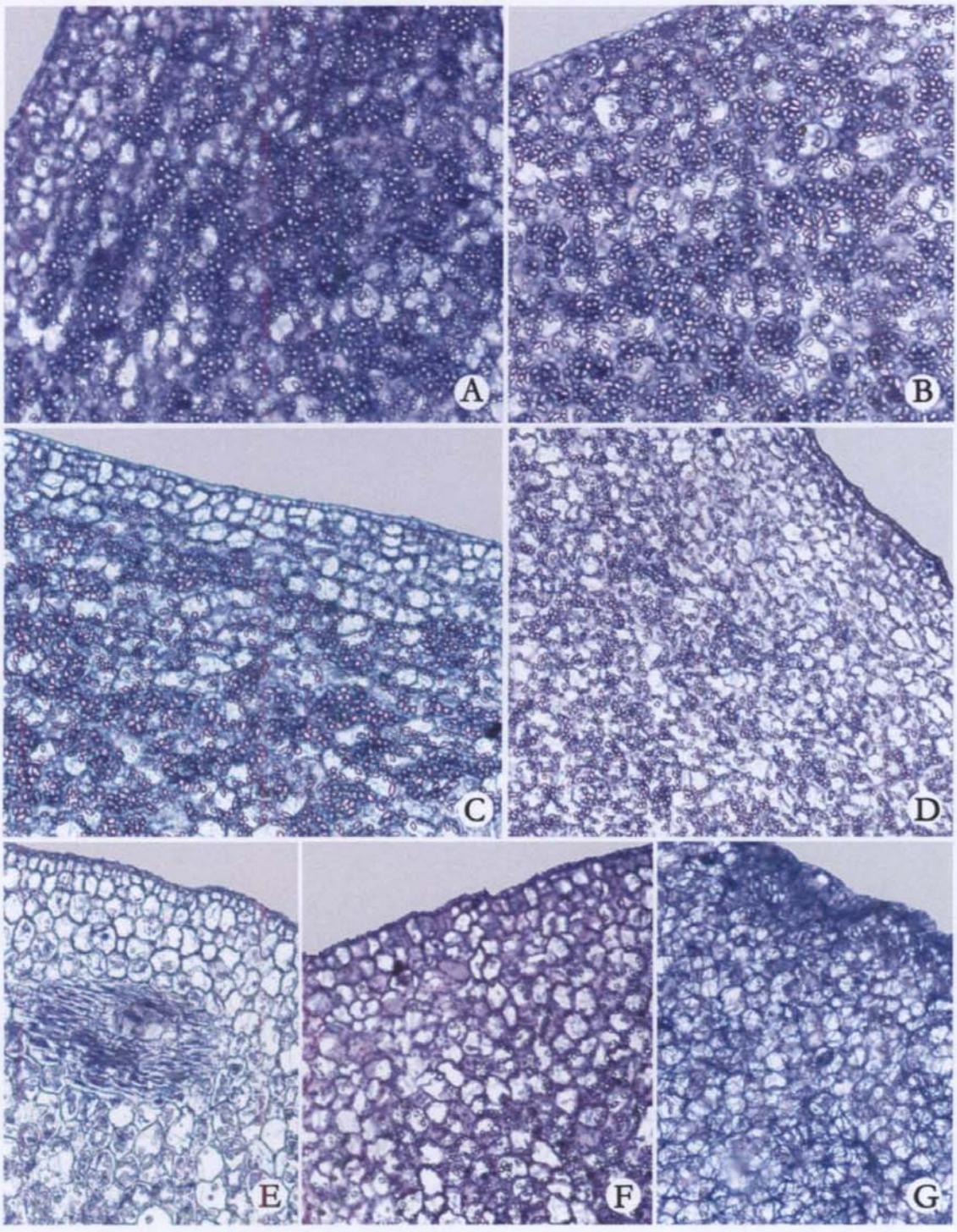


Plate 15 : Effect of desiccation on localization of protein during germination of partly ripened Mango (*Mangifera indica*) seeds. Fig. **A**: Control seeds; **B, C, D, E, F & G**: Seeds desiccated for 5, 10, 15, 20, 25, & 30 days respectively.

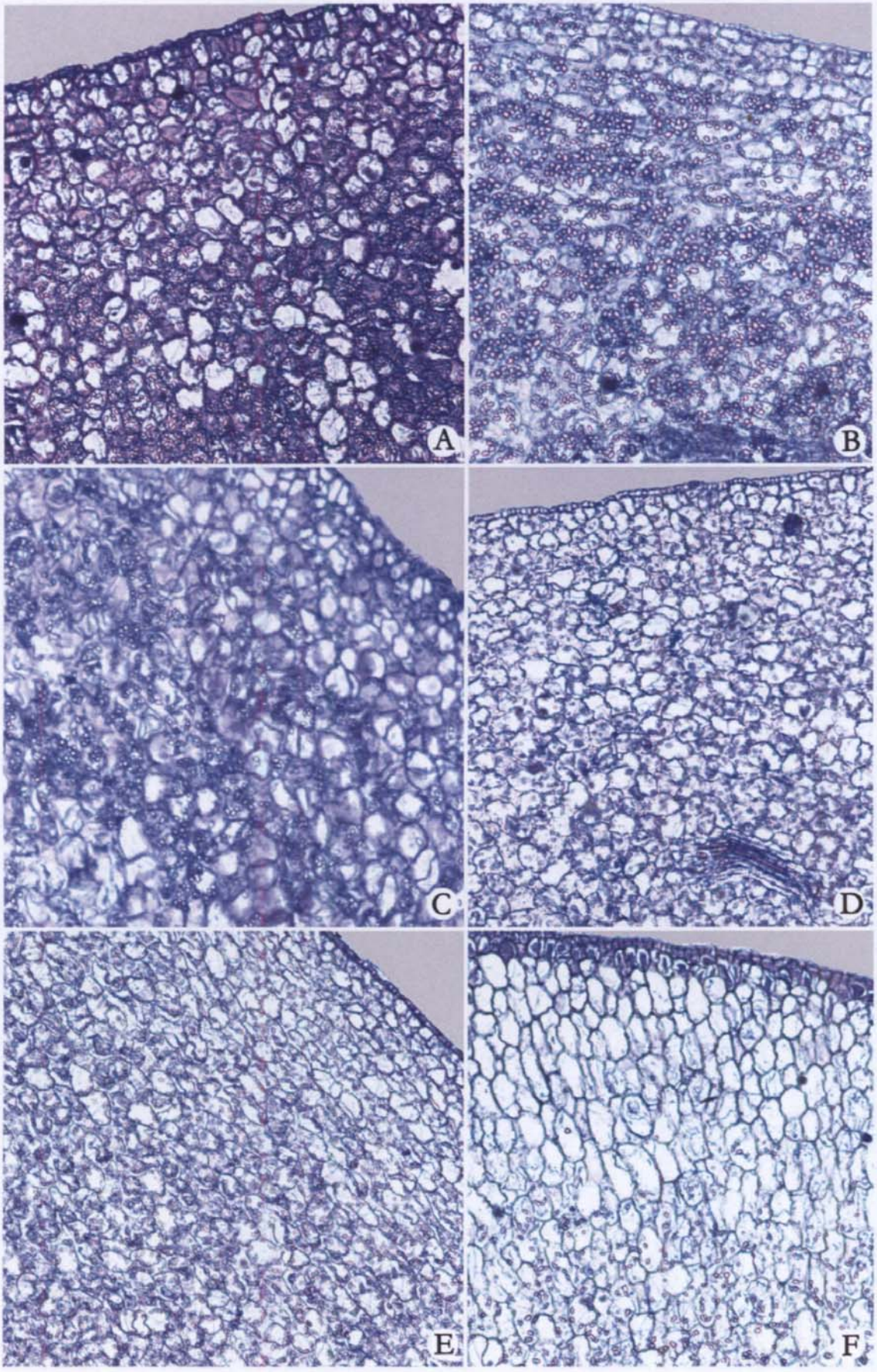


Plate 16 : Effect of desiccation on localization of protein during germination of ripened Mango (*Mangifera indica*) seeds.
Fig. **A**: Control seeds; **B, C, D, E & F** : Seeds desiccated for 5, 10, 15, 20 & 25 days respectively.

Fig. D). But the cortical cells exhibited a reduction in the staining intensity as compared to that of the epidermal cells and that of the previous stages. The frequency of protein masses in the cortical cells was lesser than the previous stages. The elongated cells of procambial strands composed of feebly stained masses of protein which was also lesser than that observed in the previous stages but slightly higher than that of cortex.

The protein content in seeds germinated after 20 days of desiccation was found decreased as compared to the previous stages (Plate 16, Fig. E). The protein masses were less in the cells of the epidermal and cortical cells and were feebly stained. In the cortical cells the protein masses were found unevenly distributed. The cells of the procambial strands were also composed of very less amount of protein masses as compared to the previous stages of desiccation and control.

The 25 days desiccated seeds on germination showed a reduction in the protein content than that of the previous stages of desiccation and control (Plate 16, Fig. F). The epidermal cells were more deeply stained than cortical cells. A few cells of the cortex composed of protein masses, other cells remain empty. The staining intensity of the procambial strands was also lesser than that of the previous stages.

6. *In vitro* studies

Very young seeds (20-25 DAA) generated callus in culture media (Plate 17, Fig. A). Cultured seed halves with the nucellus showed browning within 4 days irrespective of the culture media. Of the different concentrations of 2, 4-D, 4.52 µM was found suitable for the formation of callus and embryo (Table 26). The seed halves cultured on medium with the optimal level of 2, 4-D initiated callus between 30 days and 40 days (Plate 17, Fig. B) and was followed by the formation of embryos after 55 days (Plate 17, Fig. C). The

Table 26: Induction of callus and formation of embryos from seed halves on MS medium supplemented with different level of 2, 4-D.

2,4-D (μM)	Ovule halves inducing callus (%)	Mean embryos after 80 days
00	00	00 [c]
2.26	35	4.3 [b]
4.52	65	13.0 [a]
6.78	40	5.6 [b]
9.04	10	00 [c]
13.56	00	00 [c]
18.08	00	00 [c]

Data represents the mean of 20 replicates. Mean values followed by the same letters are not significantly different at 5% level.

callus was friable and initially cream coloured, which become brown later. The callus and the initiated embryos were confined to the micropylar region of the seed half. The medium with 4.52 μM 2, 4-D, produced a mean of 13 embryos per seed half within 80 days (Table 26). The embryos were mostly at globular and heart stages (Plate 17, Fig. C) and some embryos were progressed to cotyledonary stages (Plate 17, Fig. C). Combination of the optimal level of 2, 4-D with various concentration of BA or kinetin was not favourable for callus formation.

Friable callus with or without embryos from the initiation medium was transferred from the optimal 2, 4-D supplemented medium exhibited an enhanced frequency of callus proliferation and embryo formation (Plate 17, Fig. D). However, it depended on the concentration of 2, 4-D (Table 27). Of the different levels, MS medium with 4.52 μM 2, 4-D showed higher frequency of embryo formation, both in the case of solid as well as suspension media (Plate 17, Fig. E). About 250 mg callus induced a mean of 143 embryos. The embryos transferred to MS medium with 2.26 μM 2, 4-D facilitated secondary somatic embryogenesis (Plate 17, Fig. F). In all cases embryos of different developmental stages were present i.e., asynchronous (Plate 17, Fig. E). However, globular or heart shaped embryos were dominated. These embryos were progressed to cotyledonary stages as the culture period went on. The embryos showed variations in size, and mature embryos turned to green colour slowly and started to germinate (Plate 18, Fig. A).

6.1. Total phenolics

The estimation of total phenolic content in the embryos as well as that excreted to the medium in which the embryos were grown was 330 mg g^{-1} and 510 mg g^{-1} respectively. The total phenolics were two fold as compared to that estimated from zygotic embryos of mango.

Table 27: Induction of somatic embryos from the callii developed on MS medium with 4.52 µM 2, 4-D during first subculture to MS medium without or with different level of 2,4-D.

2,4-D (µM)	Number of somatic embryos per 250 mg callus (mean)	
	Suspension	Solid
00	10.4 [h]	7.9 [h]
0.045	18.2 [g]	12.6 [g]
0.11	27.7 [f]	19.3 [f]
0.26	38.5 [e]	24.0 [e]
0.45	56.5 [d]	37.1 [d]
1.13	98.7 [b]	54.3 [b]
2.26	143.5 [a]	98.9 [a]
3.39	76.1 [c]	45.6 [c]
4.52	19.4 [g]	13.5 [g]

Data represents the mean of 20 replicates. Mean values within columns followed by the same letters are not significantly different at 5% level. Growth period was 40 days. All media showed callus multiplication.

