PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON JACKFRUIT SEEDS (*Artocarpus heterophyllus Lam.*) DURING STORAGE AND GERMINATION

Thesis
submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

SHEELA S.

DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT

2007

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CERTIFICATE

This is to certify that the thesis entitled "**Physiological and Biochemical Studies on Jackfruit Seeds (*Artocarpus heterophyllus* Lam.) During Storage and Germination**" submitted by **Smt. SHEELA. S** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, is a bonafide record of the 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 or submitted before for any other degree.

Dr. NABEESA SALIM

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DECLARATION

I hereby declare that the thesis entitled "**Physiological and Biochemical Studies on Jackfruit Seeds (*Artocarpus heterophyllus* Lam.) During Storage and Germination**" submitted by me in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in Botany, University of Calicut, has not been submitted for any other degree.

1/2/07

Place: C.U. Campus
Date : 1-2-2007.

SHEELA. S

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Shathakodi Pranams

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Introduction

Recalcitrant seeds have been reported from a variety of species including deciduous temperate trees, aquatic grasses, mangroves, trees of semi evergreen tropical forests and tropical rain forests. Rapid germination and short period of seed life span which are the attributes of recalcitrant seeds have been mentioned by various authors in discussion of seed development, maturation, desiccation, viability, longevity and storability.

The categorization of seeds into 'orthodox' and 'recalcitrant' (Roberts, 1973) was based principally on the post-harvest behaviour such as desiccation tolerance / sensitivity, storability and germination. Recalcitrant seeds have high moisture content as they are not subjected to water loss during maturation and their life span is frequently brief and only occasionally exceed a few months. These seeds are damaged by dehydration, so desiccation sensitive and may be chilling sensitive and generally cannot be stored effectively for long periods.

Recalcitrant nature is exhibited mostly by economically important crop species of tropics and subtropics. Now it has become apparent that there is a wide range in the post-harvest responses of seeds, suggesting three categories such that post-harvest physiology may be considered as constituting a

continuum across species. Within the recalcitrant category, there are marked differences in the responses of seeds of individual species and they are classified as highly, moderately and less recalcitrant (Farrant *et al.*, 1988; Berjak *et al.*, 1989).

Recalcitrant seeds cannot be dried to moisture contents below 30% without injury (desiccation sensitive) and are unable to tolerate chilling / freezing (chilling sensitive). As a result, these seeds live for only short duration and are difficult to be stored successfully because the high moisture content encourages microbial contamination and results in rapid deterioration (short storability). It is generally observed that the recalcitrant seeds never enter into dormancy, but instead continue their development and progresses towards germination (Berjak *et al.*, 1990).

Recalcitrant seeds are highly sensitive or intolerant to desiccation because of their high moisture content at the time of shedding due to the lack of maturation drying (desiccation) on the mother plant. Desiccation sensitivity has been reported in *Hevea braziliensis* (Chin *et al.*, 1981), *Shorea* species (Nautiyal and Purohit, 1985 a; Corbineau and Come, 1988; Finch-Savage, 1992; Chaitanya *et al.*, 2000 a, b), *Avicennia* species (Farrant *et al.*, 1993; Greggains *et al.*, 2001; Le Tam *et al.*, 2004) *Artocarpus heterophyllus* (Chin *et al.*, 1984; Fu *et al.*, 1993; Chandel *et al.*, 1995; Smith *et al.*, 2001; Peran *et al.*, 2004), *Inga* species (Pritchard *et al.*, 1995; Faria *et al.*, 2004) *Theobroma cacao* (Hor *et al.*, 1984; Li and Sun, 1999) *Machilus* species (Lin and Chen, 1993, 1995; Chien and Lin, 1997), *Garcinia* species (Malik *et al.*, 2005).

Short life span or longevity / storability of recalcitrant seeds is another important characteristic which is intimately associated with moisture content distribution and / or desiccation. Decline in germination percentage due to reduced moisture content has been reported in *Hevea* (Chin *et al.*, 1981),

Shorea (Nautiyal and Purohit, 1985 a; Corbineau and Come, 1988; Finch-Savage, 1991, 1992; Chaitanya *et al.*, 2000 a), *Digitalis purpurea* (Hay *et al.*, 1997) *Gaurea* species (Connor and Bonner, 1998), *Aesculus* species (Tompsett and Pritchard, 1993, 1998) *Avicennia* species (Farrant *et al.*, 1993; Greggains *et al.*, 2001; Le Tam *et al.*, 2004).