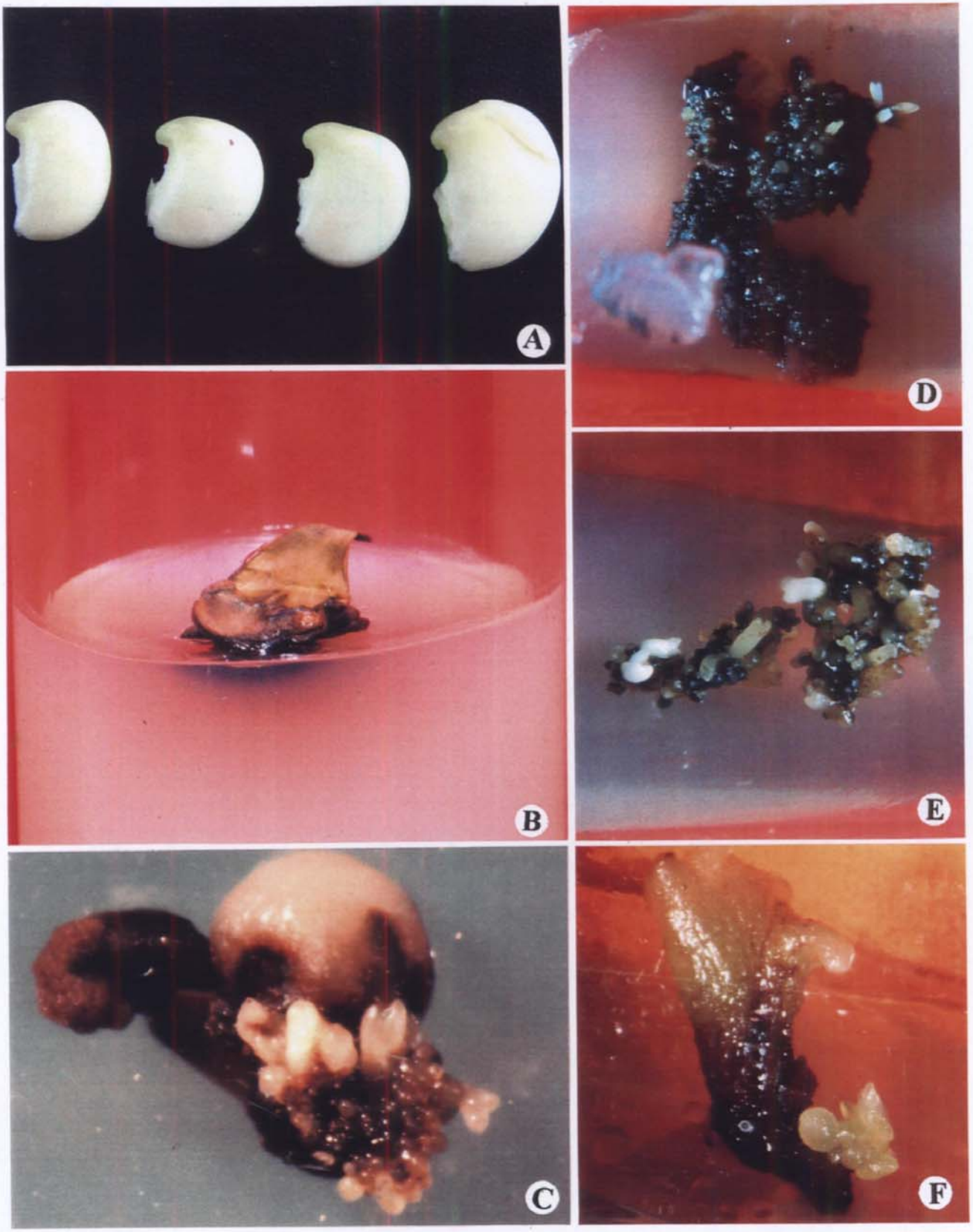


Plate17, Fig. A. Seeds used for in vitro culture (25-29 DAA), **B.** Initiation of callus from half on MS medium 4.52µ M 2,4-D after 30 days, **C.** Callus proliferation and embryo formation on MS medium with 4.52µ M 2, 4-D after 50 days, **D.** Enhanced callus and embryo multiplication during subculture on 4.52 µ M 2, 4-D, **E.** Different types of embryo on medium with 4.52 µ M 2, 4-D, **F.** Secondary somatic embryogenesis on MS medium with 2.26 µM 2, 4-D.

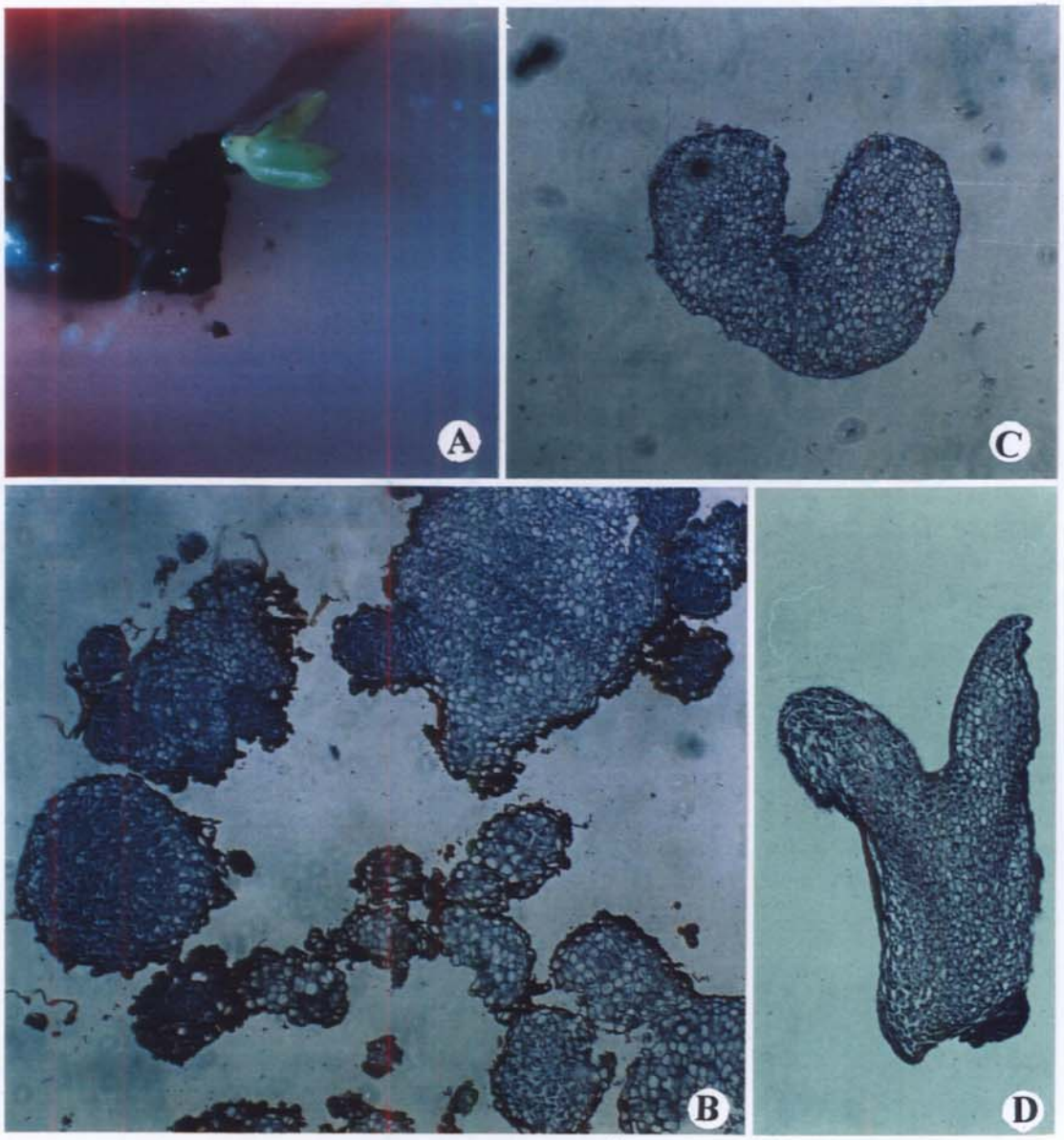


Plate 18, Fig. A. An embryo at green cotyledons.
B. Section of somatic embryos at different stages.
C. Section of a heart shaped embryo.
D. Section of cotyledonary embryo.

6.2. Histological studies

The microtome sections confirmed the embryo status. The sections showed different stages of embryos characteristic to that of dicotyledons: globular, heart, torpedo and cotyledonary stages (Plate 18, Fig. B, C and D).

Discussion

Accumulation of dry matter is a characteristic feature of orthodox seed development and the moisture content is reduced during maturation (Bewley and Black, 1985, 1994; Mayer and Poljakoff-Mayber, 1989; Baskin and Baskin, 2001). Recalcitrant seeds also undergo a phase of reserve accumulation but maturation drying is insignificant (Farrant *et al.*, 1992b, Finch-Savage and Blake, 1994). In young seeds of mango the dry weight was very low compared to other developmental stages and was found increasing in the subsequent stages of development (Table 9). Similar results were reported in *Machilus thunbergii* (Welbaum and Bradford, 1988; Lin and Chen, 1995) and in *Digitalis purpurea* seeds (Hay and Probert, 1995; Hay *et al.*, 1996) and in those plants progressive increase in dry weight during seed development and a steady decline of seed moisture content upto the period of natural seed shedding were observed. In accordance with these views in mango seeds also a steady decline in moisture content was obtained (Table 8) with a concomitant increase in dry weight.

The moisture content of mango seeds at various developmental stages such as young, mature, partly ripened and ripened varied significantly (Table 8,

Fig. 4)). Earlier studies revealed that in most recalcitrant seeds, the moisture content is getting reduced towards the end of development as observed in *Quercus robur* (Finch-Savage, 1992b; Finch-Savage and Blake, 1994), *Aesculus hippocastanum* (Tompsett and Pritchard, 1993; Farrant *et al.*, 1997), *Camellia sinensis* (Berjak *et al.*, 1993) and *Machilus thunbergii* (Lin and Chen, 1995). But Pammenter and Berjak (1999) opined that the decrease in moisture content towards the end of seed development in most of the recalcitrant seeds except *Avicennia marina* is not due to 'maturation drying' as there is no or little net loss of water. In *Avicennia marina* seeds water content remains unchanged during development because water and dry matter accumulate at the same rate due to the occurrence of soluble sugars as major reserve rather than insoluble materials (Farrant *et al.*, 1992b).

During post harvest period sensitivity towards desiccation is one of the important, in fact, well documented aspects of recalcitrant seeds (Farrant *et al.*, 1988; Berjak *et al.*, 1989). According to Pammenter and Berjak (1999) response of recalcitrant seeds to desiccation depends on the inherent characteristics of the species, developmental status of the seeds and the conditions under which the seeds are desiccated. Mango seeds collected at various stages of development, when subjected to desiccation under open room condition exhibited a decrease in moisture content with a concomitant increase in dry weight (Table 9, Fig. 5).

Since the present investigation is centered on only one species, the variability in desiccation sensitivity among seeds of different developmental/ physiological phases is elaborately studied. In recalcitrant seeds germination is considered as a continuum of development and this concept is based mainly on the observations pertaining to desiccation sensitivity and the underlying principles are firmly related to the properties of moisture content in seeds (Farrant *et al.*, 1988). According to Vertucci and Leopold (1987) and Vertucci

and Farrant (1995) the hydration level corresponding to specific water potential, the physiological status and metabolic process of recalcitrant seeds are firmly interrelated. In young seeds of mango, both initial and critical water contents were very high (Table 8, Fig. 4), but the longevity was comparatively shorter than other seed lots, presumably due to high metabolic activity resulting in dry matter accumulation at this developmental stage. Hence, desiccation stress was more in young mango seeds in which, upto 20 days of desiccation, 100% germination was noticed when the moisture content was 0.46g g^{-1}. A further reduction of moisture content resulted in adverse effect of germination.

Finch-Savage (1992a) reported that in *Quercus robur* seeds, desiccation tolerance was increased throughout development to shedding, but viability was lost at a relatively high moisture content and seeds did not pass through a fully desiccation tolerant phase. The author further stated that, desiccation sensitivity of recalcitrant seeds may be due to the premature termination of seed development, which prevents the full expression of desiccation tolerance.

According to Vertucci and Leopold (1984) and Vertucci (1989) in soybean seeds respiration rate increases with increasing water content above 15%. Conversely, Farrant *et al.,* (1997) suggested that in *Phaseolus vulgaris* decline in respiratory rate occurred due to de-differentiation of mitochondria prior to desiccation. The control seeds of young mango showed higher moisture content but as development proceded the moisture content was decreased (Table 8). A further reduction in moisture content was observed during desiccation. This result is in agreement with the views of Lin and Chen (1995), Finch-Savage (1996) and Farrant *et al.,* (1997) who stated that recalcitrant seeds are 'metabolically active' due to their higher moisture content. Those authors further suggested that in recalcitrant seeds respiratory rate is relatively higher and is reduced during desiccation. Similarly, in the present study also, desiccation is found to induce reduced respiration and

ultimately loss the viability. Berjak *et al.,* (1993) also reported that the 'metabolically active' state of recalcitrant seeds might be due to the presence of golgi bodies, polysomes and rough endoplasmic reticulum.

The occurrence of very high moisture content in mango seeds are in conformity with the views of Vertucci and Farrant (1995), who opined that at different levels of moisture content the water in seeds, has distinctly different properties and hence different chemical and metabolic processes can occur at different levels.

The metabolic processes of mature and partly ripened seeds were obviously different in such a way that biochemical changes related to senescence begin to occur in partly ripened fruits and so initial moisture content of seeds was comparatively lower. On removal of more water from partly ripened seeds critical moisture content was lower than that of mature seeds. The deleterious effect of enhanced water removal from the partly ripened seeds was accompanied by the senescence process. In other words, mature and partly ripened seeds possess different properties or mechanism of desiccation tolerance, even though the longevity is more or less similar. However, in ripened seeds viability was retained only upto 15 days of desiccation when the moisture content was reduced to 0.4g g^{-1} from 0.59g g^{-1} dry weight which is the lowest moisture content compared to the seeds of other developmental stages and these seeds were found to be more sensitive to desiccation. Similar results were obtained in *Avicennia marina* (Greggains *et al.*, 2001) and according to those authors viability declined as the seeds dried below 60% moisture content. The minimum initial moisture content of ripened mango seeds is indirectly indicative of reduced metabolism probably due to more or less completed growth and accumulation of reserve metabolites. When the seed ripens the harvest maturity is attained and hence metabolic activities

are reduced and maturation drying is getting progressed (Copeland and McDonald, 1995).

In *Machilus kusanoi* seed viability was lost when the moisture content dropped from 51.5 to 44.6 % (Chien and Lin, 1997) and Nautial and Purohit (1985a) reported that the seeds of *Shorea robusta* lost viability when initial moisture content (50%) was reduced to 25%. Similarly Anilkumar *et al.*, (1996) reported that *Aporusa lindleyana* seeds with 40% moisture content lost viability as the moisture content were reduced to 30% during a period of 36 hours storage.

Desiccation tolerance is an inherent characteristic of recalcitrant seeds of *Machilus thunbergii,* starting from the early stage of seed development (Lin and Chen, 1995). According to those authors mature seeds of *M. thunbergii* showed maximum tolerance and seeds of all developmental stages lost their viability completely during desiccation for 30 days. In mango seeds also maximum desiccation tolerance was exhibited by mature and partly ripened seeds and these seeds started to loss viability after 25 days of desiccation. Nevertheless, ripened seeds were more sensitive to desiccation. Finch-Savage (1992b) suggested that loss of viability in *Quercus robur* seeds could be resulted from ageing during drying, which is unavoidably long because of their large size and resistance to drying.

Comparatively rapid loss of viability in ripened mango seeds during desiccation in comparison with young and mature seeds can be attributed to the accelerated ageing of already aged (ripened) seeds. In addition to ageing, comparatively lower moisture content also contributes to the rapid desiccation of ripened mango seeds, whereas young and mature seeds are characterized by lack of ageing and occurrence of surplus moisture content, which require prolonged duration for drying.

Cent percentage germination in mango seeds of all the developmental stages revealed that even the young seeds acquire the physiological maturity to germinate and unlike orthodox seeds no desiccation is required to start the germination related events.

Seed vigour index (SVI) of mango seeds was found maximum on 5th day and 10th day after desiccation in young and mature seeds respectively whereas partly ripened and ripened control seeds showed maximum seed vigour index (Table 2, Fig. 2). These results revealed that at the earlier two stages of development (i.e., young and mature) the mango seeds possess high moisture content and slight desiccation is required to get more vigorous seeds. The high seed vigour index was due to the distribution of more or less uniform seed germination during a short period. This observation is in agreement with the results obtained by Xia *et al.,* (1992) and according to them the seed vigour index of just harvested lychee (*Lychi chinensis*) and longan (*Euphorbia longan*) seeds could be increased only by slight desiccation. Conversely, Chaitanya *et al.*, (2000) reported that in *Shorea robusta*, dehydration of seeds during storage resulted in loss of seed vigour due to rapid decline in protein content and subsequently the loss of viability.

Seedling vigour analysis is used to categories the seedling qualities by considering one or more quantifiable parameters that are common and sensitive to the seedlings (Copeland and McDonald, 1995). In the present study, total biomass per seedling was taken as the parameters for seedling vigour analysis. Least value of seedling vigour was shown by young seeds and desiccation resulted in only very slow increase (Table 7, Fig. 3). Partly ripened and ripened seeds exhibited maximum vigour and only negligible reduction was occurred during desiccation. Mature seeds showed intermediate values of seedling vigour. These data reveals that seed maturity is essential for the establishment of healthy seedlings in recalcitrant seeds eventhough young

seeds are cent percent germinable. Nevertheless, in all seed samples except ripened, desiccation induced an increase in seedling vigour during a period of 5 days. According to Xia *et al.*, (1992) and Pritchard *et al.*, (1995) seed vigour and germination rate may be increased during early period of desiccation in recalcitrant seeds. In mango seeds the seedling vigour is getting enhanced during early days of desiccation. After 5 days all seeds showed reduced seedling vigour probably due to seed deterioration. Hence it is concluded that seedling vigour is influenced by both maturity and desiccation in mango seeds. Seedling vigour reduction due to seed quality deterioration has been reported in recalcitrant seeds (Fu *et al.*, 1990).

In the present study it is found that as seed development proceeds, the time taken for initiation and completion of germination are decreased (Table 3, 4, 5, and 6). The control seeds of young and mature samples required same number of days to start and complete germination. But desiccation resulted in an early germination, when the period of desiccation was increased the number of days required for initiation of germination also was increased. According to Farrant *et al.,* (1985) desiccation could affect the viability of recalcitrant seeds in such a way that slow drying of *Avicennia marina* seeds could permit initiation of germination. According to Pammenter and Berjak (1999) dehydration-associated damages in desiccation-sensitive seeds may result in the increase of germination lag which may be due to damages of desiccation stress as well as damages of rehydration.

Starch content in mango seeds was found increased with maturation and ripening (Table 10, Fig. 6). Bhattacharya *et al.*, (2002) reported increased starch content in the seeds of *Camellia sinensis* and according to those authors the accumulation of starch in the seeds resulted in maximum dry weight at mature stage. Similarly, in the present investigation also the dry weight was increased with maturation and ripening and accumulation of starch also

contributes to the increase in dry weight. The seeds of all developmental stages exhibited a gradual increase in starch content when subjected to desiccation. This result is in agreement with the views of Farrant *et al.*, (1985) who noticed accumulation of starch in plastids of *Avicennia marina* seeds during desiccation.

Histochemical localization of starch during various developmental stages of mango seeds revealed that the starch content in terms of grain number and size of starch grains was increased as development proceeds (Plate 1 to 4, Fig. A). The grain number and size of young and mature seeds remain more or less same. The starch in young mango seeds was increased during desiccation in terms of grain number and size (Plate 1, Fig. A). More or less similar distribution pattern was observed in other developmental stages also. These data indicate that during late stages of development and desiccation, metabolism of mango seeds is very feeble resultantly starch and dry weight accumulation is presumed to occur due to loss of moisture content. Similarly, during desiccation total sugar content also registered only negligible fluctuation. Another interesting observation is that this pattern of carbohydrate accumulation was continued in mango seeds until the loss of viability.

Histochemical localization of insoluble polysaccharides by PAS reaction revealed that cell wall thickening and staining intensity are increased in desiccated seeds compared to the control seeds (Plate 1 to 4). This observation can be correlated with more lignified cell walls formed during desiccation (Ingram and Bartels, 1999). Phenolics are known to be increased and located in cell walls in desiccated seed tissues of *Brassica napus* (Zobel *et al.*, 1989). Hence, in mango seeds desiccation results in enhanced phenolic content (Table 17, Fig. 11) and more thickening of cell walls (Plate 1 to 4, Fig. A to G).

Fructose is the prominent sugar in young mango seeds which decreases during maturation (Table 11a). In *Machilus thunbergii,* Lin and Chen (1995)

observed a decrease in the fructose content during the late stages of seed development. Similarly, Steadman *et al.,* (1996) also suggested that very low quantity of monosaccharides occur in various recalcitrant seeds compared to orthodox seeds. Contradictory to this, the present author noticed an increase in fructose content in the late stages of seed development in mango.

On desiccation, the fructose content was increased during the early stages and then declined gradually in young seeds (Table 11a). Gradual increase in fructose content was noticed in the samples of all other subsequent stages of development throughout the period of desiccation.

Irrespective of the developmental stages, abundance of the reducing sugars, such as glucose and fructose was observed in mango seeds (Table 11a). Leprince *et al.*, (1990a) reported that soluble sugars are involved in the process of acquisition of desiccation tolerance during maturation of *Brassica campestris* seeds. In mango seeds during desiccation the sugars are getting increased and at the brim of viability loss, further increase was observed (Table 11a). This trend is in conformity with the views of Leprince *et al.,* (1993), who suggested that desiccation intolerant seeds are generally endowed with large concentration of monosaccharide and low concentration of di- and high-saccharides. According to those authors the accumulation of sucrose and oligosaccharides is related with desiccation tolerance, albeit, absence or occurrence of very low amount of monosaccharides (sugars, such as glucose and fructose) are characterized by desiccation tolerance.

Distribution profile of monosaccharide (fructose and glucose) in mango seeds during desiccation showed that all seed samples except young seeds, a continuous increase being maximum in desiccation tolerant samples after 25/30 days of desiccation. Leprince *et al.*, (1992) found a significant correlation between increased respiratory rate and increased monosaccharide concentration in germinating maize radicle and suggested that the latter could regulate the

former. According to those authors these correlation might have some significance in the loss of desiccation tolerance. Leprince *et al.*, (1990b) opined that impairment of respiration is occurred during desiccation and this process may induce electron leakage from respiratory chains which in turn could initiate lethal peroxidative damage. Free-radical generation as a consequence of dehydration during desiccation of recalcitrant seeds has been well documented (Smith and Berjak, 1995; Come and Corbineau, 1996; Pammenter and Berjak, 1999). In germinating maize seeds also loss of desiccation tolerance is controlled by a system of free-radicals and free radical processes (Leprince *et al.*, 1990b).

Monosaccharides are purely effective agents in promoting the formation of glassy cytoplasm (Koster, 1991) who suggested that glass formation depends on particular combinations of sugars as well as different temperature regimes.

In young mango seeds desiccation tolerance was maintained upto 20 days and a simultaneous increase in the quantity of raffinose family of oligosaccharides was obtained (Table 11b, Fig. 9). Distributions of raffinose family of oligosaccharides and desiccation tolerance have been reported in soybean and maize (Koster and Leopold, 1988; Chen and Burris, 1990; Blackman *et al.,* 1992). Apart from this, raffinose family of oligosaccharides has been proposed to play roles in desiccation tolerance and cold acclimatization (Bachmann *et al.,* 1994). The ratio of raffinose family of oligosaccharides to sucrose is comparatively low in young mango seeds i.e., 0.1 (Table 11). During desiccation, the raffinose family of oligosaccharides is getting increased upto 20 days of desiccation, after which the seed viability was lost and these sugars are significantly reduced resulting in an increase in the ratio of oligosaccharides to sucrose from 0.1 to 0.3. Generally, in recalcitrant seeds the ratio of oligosaccharides to sucrose comes between 0.083 to 0.143, whereas in majority of orthodox seeds the values come above 0.143 (Steadman

et al., 1996). In mango seeds it is evident that the young seeds are characterized by typical recalcitrant nature because the ratio comes around 0.1, but during desiccation this ratio is increased presumably due to "drying shock" that normally occur in orthodox seeds, where the ratio is comparatively higher. The viability loss of mango seeds was coincided again with a significant depression of the ratio of oligosaccharides to sucrose. Vastly lower values of ratio of oligosaccharides to sucrose have been reported as a result of the presence of minute quantities of oligosaccharides, in many species inclusive of *Quercus robur* and *Q. rubra* (Finch-Savage *et al.,* 1993; Sun *et al.,* 1994). According to Chen and Burris (1990), Leprince *et al.,* (1990b) and Ooms *et al.,* (1993) in developing orthodox seeds the oligosaccharides to sucrose ratio is less than 0.083 due to the lack of acquisition of desiccation tolerance.

The raffinose family of oligosaccharides in mature mango seeds exhibited a two-fold increase compared to young seeds and thus the ratio of oligosaccharides to sucrose is also increased nearly double since sucrose remained almost unchanged (Table 11a and 11b). Desiccation promoted the production of oligosaccharides significantly up to 20 days. So the ratio of oligosaccharides to sucrose also showed a significant increase. In 25 days desiccated seeds, the raffinose family of oligosaccharides was reduced to half of 20 days desiccated seeds. The ratio of oligosaccharides to sucrose declined to 0.14. This indicates that the comparatively more desiccation tolerance of mature seeds than other seed lots is presumably due to the maintenance of the ratio i.e., 0.083 to 0.143 as suggested by Steadman *et al.,* (1996). The loss of viability of mature mango seeds is found to be coincided with rapid decline of oligosaccharides to sucrose ratio (Table 11b). Disappearence of oligosaccharides and resultant reduction in seed longevity was reported in *Impatiens wallerina* and *Capsicum annuum* (Buitink *et al.*, 2000). In bean (*Phaseolus vulgaris*) seeds, ratio of oligosaccharides to sucrose was reported as unity when the seeds became tolerant to drying since the seeds are orthodox

(Bailly *et al.*, 2001). It is interesting to note that a positive correlation can be drawn between young and mature seeds in terms of moisture content and the ratio of oligosaccharide to sucrose, inspite of the difference in storability/longevity, which was more in mature seeds.