Chilling sensitivity of recalcitrant seeds has been investigated in *Theobroma cacao* (Hor *et al.*, 1984; Ruhl, 1996), *Symphonia globulifera* (Corbineau and Come, 1988), *Zizania palustris* (Kovach and Bradford, 1992, Still *et al.*, 1994), *Syzygium aromaticum* (Anilkumar *et al.*, 2000), *Shorea robusta* (Varghese *et al.*, 2004), *Avicennia alba* (Le Tam *et al.*, 2004), *Garcinia* species (Malik *et al.*, 2005).

Generally, storability of these seeds are very short and temperature dependent. A number of investigations have been undertaken on the storage behaviour of recalcitrant seeds. Examples are *Hevea* (Chin *et al.*, 1981), *Theobroma cacao* (Hor *et al.*, 1984; Li and Sun, 1999), *Quercus* species (Clatterbuck and Bonner, 1985; Hendry *et al.*, 1992; Finch-Savage, 1992; Bonner, 1996 b) *Machilus* species (Lin and Chen, 1993, 1995; Chien and Lin, 1997), *Syzygium* species (Anilkumar *et al.*, 1998, 2000) and *Myristica* species (Anilkumar *et al.*, 2002).

As recalcitrant seeds are shed from mother plant at high moisture content, they are metabolically active and undergo germination-associated changes immediately after shedding (Pammenter *et al.*, 1994; Pammenter and Berjak, 1999). Metabolic events occurring under moist storage are related to germination-associated changes.

Jackfruit (*Artocarpus heterophyllus* Lam.) is one of the most remunerative and important fruits of India. It is the largest among edible fruits and belongs to family Moraceae. Originally it is a native of India and

presently cultivated throughout tropical low land in both hemispheres. Being a cross pollinated and mostly seed propagated plant, existing populations of jackfruit trees show a wide variation of morphological characters. Cultivated types are broadly classified into two groups like soft flesh type and firm flesh type. The sweet and firm flesh variety is called as kappa and the soft, mucilaginous variety as barka (Anonymous, 1994). Genetic diversity and canopy management study in Jackfruit (*Artocarpus heterophyllus* Lam.) revealed that there was no significant difference between soft and firm fleshed types in terms of morphological, anatomical and biochemical characters of leaves studied (Muthulakshmi, 2003). Protein profile analysis of Jackfruit seeds during developmental stages was carried out by Kabir and Daar (1995) and an immunoglobulin A binding protein was identified in developing seeds.

The flesh of Jackfruit is rich in beta carotene, calcium and riboflavin while the seeds are rich in phosphorus, calcium, iron, thiamine and vitamin-C (Anonymous, 2003). Seeds mostly starchy and contain fair amount of protein and have good pectin content. Jackfruit seeds are widely used as a rich source of lectins like jacalin, artocarpin, HN jakelin, KM etc. (Anonymous, 2000).

Jackfruit seeds have been included under recalcitrant category based on their storage behaviour (Chin *et al.*, 1984; Fu *et al.*, 1993; Chandel *et al.*, 1995; Smith *et al.*, 2001; Peran *et al.*, 2004). According to Chin *et al.*, (1984), *Artocarpus heterophyllus* seeds were killed on drying even to a still high level of 43% moisture content, a decrease of 10% from their original 53% moisture content. The drying of excised embryonic axes of Jackfruit (*Artocarpus heterophyllus*) seeds revealed that the moisture content of excised Jackfruit seed embryonic axes could safely reach 16% when dried with silica gel and when these were incubated in MS medium containing Arginine and NAA for 20 days, they differentiated to seedlings (Fu *et al.*, 1993). Chandel *et a/.*, (1995) pointed out that embryonic axes of partially mature and mature Jack

fruit seeds could be desiccated to lower moisture level of 14% and 7% respectively. Those authors successfully cryopreserved partially and fully mature embryonic axes of jackfruit seeds with survival of 30% and 25% respectively. According to them long term conservation of germplasm of the jackfruit seeds was possible through cryopreservation of excised embryonic axes.