Maximum content of raffinose family of oligosaccharides was observed in partly ripened control seeds and was increased during desiccation (Table 11a). The ratio of oligosaccharides to sucrose is 0.13 in control seeds (Table 11b) and this is in accordance with the views of Steadman *et al.*, (1996). Due to desiccation, the increase of oligosaccharides in partly ripened seeds was resulted in a higher ratio of oligosaccharides to sucrose after 25 days and this finding is positively related to maximum longevity observed in this seed sample. A more or less similar trend in the distribution of oligosaccharides to sucrose ratio was observed in ripened seed samples upto 25 days of desiccation and afterwards sudden fall in the ratio coinciding with a complete loss of viability was occurred.

Accumulation of raffinose is known to be associated with desiccation tolerance in seeds (Buchanan *et al.*, 2001). In mango seeds raffinose content is getting increased during desiccation (Fig. 8) but when the viability is lost, significant reduction of raffinose occurs in all seed samples. Those authors suggested that for prolonged longevity of mature seeds, accumulation of raffinose is essential. The ratio of sucrose to raffinose might be a determining factor for maintenance of desiccation tolerance, the ratio must be not greater than 20:1. In the present study, the ratio of sucrose to raffinose is coming below this value (Table 11b). But it is evident that when viability is completely lost (25 and/or 30 days desiccated seeds) due to desiccation intolerance and occurrence of very high ratio of sucrose to raffinose in young and ripened mango seeds correlated with shorter longevity. As mentioned earlier mature and partly ripened seeds are having maximum desiccation

tolerance and the ratio of sucrose to raffinose is falling under 20:1. Raffinose appears to prevent crystallization of sucrose during cell dehydration allowing the cytoplasm to retain a stable, glassy state (Leopold and Vertucci, 1986; Leopold *et al.*, 1994).

More or less uniform or regular increase in sucrose content was observed in all seed samples except ripened seeds during desiccation (Table 11b, Fig. 7). Sucrose can assume a protective role by disaccharide-lipid interaction as shown in artificial membrane systems (Crowe *et al.*, 1986, 1987, 1988). The induced synthesis of sucrose in mango seeds during desiccation over a period of several days indicates that some maturation-specific metabolic activities are quickly induced and may be a common phenomenon of recalcitrant and orthodox seeds. In *Machilus thunbergii* glucose and fructose were decreased during late stages of seed development and sucrose showed accumulation in the last two stages (Lin and Chen, 1995) owing to the acquisition of desiccation tolerance as reported in orthodox seeds (Leprince *et al.*, 1993). This condition is similar to the induction of sucrose in developing seeds of soybean (Blackman *et al.*, 1992).

During desiccation of young mango seeds a continuous increase of sucrose content was noticed with a ten-fold increase in seeds desiccated for 5 days (Fig. 7). Physiological significance of sucrose synthesis in developing seeds of soybean undergoing slow drying has been investigated (Blackman *et al.*, 1992). According to Lahuta *et al.*, (2000) in orthodox seeds like *Vicia faba* an increased production of sucrose was occurred during desiccation.

Sugars in general, sucrose in particular have bean proposed to play an important role in conferring desiccation tolerance in seeds (Lin and Chen, 1995). Those authors further stated that eventhough the significance of sucrose induction during slow drying is not clear. Sucrose can assume a protective role against desiccation injury and this is in agreement with the views of Crowe *et*

al., (1986, 1987, 1988) and Hoekstra *et al.*, (1990) who suggested that in artificial membrane systems sucrose plays a protective role by the interaction of this disaccharide with lipids by hydrogen bonding of -OH group with the polar head groups of membrane phospholipids. Studies on the comparative effect of sugars on desiccation tolerance showed that soluble sugars such as trehalose was found to be the best protective role in animal tissues (Crowe *et al.*, 1988). According to Koster and Leopold (1988) and Leprince *et al.*, (1990a) given the absence of trehalose in seeds, other soluble sugars such as sucrose, glucose and oligosaccharides assume the same protective role in membrane as reported earlier (Steadman *et al.*, 1996). Disaccharides including sucrose, maltose and raffinose are found to be better protective agents of membranes during desiccation. These protective roles of sugars during desiccation include protein stabilization and protein sugar interaction (Carpenter *et al.*, 1987, 1990).

In addition to the protection mechanism played by sugars on membrane integrity, cytoplasm also is subjected to be protected by the sugar content. According to Leopold (1990) extreme desiccation of cytoplasm could result in crystallization of proteins and solutes which induce severe injury to the cell. Burke (1986) suggested a hypothesis for cytoplasmic protection by vitrification or glass formation within the cytoplasm. Due to virtificcation during desiccation, molecular diffusion is impeded, so chemical reactions are strongly slowed if at all inhibited. Consequently, degradative processes are prevented. Vitrification prevents crystallization of cytoplasmic solutes and prevents thermal changes of water (Leopold, 1990).

Distribution of sucrose content and the pattern of accumulation during desiccation in mango seeds are agreeable with the hypothesis that, sucrose serves as a protective agent against desiccation stress. Sucrose has been found to be associated with desiccation tolerance in pollen and seeds (Hoekstra *et al.*,

1989, 1994). In young mango seeds the induction of sucrose synthesis during the early days of desiccation is found to be directly related to desiccation tolerance and this accumulated condition of sucrose is continued until the viability is lost. Similarly, in mature seeds also the same trend is seen except the absence of sudden induction during desiccation for 5 days and an enhancement of sucrose content compared to the young seeds throughout the desiccation period. Here a positive correlation of longevity and sucrose content in mature mango seeds is very evident. Control samples of partly ripened seeds contained very high sucrose content compared to the previous stages but during desiccation an induction of sucrose synthesis is not obvious, albeit, slight reduction occurred during viability loss. Contradictory to these developmental stages, in ripened mango seeds sucrose content was comparatively low and only slight changes were noted during desiccation. In this case correlation can only be drawn between the lower sucrose content and shorter period of longevity.

The protein content in mango seeds was comparatively low in control seeds, maximum protein content was detected in the mature seeds and a decrease was noticed during ripening (Table 12, Fig. 10). Studies on a variety of desiccation tolerant seeds, such as barley (Bartels *et al.*, 1988), maize (Bochicchio *et al.*, 1988) and soybean (Blackman *et al.*, 1991) showed that a set of dehydration inducible, hydrophilic proteins (Late Embryogenesis Abundant proteins or dehydrin-like proteins) are produced and accumulated during the late stages of seed development. Some of these proteins have been implicated in the mechanism of seed desiccation tolerance prior to maturation drying in orthodox seeds (Kermode, 1990; Galau *et al.*, 1991; Ried and Walker-Simmons, 1993; Oliver and Bewley, 1997). The occurrence of Late Embryogenesis Abundant proteins and dehydrin-like proteins in recalcitrant seeds is anomalous. Their presence in some recalcitrant seeds such as *Quercus robur* (Finch-Savage and Blake, 1994; Gee *et al.*, 1994; Farrant *et al.*, 1996)

has been detected. Similarly, Bradford and Chandler (1992) and Still *et al.*, (1994) identified the Late Embryogenesis Abundant proteins or dehydrin-like proteins in seeds of *Zizania palustris*, a temperate species, which is considered to be recalcitrant (Vertucci *et al.*, 1994).

In contrast, no new proteins are produced during late stages of development in the highly desiccation sensitive seeds of *Avicennia marina* (Farrant *et al.*, 1992a) and dehydrin-like proteins could not be demonstrated in the recalcitrant seeds of 10 tropical wetland species (Farrant *et al.*, 1996), eventhough those authors did identify dehydrin-like proteins in the recalcitrant seeds of a number of tropical and temperate non-wetland species. But Han *et al.*, (1997) observed that dehydrin-like proteins are absent in the seeds of *Trichilia dregeana*, a tropical recalcitrant tree, during development. In the present investigation also the occurrence of reduced protein content in the ripened seeds compared to the other developmental stages, may be due to the lack of the synthesis of Late Embryogenesis Abundant proteins (dehydrin-like proteins) at this stage.

Irrespective of the stages of development, mango seeds produced more protein during desiccation (Table 12, Fig. 10). This indicates that some specific metabolic activities are occurred on desiccation. Lin and Chen (1995), suggested that in *Machilus thunbergii* the increased production of protein during desiccation over a period of several days, indicate that some maturation specific metabolic activities are quickly achieved and may be a common phenomenon of recalcitrant seeds. Contrary to this Nautiyal and Purohit (1985b) and Chaitanya *et al.*, (2000) observed a rapid decline in protein content in seeds of *Shorea robusta* under desiccation and ultimate loss of viability. Farrant *et al.*, (1985) reported an increased protein synthesis in recalcitrant seeds of *Avicennia marina* immediately after shedding and the metabolic

activities in recalcitrant seeds in general, at the stage of shedding are similar to those occurring during early stages of germination.

As mentioned earlier, some specific dehydrin-like proteins inclusive of Late Embryogenesis Abundant proteins are synthesized during maturation drying in orthodox seeds. But in recalcitrant seeds, maturation drying does not occur normally. The embryonic axes of *Zizania palustris* seeds accumulate dehydrin-like proteins during dehydration at 5°C, a treatment which is detrimental to the survival (Bradford and Chandler, 1992). According to those authors, the presence of such proteins alone was not sufficient to avoid desiccation induced injury. Similarly, Blackman *et al.*, (1991) came to a conclusion that boiling stable proteins in soybean seeds was accumulated when the seeds under high relative humid conditions, which enabled them to be intolerant to desiccation. But according to Finch-Savage and Blake, (1994) the increase in desiccation tolerance in *Quercus robur* seeds might be due to the presence of dehydrin-like protein. In the present study, total protein content of the seed samples of all stages of development was increased during desiccation (Table 12). Eventhough individual protein profile was not elucidated, the enhancement of total proteins may be due to the *de novo* synthesis of proteins which never induce the seeds to be intolerant to desiccation after 25 days of storage.

Senaratna *et al.*, (1987) reported that desiccation resulted in a decrease of protein content in soybean seeds and qualitative changes also have been detected during lethal desiccation, leading in loss of thiol groups of membrane proteins. According to those authors apart from changes in lipid membrane protein, qualitative and quantitative changes of proteins have been associated with desiccation induced injury in seeds.

Histochemical localization of proteins of desiccated mango seeds revealed that irrespective of the developmental stage, the staining intensity for proteins

was found increased throughout the period of desiccation (Plate 5 to 8). This is in accordance with the results obtained in *Zizania palustris* (Bradford and Chandler, 1992) and *Machilus thunbergii* (Lin and Chen, 1995), who suggested an increased production of protein during desiccation. This indicates that desiccation promotes the production/synthesis of some specific proteins pertaining desiccation tolerance.

Generally, amino acid distribution in mango seeds during different stages of development did not show any significant qualitative or quantitative variations (Table 13 to 16). In young mango seeds, free amino acid distribution is very low and protein content also is very low. As mentioned earlier, these tissues are metabolically very active despite the attainment of physiological maturity. So comparatively lower concentration of free amino acids and total protein content is due to the high metabolic activity. High protein and amino acid content at maturity can also be correlated with regulation in metabolic activities, whereas in partly ripened seeds maximum amino acid content and comparatively low protein content were observed. These observations are indicative of the protein degradation resulting in the accumulation of free amino acids, which are apparently not catabolized. In ripened seeds both free amino acids and total proteins are very low, probably this is related to metabolic activities of senescence during the ripening processes.

When a comparison is made between mature and partly ripened seeds in the distribution of free amino acids, almost similar pattern, except the disappearance of threonine and tyrosine in mature seeds and phenylalanine in the partly ripened seeds is observed towards the late stages of desiccation. Since, both the seeds are having similar desiccation tolerance, the occurrence of free amino acid distribution is presumed to be involved in this process. The mechanism of this process is correlated with the distribution of phenolics and is

being discussed under phenolics. Almost the same correlation can be drawn in young and ripened seeds, where desiccation tolerance is more or less uniform. In the present study a remarkable observation is the disappearance of cystein, histidine, phenylalanine, threonine and tyrosine in young seeds and cystein, histidine, phenylalanine and tyrosine in ripened seeds.

Proline is presumed to have a vital role in desiccation tolerance of plant tissues in general. In the present study progressive reduction of proline content was observed in all seed samples during desiccation (Table 13, 14, 15 and 16). One of the reasons may be the impaired synthesis of proline in seeds desiccated during the period of 20-30 days, due to low water potential, as reported by Poulsen and Eriksen (1992), according to whom, the reduced proline content is involved in drying process of plant tissues. The involvement of proline in the maintenance of desiccation tolerance is not at all relevant in mango seeds, since only significant reduction is occurred during desiccation. The lack of accumulation of proline may be one of the reasons for the recalcitrant nature of mango seeds.

Phenolics are heterogonous group of secondary metabolites which are originated in the endoplasmic reticulum and located in cell vacuoles (Parhan and Kaustinen, 1977) and also located in cell walls (Zobel *et al.*, 1989). Considerable amount of phenolics was present in the control seeds of all the developmental stages of mango and maximum in mature seeds (Table 17, Fig. 11). In young and ripened seeds during desiccation gradual increase was observed, maximum in seeds desiccated for 25 - 30 days. Free amino acids such as, phenylalanine and tyrosine are found to be disappeared during desiccation of these seeds and so a correlation can be drawn between the disappearance of phenylalanine and tyrosine, since phenolics are derived from these amino acids through phenyl propanoid pathway. Phenolics can help defense against various stresses/pathogens and act as antioxidants in seeds and

enhanced phenolics content in the cotyledon and maximum content was present in nonviable seeds (Anilkumar, 1998).

Studies on germination/reserve mobilization in recalcitrant seeds are scanty whereas in orthodox seeds metabolism of seed germination is well documented (Khan, 1977; Bewley and Black, 1983, 1985, 1994; Mayer and Poljakoff-Mayber, 1989; Copeland and McDonald, 1995; Baskin and Baskin, 2001). Nevertheless, the present author made an attempt to elucidate the impact of desiccation on the metabolism of germination in mango seeds which is included under recalcitrant category. A detailed study of germination process at comparable intervals is not attempted because the main objective of this investigation is to elucidate the effect of desiccation on the distribution of metabolites in mango seeds.

Sample collection of germinated seeds (seedlings) was done only once i.e., 10^{th} day after germination and since the seedling was only differentiated into plumule, radicle and cotyledon, only cotyledons were sampled to compare the reserve mobilization pattern of control and desiccated seeds.

The starch content of germinated control samples of all developmental stages of mango seeds did not show much variation compared to its control seeds (Table 18, Fig. 12). During germination in orthodox seeds starch is depleted due to hydrolytic reactions. In fresh seeds of mango (control) germination associated changes are presumed to be occurred. Since mango seeds are categorized as recalcitrant in which germination is continuum of development (Farrant *et al.*, 1988). According to Berjak *et al.*, (1993) seeds like *Camellia sinensis* are metabolically active because of the hydrated condition of the seeds and the presence of golgi bodies, polysomes and rough endoplasmic reticulum. The histochemical localization of starch also confirmed more or less uniform distribution in control and germinated seeds (Plate 9 to 12). The desiccated seeds during germination exhibited a decrease in starch

seeds (Table 18, Fig. 12). During germination in orthodox seeds starch is depleted due to hydrolytic reactions. In fresh seeds of mango (control) germination associated changes are presumed to be occurred. Since mango seeds are categorized as recalcitrant in which germination is continuum of development (Farrant *et al.*, 1988). According to Berjak *et al.*, (1993) seeds like *Camellia sinensis* are metabolically active because of the hydrated condition of the seeds and the presence of golgi bodies, polysomes and rough endoplasmic reticulum. The histochemical localization of starch also confirmed more or less uniform distribution in control and germinated seeds (Plate 9 to 12). The desiccated seeds during germination exhibited a decrease in starch content, which was very significant in comparison with that of the respective control seeds (Plate 9 to 12, Fig. A). Similar results were obtained in *Camellia sinensis* (Monneri *et al.*, 1986) and *Hopea parviflora* (Kamarudeenkunju, 2003). Histochemical localization of starch grains in the cotyledonary tissues of desiccated seed when germinated showed a reduction in starch grain size and number, which were found to be increased during desiccation. The synthesis of starch in mango seeds during desiccation may be due to the reduction of moisture content leading to an increased osmoticum, which may imbalance the cellular activity. Hence, starch synthesis involves the utilization of sugars and osmoticum is maintained. But during germination due to rehydration starch hydrolysis and resultant sugar synthesis is occurred leading to starch depletion.

During 10^{th} day of germination of young control seeds, maltose and oligosaccharides like stachyose and rhamnose were increased. This result is agreeable with the views of Bewley and Black (1994). Contrary to earlier reports of sucrose depletion during germination (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994), sucrose content in mango seeds is increased during germination. This may be either due to feeble mobilization or enhanced synthesis of sucrose in the cotyledons. A concomitant reduction of starch is also observed (Table 18).

The young seeds desiccated for 10 to 30 days exhibited a trend of reduction in fructose, glucose, stachyose and sucrose, whereas rhamnose content was increased compared to their respective controls (desiccated). The significant reduction of sugar during germination of desiccated seeds reveals the enhanced catabolism due to desiccation-induced stresses leading to ageing (Pammenter and Berjak, 1999). The distribution of starch in mango seeds also showed more or less similar reduction (Table 18, Fig. 12). Raffinose, which was abundant in desiccated samples was completely absent in germinated seeds (Table 19). The role of raffinose in desiccation stress is well documented owing to their function as prevention of sucrose crystallization and glassy state of cytoplasm (Crowe *et al.*, 1986, 1987, 1988; Koster and Leopold 1988; Steadman *et al.*, 1996). Disappearance of this sugar during germination reveals the removal of desiccation stress due to rehydration prior to germination. Absence of raffinose is reported in many cereals and legumes during germination (Bewley and Black, 1994).

Desiccated samples of mature seeds during germination behaved almost similar to that of young seeds, except in the reduction of sucrose and absence of stachyose (Table 19). This reveals the more enhanced mobilization to the growing axes and this coincides with the establishment of more vigorous seedlings (Table 4). The disappearance of stachyose indicates manifestation of desiccation intolerance occurred during germination as reported in many seeds (Bewley and Black, 1994).

In ripened mango seeds, desiccation resulted in significant reduction of all soluble sugars inclusive of raffinose family of oligosaccharides. During germination of this desiccated seeds, all soluble sugars except sucrose were disappeared presumably revealing the utilization as respiratory substrates and/or mobilization of these soluble sugars to the growing seedling. In other words, it is clear that maximum starch content is getting utilized (mobilized)

and all soluble sugars are exhausted during the germination of desiccated seeds in which seedling vigour was continuously reduced during a very short period of 5 days of desiccation and longevity was found to be minimum.

During germination, a major event in the storage tissue is the mobilization of stored reserves, which require participation of many enzymes. For the mobilization of storage proteins, they must be hydrolyzed and the enzyme required for the hydrolysis is present in the seeds or synthesized *de novo* (Bewley and Black, 1994). The liberated amino acids are transported from the cotyledons to the growing seedlings.

Mango seeds are not protein rich, but during desiccation protein content is increased (Table 12). Notwithstanding, mobilization of protein during germination for a period of 10 days is getting retarded in desiccated seeds compared to the control (Table 20, Fig.13). When a comparison is made during germination of desiccated and their respective control seeds (ungerminated), a gradual increase in the ratio of protein content clearly indicates the significant enhancement of protein mobilization in desiccated seeds. Protein content of nonviable seeds also is very low probably due to more catabolic reactions leading to ageing and death (Pammenter and Berjak, 1999).

The histochemical localization of proteins of germinated mango seeds during desiccation showed a gradual reduction (Plate 13 to 16). This may be either due to mobilization and/or leaching out of nitrogenous compounds by the disintegration of cell wall on desiccation. Nautial and Purohit (1985a) opined that desiccation results in the loss of membrane permeability and hence more leachate constituting of sugars, proteins and inorganic phosphates are extruded at a fast rate. In the present study also the author observed loss of membrane integrity in the late stages of desiccation of all the developmental stages of mango seeds.

After 10 days of germination the amino acid content of young control seeds showed only negligible qualitative and quantitative changes (Table 21). But seeds desiccated for 5 days exhibited a significant quantitative increase. More or less same trend was observed upto 15 days of desiccation. During the entire period of desiccation upto 30 days cysteine and aromatic amino acids such as phenyl alanine, threonine and tyrosine were absent, but reappeared during germination. During the period i.e., when desiccation sensitivity was expressed, significant quantitative increase of total free amino acids were occurred. The reason for reappearance of the above amino acids such as cysteine, phenylalanine, threonine and tyrosine is their synthesis during rehydration of desiccated seeds prior to germination. Another important observation in the distribution of total amino acids during germination is significant increase of free amino acids, which may be formed due to hydrolysis of abundant quantity of proteins, which are presumed to be accumulated during desiccation (Table 12). The accumulation of the free amino acids may also be due to inhibition of mobilization as a result of reduced vigour already observed during desiccation.

Almost same pattern of distribution of amino acids are seen in ripened mango seeds on germination also (Table 24), whereas mature and partly ripened seeds showed only quantitative increase during germination (Table 22 and 23). These seeds are comparatively desiccation tolerant in terms of longevity, germination percentage and seed vigour index. But the significant increase of free amino acids in desiccated samples of mature and partly ripened seeds indicates the impaired mobilization of amino acids during germination inspite of their desiccation tolerance.

The desiccated seeds on germination exhibited sharp decline in the concentration of phenolic content of mango seeds in comparison with their respective ungerminated controls i.e., desiccated seeds (Table 25, Fig. 14). This may be due to leaching of phenolic compounds into the surrounding

medium. Bewley and Black (1994) reported that phenolic compounds such as coumarin and chlorogenic acid and their derivatives occur in the coats of many seeds. According to Taylor *et al.*, (1988) leakage of a fluorescent compound namely Sinapine was observed only around imbibed samples of heat killed cabbage seeds.

In the present study the somatic embryogenesis was of the IEDCs (Induced Embryogenic Determined Cells) type (Plate 17 & 18). The potential of 2,4-D in the induction of callus and subsequent somatic embryogenesis has been well documented in different cultivars of mango such as Keitt, Parris, Irwin, Peach, Tommy Atkins (Litz and Schaffer, 1987), Amrapali (Laxmi *et al.*, 1999; Ara *et al.*, 2000), and Chausa (Ara and Jaiswal, 2000). However, induction of direct somatic embryos from nucellus of mango cv. Bappakai by medium modified with BA and NAA has also been reported (Chaturvedi *et al.*, 2004). Induction of direct somatic embryogenesis from cotyledons of 14 mango cultivars using IBA has been accomplished (Huang *et al.*, 2000).

An enhancement of somatic embryogenic frequency upon transfer to lower level of 2, 4-D as in the present study (Table 26) has been reported in different mango cultivars (Litz and Schaffer, 1987). In view of Zimmerman (1993) the pro-embryogenic mass in an auxin containing medium generally synthesize all genes necessary to complete the globular stage of embryos. During the present study, embryos transferred from the 2, 4-D supplemented medium to 2, 4-D free medium facilitated maturation of >80% embryos (Table 27). The removal of auxin from the culture is therefore considered to be essential for the inactivation of several genes or synthesis of new gene-products for completion of embryo development (Zimmerman, 1993). According to Stuart *et al.,* (1985), the embryos exposed to auxin during development fail to accumulate storage protein. The low frequency of germination may be due to the persistence of auxin and thereby the failure of accumulation of storage products.

Difficulty of somatic embryo germination has been reported in many cultivars of mango and is considered as one of the bottlenecks for high frequency plantlet retrieval. In the present study, the germination was not successful. The phenolic content of the somatic embryogenic cultures was doubled as compared to that of zygotic embryos. The lack of germination of somatic embryos may be due to the accumulation of higher amount of phenolic compounds in the *in vitro* medium. This is in accordance with the views of Bewley and Black (1994), who suggested that the dormant seeds when placed for germination secrete phenolic compounds into the surrounding medium and which may inhibit germination of neighbouring seeds. However, successful germination has been reported in mango cultivars like Amrapali (Laxmi *et al.*, 1999; Ara *et al.*, 2000), Bappakai (Chaturvedi *et al.*, 2004) and in 14 other cultivars (Huang *et al.*, 2000). The genotypic difference may be one of the reasons for inhibition of somatic embryo germination in Natumanga. However, the accomplishment of high frequency embryo germination allows rapid propagation of this cultivar of Kerala State at affordable prices.

Conclusions

The recalcitrant nature of *Mangifera indica* seeds is established due to the following characters:

1. Desiccation intolerance.
2. Short life span (longevity).
3. Germinability of young seeds due to acquisition of physiological maturity, much earlier than mass maturity.
4. Critical water content for viability ranges from 38-46 percentage, with slight variation depending on maturity of seeds.
5. Germination-associated metabolic changes in control (fresh) seeds of all developmental stages.

High seed vigour index values are shown by fresh seeds of partly ripened and ripened seeds. Storability of young and ripened seeds is only 20

days and 15 days respectively, but the mature and partly ripened seeds are viable upto 30 days and so are more tolerant to desiccation.

In young, mature and partly ripened seeds simultaneous accumulation of sucrose and raffinose occurs upto 20-25 days afterwards, raffinose is getting reduced and concomitantly viability is lost. The role of raffinose is established in the inhibition of crystallization of sucrose, which is essential for the maintenance of desiccation tolerance. In ripened seeds sucrose is getting depleted along with viability loss, eventhough the raffinose is getting accumulated.

In recalcitrant seeds, for the maintenance of desiccation tolerance the ratio of oligosaccharides to sucrose is between 0.083 to 0.14. Irrespective of the developmental stages, in mango seeds the ratio is maintained in desiccation tolerant stages upto 15-25 days and later the ratio is getting reduced indicating the appearance of desiccation sensitivity.

The ratio of sucrose to raffinose is reported to be within the range of 20:1 for the maintenance of desiccation tolerance. In mango seeds this ratio also agrees with the desiccation tolerance/sensitivity.

Total protein and free amino acid distribution during desiccation of mango seeds of all stages of development reveals desiccation induced accumulation due to impaired metabolism.

Mango seeds of all stages of development show accumulation of phenolics during desiccation leading to desiccation intolerance and loss of viability.

Histochemical localization of starch and proteins confirm the accumulation of starch and protein in seeds of all stages of development. Cell

wall thickening of cotyledonary cells is another characteristic of desiccation stress.

Depletion of starch and sugars which are accumulated during desiccation in the seeds of all developmental stages indicates the enhanced mobilization of these metabolites during germination.

The decrease of protein content during germination of desiccated seeds results in the enhanced production of free amino acids.

Depletion of phenolic compounds during germination of desiccated seeds is either due to catabolism or leaching during rehydration.

Histochemical studies of desiccated seeds during germination showed reduction of starch grain number and size, protein staining intensity and these observations confirm the quantitative (analytical) data.

The *in vitro* studies of young dēembryonated seeds result in somatic embryogenesis. The germination of somatic embryos is not successful and this may be due to the abundant occurrence of phenolic compounds in the medium oozed out from the explant.

References

Andrade, A.C.S., Cunha, R., Souza, A.F., Reis, R.B. and Almeidia, K.J. 2003. Physiological and morphological aspects of seed viability of a neo-tropical savannah tree, *Eugenia dystenterica* DC. ***Seed Sci. Technol.*** **31:** 125-137.

Anil Kumar, C., Babu, K.P. and Krishnan, P.N. 2002. Seed storage and viability of *Myristica malabarica* Lam. an endemic species of Southern Western Ghats (India). ***Seed Sci. Technol.*** **30:** 651-657.

Anilkumar, C. 1998. **Physiological and biochemical aspects of seed storage and viability in *Syzigium* species.** Ph.D. Thesis, University of Calicut.

Anilkumar, C., Thomas, J. and Pushpangadan, P. 1996. Storage and germination of seeds of *Aporusa lindleyana* (Wight) Baillon, an economically important plant of Western Ghats (India). ***Seed Sci. Technol.*** **25**:1-6.

Ara, H., Jaiswal, U. and Jaiswal, V.S. 2000. Somatic embryogenesis and plantlet regeneration in Amrapali and Chausa cultivars of mango (*Mangifera indica* L.). ***Curr. Sci.*** **78**: 164-169.

Arogba, S.S., Ajiboye, O.L., Ugboko, L.A., Essienette, S.Y. and Afolabi, P.O. 1998. Properties of polyphenol oxidase in mango (*Mangifera indica*) kernel. ***J. Sci. Food Agric.*** **77**: 459-462.

Augustin, M.A. and Ling, E.T. 1987. Composition of mango seed kernel. ***Pertanika.*** **10**: 53-60.

Bachmann, M., Matile, P. and Keller, F. 1994. Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga raptans* L. Cold accumulation, translocation and sink to source transition: discovery of chain elongation enzyme. ***Plant Physiol.*** **105:** 1335-1345.

Bailly, C., Audigier, C., Ladonne, F., Wagner, M.H., Coste, F., Corbineau, F. and Come, D. 2001. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. ***J. Exp. Bot.*** **52:** 701-708.

Bartels, D., Singh, M. and Salamini, F. 1988. Onset of desiccation-tolerance during development of the barley embryo. ***Planta*** **175:** 485-492.

Baskin, C.C. and Baskin, J.M.C. 2001. ***Seed: Ecology, Biogeography and Evolution of Dormancy and Germination.*** Academic Press. San Diego. New York. pp. 666.

Berjak, P., Farrant, J.M. and Pammenter, N.W. 1989. The basis of recalcitrant seed behaviour. In: R.B. Taylorson. (Ed.) ***Recent Advances in the Development and Germination of Seeds.*** Plenum Press. New York. 89-108.

Berjak, P., Vertucci, C.W. and Pammenter, N. W. 1993. Effects of development status and dehydration rate on characteristics of water and desiccation-sensitivity in recalcitrant seeds of *Camellia sinensis.* ***Seed Sci. Res.*** **3:** 155-166.

Berlyn, G.P. and Miksche, J.P. 1976. ***Biochemical Technique and Cytochemistry.*** The Iowa State University Press, Ames, Iowa. pp. 326.

Bewley, J.D. and Black, M. 1983. ***Physiology and Biochemistry of Seeds in Relation to Germination,*** Vol 1, ***Development, Germination and Growth.*** Springer-Verlag, New York. pp. 306.

Bewley, J.D. and Black, M. 1985. ***Seeds: Physiology of Development and Germination.*** Plenum Press, New York. pp. 367.

Bewley, J.D. and Black, M. 1994. ***Seeds: Physiology of Development and Germination.*** 2nd Edn. Plenum Press. New York. pp. 445.

Bhattacharya, A., Nagar, P.K. and Ahuja, P.S. 2002. Seed development in *Camellia sinensis* (L). O. Kuntze. ***Seed Sci. Res.*** **12:** 39-46.

Blackman, S.A., Obendorf, R.L. and Leopold, A.C. 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. ***Plant Physiol.*** **100:** 225-230.

Blakman, S.A., Wettlaufer, S.H., Obendorf, R.L. and Leopold, A.C. 1991. Maturation proteins associated with desiccation tolerance in soybean. ***Plant Physiol.*** **96:** 868-874.

Boby, V. and Naithani, S.C. 2002. Desiccation-induced changes in lipid peroxidation, superoxide level and antioxident enzyme activity in neem *(Azadirachta indica* A. Juss) seeds. ***Acta Physiol. Plant.*** **24:** 79-87.

Bochicchio, A., Vazzana, C., Raschi, A., Bartels, D., and Salamini, F. 1988. Effect of desiccation on isolated embryos of maize. Onset of desiccation tolerance during development. ***Agronomie.*** **8:** 29-36.

Bonner, F.T. 1996. Reponses to drying of recalcitrant seeds of *Quercus nigra* L. ***Ann. Bot.*** **78:** 181-187.

Boubriak, I., Dini, M., Berjak, P. and Osborne, D.J. 2000. Desiccation and survival in the recalcitrant seeds of *Avicennia marina*: DNA replication, DNA repair and protein synthesis. ***Seed Sci. Res.*** **10:** 307-315.

Bradford, K.J. and Chandler, P.M. 1992. Expression of dehydrin-like proteins in embryos and seedlings of *Zizania palustris* and *Oryza sativa* during dehydration. ***Plant physiol.*** **99:** 488-494.

Buchanan, B.B., Cruissen, W. and Jones, R.L. 2001. ***Biochemistry and Molecular Biology of Plants.*** American Society of Plant Physiologists. Maryland. pp.1367.

Buitink, J., Hemminga, M.A. and Hoekstra, F. A. 2000. Is there a role for oligosaccharides in seed longevity? An assessment of intracellular glass stability. ***Plant Physiol.*** **122:** 1217-1224.

Burke, M.J. 1986. The glassy state and survival of anhydrous biological systems. In: A.C. Leopold. (Ed.) ***Membrane, Metabolism and Dry Organisms***. Cornell University Press. Ithaca. 358-363.

Carpenter, J.F., Crowe, L.M. and Crowe, J.H. 1987. Stabilization of phosphofructokinase with sugars during freeze-drying:characterization of enhanced protection in the presence of divalent cations. ***Biochim. Biophys. Acta.*** **923:** 109-115.

Carpenter, J.F., Crowe, J.H. and Arakawa, T. 1990. Comparison of solute-induced protein stabilization in aqueous solution and in the frozen and dried states. ***J. Diary Sci.*** **73:** 3627-3636.

Chaitanya, K.S.K., Keshavkant, S. and Naithani, S.C. 2000. Changes in total protein and protease activity in dehydrating recalcitrant sal (*Shorea robusta*) seeds. ***Silva Fenn.*** **34:** 71-77.

Chambrillange, N., Dussert, S., Englman, F., Doulbeau, S. and Hamon, S. 2000. Desiccation tolerance in relation to soluble sugar contents in seeds of ten Coffee (*Coffea* L.) species. ***Seed Sci. Res.*** **10:** 393-396.

Chandel, K.P.S., Choudhury, R., Radhamani, J. and Malik, S.K. 1995. Desiccation and freezing sensitivity in recalcitrant seeds of tea, cocoa and jack fruit. ***Ann. Bot.*** **76:** 443-450.

Chandra, N. 1980. Some physiological changes accompanying loss of viability of the seeds of *Mangifera indica* L. ***Plant Biochem. J.*** **7:** 105-109.

Chaturvedi, H.C., Sharma, M., Agnihotri, S., Sharma, A.K., Jain, M., Chourasia, A., Kidwai, N.R. and Gupta, P. 2004. *In vitro* development and convertibility of nucellar embryos of *Mangifera indica* L. Bappakai, A dwarfing rootstock. ***Plant Cell Biotec. Mole. Biol.*** **5:** 7-14.

Chen, Y. and Burris, J.S. 1990. Role of carbohydrates in desiccation tolerance and membrane behaviour in maturing maize seed. ***Crop Sci.*** **30:** 971-975.

Chien, C.T. and Lin, T.P. 1997. Effect of harvest date on the storability of desiccation-sensitive seeds of *Machilus kusanoi* Hay. ***Seed Sci. Technol.*** **25:** 361-371.

Chin, H.F. 1988. ***Recalcitrant Seeds.*** **A Status Report.** IBPGR. Rome, pp. 28.

Chin, H.F., Aziz, M., Ang., B.B. and Hamzah, S. 1981. The effect of moisture and temperature on the ultra structure and viability of seeds of *Hevea braziliensis.* ***Seed Sci. Technol.*** **9:** 411-422.

Chin, H.F., Hor, Y.L. and Liassim, M.B. 1984. Identification of recalcitrant seeds. ***Seed Sci. Technol.*** **9:** 411-422.

Clutterbuck, W.K. and Bonner, F.T. 1985. Utilization of food reserves in *Quercus* seeds during storage. ***Seed Sci. Technol.*** **13:** 121-128.

Come, D. and Corbineau, F. 1996. Metabolic damage related to desiccation sensitivity. In: A.S. Ouedraogo., K. Poulsen. and F.Stubsgaard. (Eds.)

Intermediate/Recalcitrant Tropical Forest Tree Seed. IPGRI. Rome. 83-97.

Connor, K.F. and Bonner, F.T. 2001. The effect of desiccation on seeds of *Acer saccharinum* and *Aesculus pavia*: recalcitrance in temperate tree seeds. ***Trees.*** **15:**131-136.

Connor, K.F. and Sowa, S. 2003. Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. ***Tree physiol.*** **23:** 1147-1152.

Copeland, L.O. and McDonald, M.B. 1995. ***Seed Science Technology***. 3rd Edn. Chapman and Hall. pp. 409.

Corbineau, F. and Come, D. 1988. Storage of recalcitrant seeds of four tropical species. ***Seed Sci. Technol.*** **16:** 97-103.

Corbineau, F., Kante, M. and Come, D. 1986. Seed germination and seedling development in the mango (*Mangifera indica* L.). ***Tree Physiol.*** **1:** 151-160.

Crowe, J.H., Crowe, L.M., Carpenter, J.F. and Wistrom, C.A. 1987. Stabilization of dry phospholipids bilayers and proteins by sugars. ***Biochem. J.*** **242:** 1-10.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J. and Anchordoguy, T.J. 1988. Interactions of sugars with membranes. ***Biochim. Biophys. Acta.*** **947:** 367-384.

Crowe, L.M. Womersley, C., Crowe, J.H., Reid, D., Appel, L. and Rudolph, A.S. 1986. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. ***Biochim. Biophys. Acta.*** **861:** 131-140.

Danthu, P., Gueye, A., Boye, D.B. and Sarr, A. 2000. Seed storage behaviour of four Sahelian and Sudanian tree species (*Boscia senegalensis, Butyrospermum parkii, Cordyla pinnata* and *Saba senegalensis*). ***Seed Sci. Res.*** **10:** 183-187.

Davies, R.I. and Pritchard, H.W. 1998. Seed storage and germination of palms *Hyphaene thebaica, H. petersiana* and *Medemia argun.* ***Seed Sci. Technol.*** **26:** 823- 827.

Decruse, S.W. and Seeni, S. 2003. Seed cryopreservation is a suitable storage procedure for a range of *Piper* species. ***Seed Sci. Technol.*** **31:** 213-217.

DeWald, S.G., Litz, R.E. and Moore, G.A. 1989. Maturation and germination of mango somatic embryos. ***J. Amer. Soc. Hort. Sci.*** **114:** 837-841.

Diaz, A., Zayas, J.P. and Sanchez, R. 1982. Chemical characterization of seeds of some commercial mango (*Mangifera indica*) cultivars, Govan, Reina de Mexico and super Haden. ***Ciencia-y-Teenica-en-la-Agricultural, citricos-y-Otros-Frutales.*** **5:** 25-35.

Drew, P.J., Pammenter, N.W. and Berjak, P. 2000. 'Subimbibed' storage is not an option for extending longevity of recalcitrant seeds of the tropical species, *Trichilia dregeana.* ***Seed Sci. Res.*** **10:** 355-365.

Dussert, S., Chambrillange, N., Engelmann, F. and Hamon, S. 1999. Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Ceffea* L. ***Seed Sci. Res.*** **9:** 135-144.

Eira, M.T.S., Walters, C. and Caldas, L.S. 1999. Water sorption properties in *Coffea* spp. seeds and embryos. ***Seed Sci. Res.*** **9:** 321-330.

Farrant, J.M., Berjack, P. and Pammenter, N.W. 1992b. Proteins in development and germination of a desiccation sensitive (recalcitrant) seed species. ***Plant Growth Regul.*** **11:** 257-265.

Farrant, J.M., Berjak, P. and Pammenter, N.W. 1985. The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*. ***South African J. Bot.*** **51:** 432-438.

Farrant, J.M., Pammenter, N.W. and Berjak, P. 1992a. Development of the recalcitrant (Homoiohydrous) seeds of *Avicennia marina*: Anatomical, ultrastructural and biochemical events associated with development from histodifferentiation to maturation. ***Ann. Bot.*** **70:** 75-86.

Farrant, J.M., Pammenter, N.W. and Berjak, P. 1988. Recalcitrance: a current assessment. ***Seed Sci. Tehnol.*** **16:** 155-166.

Farrant, J.M., Pammenter, N.W. and Berjak, P. 1989. Germination associated events and the desiccation sensitivity of recalcitrant seeds-a sytudy on tree unrelated species. ***Planta.*** **178:** 189-198.

Farrant, J.M., Pammenter, N.W. and Berjak, P., Farnsworth, E.J. and Vertucci, C.W. 1996. Presence of dehydrin-like proteins and levels of abscisic acid in recalcitrant (desiccation sensitive) seeds may be related to habitat. ***Seed Sci. Res.*** **6:**175-182.

Farrant, J.M., Pammenter, N.W., Berjak, P. and Walters, C. 1997. Subcellular organization and metabolic activity during the development of seeds that attain different levels of desiccation tolerance. ***Seed Sci. Res.*** **7:** 135-144.

Finch–Savage, W.E. 1992a. Embryo water status and survival in the recalcitrant species *Querus* robur L.: Evidence for critical moisture content. ***J. Exp. Bot.* 43:** 671-679.

Finch–Savage, W.E. 1992b. Seed development in the recalcitrant species *Querus robur* L.: Development of germinability and desiccation tolerance. ***Seed Sci. Res.* 2:** 17-22.

Finch-Savage, W.E. 1996. The role of developmental studies in research on recalcitrant and intermediate seeds. In: A.S. Ouedraogo. K. Poulsen. and F. Stubsgaard. (Eds.) ***Intermediate/Recalcitrant Tropical Forest Tree Seeds.*** IPGRI. Rome. 83-97

Finch-Savage, W.E. and Blake, P.S. 1994. Indeterminate development in desiccation-sensitive seeds of *Quercus robur* L. ***Seed Sci. Res.* 4:** 127-133.

Finch-Savage, W.E., Grange, R.I., Hendry, G.A.F. and Atherton, N.M. 1993. Embryo water status and loss of viability during desiccation in the recalcitrant seed species *Quercus robur* L. In: D. Come. and F. Corbineau. (Eds.) ***Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology.*** ASFIS, Paris. 723-730.

Fletcher, R.S. and Kott, L.S. 1999. Phenolics and cold tolerance of *Brassica napus*. 10th International Rape seed Congress. Canberra, Australia.

Folin, O. and Denis, W. 1915. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. ***J. Biol. Chem.* 22:** 305-308.

Fu, J.R., Xia, Q.H. and Tang, L.F. 1993. Effect of desiccation on excised embroyonic axes of three recalcitrant seeds and studies on cryopreservation. ***Seed Sci. Technol.* 21:** 85-95.

Fu, J.R., Zhang, B.Z., Wang, X.P. and Qiao, Y.Z. 1989. Desiccation and storage study on four recalcitrant seeds. International Seed Testing Congress. Edinburg.

Fu, J.R., Zhang, B.Z., Wang, X.P., Qiao, Y.Z. and Huang, X.L. (1990). Physiological studies on desiccation, wet storage and cryopreservation of recalcitrant seeds of three fruit species and their excised embryonic axis. ***Seed Sci. Technol.*** **18**: 743-754.

Galau, G.A., Jakobsen, K.S. and Hughes, D.W. 1991. The controls of late dicot embryogenesis and early germination. ***Physiol. Plant.*** **81**:280-288.

Gee, O.H., Probert, R.J. and Coomber, S.A. 1994. 'Dehydrin-like' proteins and desiccation tolerance in seeds. ***Seed Sci. Res.*** **4**: 135-141.

Greggains, V., Finch-Savage, W.E., Atherton, N.M. and Berjak, P. 2001. Viability loss and free radical processes during desiccation of recalcitrant *Avicennia marina* seeds. ***Seed Sci. Res.*** **11:** 235-242.

Han, B., Berjakk, P., Pammenter, N.W., Farrant, J.M. and Kermode, A.R. 1997. The recalcitrant plant species, *Castanospermum australe* and *Trichilia dregeana*, differ in their ability to produce dehydrin-related stresses to ABA or water-deficit-related stresses. ***J. Exp. Bot.*** **48**:1717-1726.

Hare, P.D. and Cress, W.A. 1997. Metabolic implications of stress-induced proline accumulation in plants. ***Plant Growth Regul.*** **21**: 79-102.

Hay, F.R. and Probert, R.J. 1995. Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in Foxglove (*Digitalis purpurea* L.). ***Ann. Bot.*** **76**: 639-647.

Hay, F.R., Probert, R.J. and Coomber, S.A. 1996. Development of desiccation tolerance and longevity in seeds from detached capsules of Foxglove (*Digitalis purpurea* L.). ***Ann. Bot.*** **79:** 419-427.

Hendry, G.A.F., Finch-Savage, W.F., Thorpe, P.C., Atherton, N.M., Buckland, S.M., Nilsson, K.A. and Seel, W.E. 1992. Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. ***New Phytol.*** **122:** 273-279.

Hilhorst, H.W.M., Faria, J.M.R. and van Lammeren, A.A.M. 2004. Desiccation sensitivity and cell cycle aspects in seeds of *Inga vera* subsp. *affinis*. ***Seed Sci. Res.*** **14:** 165-178.

Hoekstra, F.A., Crowe, J.H. and Crowe, L.M. 1990. Membrane behaviour in drought and its physiological significance. In: R.B. Taylorson. (Ed.) ***Recent Advances in the Development and Germination of Seeds.*** Plemum Press. New York. 71-126.

Hoekstra, F.A., Crowe, L.M. and Crowe, J.H. 1989. Differential desiccation sensitivity of corn and *Pennisetum* pollen linked to their sucrose contents. ***Plant Cell Environ.*** **12:** 83-91.

Hoekstra, F.A., Haigh, A.M., Tetteroo, F.A.A. and van Roekel, T. 1994. Changes in soluble sugars in relation to desiccation tolerance in cauliflower seeds. ***Seed Sci. Res.*** **4:**143-147.

Hong, T.D. and Ellis, R.H. 1992. Development of desiccation tolerance in Norway maple (*Acer platanoides* L.) seeds during maturation drying. ***Seed Sci. Res.*** **2:** 169-172.

Hong, T.D. and Ellis, R.H. 2002. Optimum moisture status for the exceptional survival of seeds of arabica coffee (*Coffea arabica* L.) in medium-term storage at 20°C. ***Seed Sci. Technol.*** **30:**131-136.

Hong, T.D., Gedebo, A. and Ellis, R.H. 2000. Accumulation of sugars during the onset and development of desiccation tolerance in immature seeds of Norway maple (*Acer platanoides* L.) stored moist. ***Seed Sci. Res.*** **10:** 147-152.

Hor, Y.L., Chin, H.F. and Mohamed, Z.K. 1984. The effect of seed moisture and storage temperature on the stability of cocoa (*Theobroma cacao*) seeds. ***Seed Sci. Technol.*** **12:** 415-420.

Huang, X.L., Xiao, J.N., Wu, Y.J., Li, X.J., Zhou, M.D. and Engelmann, F. 2000. Direct somatic embryogenesis induced from cotyledons of mango immature zygotic embryos. ***In Vitro Cell. Dev. Biol.- Plant.*** **40:** 196-199.

Ingram, J. and Bartels, D. 1999. The molecular basis of dehydration tolerance in plants. ***Annu. Rev. Plant Physiol. Plant Mol. Biol.*** **47:** 377-403.

International Seed Testing Association. 1985. International rules for seed testing. Rules 1985. ***Seed Sci. Technol.*** **13:** 365-513.

Johanson, D.A. 1940. ***Plant Microtechnique***. Mc Graw Hill, New York. pp. 523.

Kamarudeenkunnju, M. 2003. **Phisiological and Biochemical studies on seeds of *Hopea parviflora* Bedd. and *Vateria indica* L. Two economically important endemic trees of Western Ghats**. Ph.D. Thesis, University of Kerala.

Kermode, A.R. 1990. Regulatory mechanisms involved in the transition from seed development to germination. ***Crit. Rev. Plant Sci.*** **9:** 155-195.

Khanna, S.K., Matto, R.L., Viswanathan, P.N., Tewari, C.P. and Sanwal, G.G. 1969. Calorimetric determination of protein and orthophosphate in plant tissue rich in phenolics. ***Indian J. Plant Physiol.*** **6:** 21-25.

Khasim, S.M. 2002. ***Botanical Microtechnique: Principles and Practice.*** Capital Publishing Company. pp. 264.

King, M.W. and Roberts, E.H. 1980. A strategy for future research into the storage of recalcitrant seeds. In: H.F. Chin. and E.H. Roberts. (Eds.) ***Recalcitrant Crop Seeds***. Tropical Press, Kulala Lumpur. 99-110.

Koster, K.L. 1991. Glass formation and desiccation tolerance in seed. ***Plant Physiol.*** **96:** 302-304.

Koster, K.L. and Leopold, A.C. 1988. Sugars and desiccation tolerance in seeds. ***Plant Physiol.*** **88:** 829-832.

Kozeko, L.E. and Troyan, V.M. 2000. The relationship between the mitotic activity and moisture content of recalcitrant seeds of *Acer saccharinum* L. during maturation, post-maturation drying and germination. ***Seed Sci. Res.*** **10:** 225-232.

Lahuta, L.B., Login, A., Rajowski, A., Socha, A. and Zalewaski, K. 2000. Influence of water deficit on the accumulation of sugars in developing field bean (*Vicia faba* var. minor) seeds. ***Seed Sci. Technol.*** **28:** 101-113.

Laidman, D.L. 1982. Control mechanisms in the mobilization of stored nutrients in germinating cereals. In: A.A. Khan. (Ed.) ***Physiology and***

Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press. The Netharlands. 371-405.

Lasztity, R., el Shafei, M.A., Abdel Samei, M.B., Hatour, F.S. and Labib, M. 1988. Biochemical studies of some non conventional sources of protein. Part 4. The proteins of mango waste stone kernels. ***Nahrung.*** **32:** 867-873.

Laxmi, D.V., Sharma, H.C., Kirti, P.B. and Mohan, M.L. 1999. Somatic embryogenesis in Mango (*Mangifera indica*) cv. Amarapali. ***Cur. Sci.*** **77:** 1355-1358.

Leopold, A.C. 1990. Coping with desiccation. In: R.G. Alscher. and J.R. Cumming. (Eds.) ***Membranes, Metabolism and Dry Organisms.*** Cornell University Press. Ithaca. 57-86.

Leopold, A.C. and Vertucci, C.W. 1986. Physical attributes of desiccated seeds. In A.C. Leopold. (Ed.) ***Membranes, metabolism and dry organisms***. London. Comstock. Ithaca. 22-34.

Leopold, A.C. Sun, W.Q. and Bernal-Lugo, I. 1994. The glassy state in seeds: analysis and function. ***Seed Sci. Res.*** **4:** 267-274.

Leprince, O., Bronchart, R. and Deltour, R. 1990a. Changes in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of *Brassica campestris* seeds. ***Plant cell Environ.*** **13:** 539-546.

Leprince, O., Buitink, J. and Hoekstra, F.A. 1999. Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. Exhibit contrasting responses of respiration to drying in relation to desiccation sensitivity. ***J. Exp. Bot.*** **50:** 1515-1524.

Leprince, O., Deltour, R., Thorpe, P.C., Atherton, N.M. and Hendry, G.A.F. 1990b. The role of free radical and radical processing systems in loss of desiccation tolerance in germinating maize (*Zea mays* L.). ***New Phytol.*** **116:** 573-580.

Leprince, O., Hendry, G.A.F. and Mc Kersie, B.D. 1993. The mechanisms of desiccation tolerance in developing seeds. ***Seed Sci. Res.*** **3:** 231-246.

Leprince, O., vander Werf, A., Deltour, R. and Lambers, H. 1992. Respiratory pathways in germinating maize radicles correleated with desiccation tolerance and soluble sugars. ***Physiol. Plant.*** **85:** 581-588.

Liang, Y. and Sun, W.Q. 2000. Desiccation tolerance of recalcitrant *Theobroma cacao* embryonic axes: the optimal drying rate and its physiological basis. ***J. Exp. Bot.*** **51:** 1911-1919.

Lin, T.P. and Chen, M.H. 1995. Biochemical characteristics associated with the development of the desiccation-sensitive seeds of *Machilus thunbergii* Sieb and Zucc. ***Ann. Bot.*** **76:** 381-387.

Litz, R.E. 1985. Somatic embryogenesis in tropical fruit trees. In: R.R. Henke., K.W. Hughes., M.J. Constantin. and A. Hollaender. (Eds.) ***Tissue Culture in Forestry and Agriculture.*** Plenum Publishing Co. New York. 179-193.

Litz, R.E. and Schaffer, B. 1987. Polyamines in adventitious and somatic embryogenesis in mango (*Mangifera indica* L.). ***J. Plant Physiol.*** **128:** 251-258.

Litz, R.E., Knight, R.J. and Gazit, S. 1984. *In vitro* somatic embryogenesis from *Mangifera indica* L. callus. ***Sci. Hortic.*** **22:** 233-240.

Litz, R.E., Mathews, V.H., Moon, P.A., Pliego-Alfaro, F., Yurgalevitch, C. and DeWald, S.G. 1993. Somatic embryos of mango (*Mangifera indica* L.). In: K. Redenbaugh. (Ed.) ***Synseeds Application of Synthetic Seeds to Crop Improvement.*** CRC Press. Boca Raton. 409-425.

Lobos, L.P. and Ellis, R.H. 2002. Seed storage behaviour of *Fagus sylvatica* and *Fagus crenata.* ***Seed Sci. Res.*** **12:** 31-37.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin-Phenol reagent. ***J. Biol. Chem.*** **193:** 265-275.

Mayer, A.M. and Poljakoff–Mayber, A. 1989. ***Germination of Seeds.*** 4th Edn. Pergamon Press. New York. pp. 270.

Merouani, H., Apolinario, L.M., Almedia, M.H. and Pereira, J.S. 2003. Morphological and Physiological maturation of acorns of cork oak (*Quercus suber* L.). ***Seed Sci. Technol.*** **31:** 111-124.

Monneri, C., Gercia, L. and Guardiola, J.L. 1986. Sugar and starch changes in pea cotyledons during germination. ***Physiol. Plant.*** **67:** 49-54.

Montgomery, R. 1957. Determination of glycogen. ***Arch. Biochem. Biophys.*** **67:** 378-386.

Morton, J. 1987. Mango. In: J. F. Morton. and F.L. Miami. (Eds.) ***Fruits of warm climates.*** 221-239.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. ***Physiol. Plant.*** **15:** 473-497.

Nautiyal, A.R. and Purohit, A.N. 1985a. Seed viability in Sal II. Membrane disruption in ageing seeds of *Shorea robusta.* ***Seed Sci. Technol.*** **13:** 77-82.

Nautiyal, A.R. and Purohit, A.N. 1985b. Seed viability in Sal II. Physiological and Biochemical aspects of ageing in seeds of *Shorea robusta.* ***Seed Sci. Technol.*** **13:** 69-76.

Oliver, M.J. and Bewley, J.D. 1997. Desiccation-tolerance of plant tissues: A mechanistic overview. ***Hortic. Rev.*** **18:** 171-213.

Ooms, J.J.J., Leon-Kloosterziel, K.M., Bartels, D., Koorneef, M. and Karssen, C.M. 1993. Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana.* ***Plant Physiol.*** **102:** 1185-1191.

Pammenter, N.W. and Berjak, P. 1999. A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. ***Seed sci. Res.*** **9:** 13-37.

Pammenter, N.W., Berjak, P., Farrant, J.M., Smith, M.T. and Ross, G. 1994. Why do stored, hydrated recalcitrant seeds die? ***Seed Sci. Res.*** **4:** 187-191.

Pammenter, N.W., Peran, R., Naicker, J. and Berjak, P. 2004. The influence of rehydration technique on the response of recalcitrant seed embryos to desiccation. ***Seed Sci. Res.*** **14:** 179-184.

Pammenter, N.W., Vertucci, C.W. and Berjak, P. 1993. Responses to dehydration in relation to non-tolerant seeds. In: D. Come. and F. Corbineau. (Eds.) ***Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology.*** Paris: Universite Pierre and Marie Curie. 867-872.

Parhan , R.A. and Kaustinen, H.M. 1977. On the site of tannins synthesis in plant cells. ***Bot. Gaz.* 138:** 465-467.

Peterbauer, T. and Richter, A. 2001. Biochemistry and Physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. ***Seed Sci. Res.* 11:** 185-199.

Poulsen, K.M. and Eriksen, E.N. 1992. Physiological aspects of recalcitrance in embryonic axes of *Quercus robur* L. ***Seed Sci. Res.* 2:** 215-221.

Pritchard, H.W. and Manger, K.R. 1998. A calorimetric perspective on desiccation stress during preservation procedures with recalcitrant seeds of *Quercus robur* L. ***Cryo Lett.* 19** (Supplement 1)**:** 23-30.

Pritchard, H.W., Tompsett, P.B., Manger, K. and Smidt, W.J. 1995. The effect of moisture content on the low temperature responses of *Auracaria hunsteinei* seed and embryos. ***Ann. Bot.* 76:** 79-88.

Pucher, G.W., Levenworth, C.S. and Vickery, H.B. 1948. Determination of starch in plant tissues. ***Anal. Chem.* 20:** 850-853.

Purdy, S.J. and Truter, E.V. 1962. Spot-area measurement. ***Analyst.* 87:** 807.

Ried, J.L. and Walker-Simmons, M.K. 1993. Group 3 late embryogenesis abundant proteins in desiccation-tolerant seedlings of wheat (*Triticum aestivum* L.). ***Plant Physiol.* 102**: 125-131.

Roberts, E.H. 1973. Predicting the storage life of seeds. ***Seed Sci. Technol.* 1:** 499-514.

Ryszard, J.G., Agnieszka, D.C. and Ralph, L.O. 1997. Soluble sugars and flatulence-producing oligosaccharides in mature yellow lupin *(Lupinus luteus* L.) seeds. ***Seed Sci. Res.* 7:** 185-193.

Sacande, M., Buitink, J. and Hoekstra, F.A. 2000. A study of water relations in neem (*Azadirachta indica*) seeds that is characterized by complex storage behaviour. ***J. Exp. Bot.*** **51:** 635-643.

Sanjeev, K.C., Khajuria, H.N., Gill, S.S., Manoj, K.G. and Bajwa, V. 2002. Storage of Neem seeds (*Azadirachta indica*). ***Seed Sci. Res.*** **60:** 204-210.

Senaratna, T., McKersie, B.D. and Borochov, A. 1987. Desiccation and free radical mediated changes in plant membranes. ***J. Exp. Bot.*** **38:** 2005-2014

Smith, M.T. and Berjak, P. 1995. Deteriorative changes associated with loss of viability of stored desiccation-tolerant and sensitive seeds. In: J. Kingel. and G. Galili. (Eds.) ***Seed Development and Germination.*** Marcel. Dekker Inc. New York. 701-746.

Stahl, E. 1969. ***Thin-Layer Chromatography - A Laboratory Handbook.*** 2nd Edn. Springer-Verlag. Heidelberg. New York. pp.1041.

Steadman, K.J., Pritchard, H.W. and Dey, P.M. 1996. Tissue-specific soluble sugars in seeds as indicators of storage category. ***Ann. Bot.*** **77:** 667-664.

Stephane, D., Nathalie, C., Florcent, E., Francois, A., Jacques, L. and Serge, H. 2000. Relationship between seed desiccation sensitivity, seed water content at maturity and climatic characteristics of Native environments of nine *coffea* L. species. ***Seed Sci. Res.*** **10:** 293-300.

Still, D.W., Kovach, D.A. and Bradford, K.J. 1994. Development of desiccation tolerance in rice (*Oryza sativa*) and wild rice (*Zizania*

palustris). Dehydrin expression, abscisic acid content, and sucrose accumulation. ***Plant Physiol.*** **104:** 431-438.

Stuart, P.A., Nelson, J., McCall, C.G., Strickland, S.G., Walker, K.A. 1985. Physiology of the development of somatic embryos in cell cultures of alfalfa and celery. In: M. Zaitlin., P. Day. and A. Hollaender. (Eds.) ***Biotechnology in Plant Science.*** New York: Academic Press. 35-47.

Sun, W.Q., Irving, T.C. and Leopold, A.C. 1994. The role of sugar, vitrification and membrane phase transition in seed desiccation tolerance. ***Physiol. Plant.*** **90:** 621-628.

Sun, W.Q., Leopold, A.C., Crowe, L.M. and Crowe, J.H. 1997. Cytoplasmic vitrification and survival of anhydrobiotic organisms. ***Comp. Biochem Physiol.*** **117:** 327-333.

Taylor, A.G., Huang, X.L. and Hill, H.J. 1988. Sinapine leakage from non-viable cabbage seeds. ***J. Exp. Bot.*** **39:** 1433-1438.

Tompsett, P.B. 1992. A review of the literature on storage of dipterocarp seeds. ***Seed Sci. Technol.*** **20:** 251-267.

Tompsett, P.B. and Pritchard, H.W. 1993. Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds. ***Ann. Bot.*** **71:** 107-116.

Varghese, B., George von. W. and Naithani, S.C. 2004. Effect of differential rate of drying on viability and storability in recalcitrant sal (*Shorea robusta* C.F. Gaertn) seeds. ***Seed Technol.*** **26:** 51-64.

Varghese, B., Naithani, R., Dulloo, M.E. and Naithani, S.C. 2002. Seed storage behaviour in *Madhuca indica* J.F. Gmel. ***Seed Sci. Technol.*** **30:** 107-117.

Vertucci, C.W. 1989. The effects of low water contents on physiological activities of seeds. ***Physiol. Plant.*** **77:** 172-176.

Vertucci, C.W. and Farrant, J.M. 1995. Acquisition and loss of desiccation tolerance. In: J. Kigel. and G. Galili. (Eds.) ***Seed Development and Germination.*** Marcel Dekker Inc. New York. 237-271

Vertucci, C.W. and Leopold, A.C. 1984. Bound water in soybean seed and its relation to respiration and imbibitional damage. ***Plant Physiol.*** **75:** 114-117.

Vertucci, C.W. and Leopold, A.C. 1987. Water binding in legume seeds. ***Plant Physiol.*** **85:** 224-231.

Vertucci, C.W., Crane, J., Porter, R.A. and Oelke, E.A. 1994. Physical properties of water in *Zizania* embryos in relation to maturity status, water content and temperature. ***Seed Sci. Res.*** **4:** 211-224.

Walters, C., Pammenter, N.W., Berjak, P. and Crane, J. 2001. Desiccation damage, accelerated ageing and respiration in desiccation tolerant and sensitive seeds. ***Seed Sci. Res.*** **11:** 135-148.

Welbaum, G.E. and Bradford, K.J. 1988. Water relation of seed development and germination in muskmelon (*Cucumis melo* L.) I. water relations of seed and fruit development. ***Plant physiol.*** **86:** 406-411.

Whelan, W.J. 1955. Starch, glycogen, fructosans and similar polysaccharides. In: K. Peach. and M.V. Tracy. (Eds.) ***Modern Methods of Plant Analysis.*** Vol. 2. Springer-Verlag. Berlin. 145-196.

Wilson, D.O. Jr. and McDonald, M.B. Jr. 1986. Lipid peroxidation model of seed ageing. ***Seed Sci. Technol.*** **14:** 269-300.

Xia, Q.H., Chen, R.Z. and Fu, J.P. 1992. Effects of desiccation, temperature and other factors on the germination of lychee *(Litchi chinensis* Sonn.*)* and longan *(Euphorbia longan* Steud.) seeds. ***Seed Sci. Technol.* 20:** 119-127.

Yang, R. and Shetty, K. 1998. Stimulation of rosmarinic acid in shoot culture of oregano (*Origanum vulgare*) colonel line in response to proline, proline analog and proline precursors. ***J. Agric. Food Chem.* 46:** 2888-2893.

Zimmerman, J.L. 1993. Somatic embryogenesis. A model for early development in higher plants. ***Plant Cell.* 5:**1411-1423.

Zobel, A.M., Kuras, M. and Tykarska, T. 1989. Cytoplasmic and apoplastic localization of phenolic compounds in the covering tissues of *Brassica napus* radicle between embryogenesis and germination. ***Ann. Bot.* 64:** 149-157.