Smith *et al.,* (2001) studied the effects of two drying rates on desiccation tolerance of embryonic axes of jackfruit (*Artocarpus heterophyllus*) seeds and suggested that rapid drying conferred greater tolerance to drying with 100% viability at approximately 0.4g g^{-1} moisture content. The influence of rehydration technique on the response of recalcitrant seed embryos to desiccation was studied in *Artocarpus heterophyllus* (Peran *et al.,* 2004). The excised embryonic axes were rapidly dried to safe moisture contents and direct re-imbibition in water resulted in higher survival than either of the slow rehydration techniques.

All the studies referred above are centered on the embryonic axis subjected to various storage techniques inclusive of cryopreservation and parameters like desiccation sensitivity, critical moisture content, chilling injury, reimbibition and rehydration rate etc. were taken into consideration to evaluate / assess the storage behaviour and recalcitrant nature of Jackfruit seeds.

Several studies have inferred that recalcitrant seeds are sensitive to desiccation and lose viability by a short period of storage. Germination of recalcitrant seeds is considered as a continuum of development and germination-associated changes occur during storage of recalcitrant seeds (Farrant *et al.*, 1988, Pammenter *et al.*, 1994). But so far no physiological / biochemical aspects of desiccation and germination of recalcitrant seeds have been reported. Similarly, the physiological basis for the loss of viability

during desiccation and storage remains uncertain. So the present investigation is an attempt to analyse the metabolic changes occurring during desiccation, storage and germination and their inter relationships in Jackfruit seeds which are starch rich.

First and foremost objective of the present investigation is the determination of desiccation rate and moisture content which are the vital factors in assessing the degree of desiccation sensitivity and the critical moisture content below which seed viability is lost. This study is proposed to highlight the effect of desiccation by gradual drying of seeds under open air condition at room temperature (30±3°C). Estimation of moisture content is proposed to be undertaken at comparable intervals during desiccation / storage period to calculate the critical moisture content.

Another important objective of the present study is to elucidate the physiological and biochemical changes of Jackfruit seeds when they are desiccated under different conditions inclusive of open-air storage by exposing to direct desiccation and storage of seeds in air tight polythene bags keeping under room temperature (30±3°C). Storage under refrigerator condition is also proposed to be conducted.

Eventhough desiccation sensitivity, chilling injury and storability under different conditions have been reported in a large number of recalcitrant seeds inclusive of Jackfruit (*Artocarpus heterophyllus* Lam.), the physiological and biochemical changes during these processes of storage remain to be studied. In the present project, the author has tried to focus on the metabolic changes in general and carbohydrate metabolism in particular of Jackfruit seeds which are starch rich with very short longevity.

Recalcitrant seeds are characterized by initiation of germination in continuum with development and so far germination has been considered as

an index to explain desiccation stress, chilling injury and storability. Nevertheless, studies on the germination metabolism and reserve mobilization pattern are not yet elucidated in any of the recalcitrant seeds so far. Hence, in the present study attempts are made to understand metabolic changes in terms of biomolecules or cell constituents distribution during germination of fresh seeds and the pattern of reserve mobilization in the seedlings grown under laboratory conditions.

Carbohydrate metabolism during desiccation and storage is proposed to be elucidated by estimating starch, metabolisable soluble sugars, enzymes (amylase activities) and protein. Reserve mobilization during germination and seedling growth also is included to elucidate the starch hydrolysis and mobilization of sugars during a period of 50 days of seedling growth. The physiology of germination and reserve mobilization of recalcitrant Jackfruit seeds compared to that of orthodox seeds which has been investigated and interpreted elaborately may help in monitoring the high metabolic activity as an important character of recalcitrant seeds.

Histochemical studies of starch and protein in the cotyledons and embryonic axes of Jackfruit seeds during desiccation enable to pinpoint the localization of the metabolites in various parts of seeds particularly the radicle tip because radicle tip is most vulnerable tissue to be exposed and damaged during desiccation stress. Histochemical study is the most appropriate parameter to observe the mobilization or disappearance of starch grains of the embryonic axes and cotyledons during desiccation/storage and germination of seeds.

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Review of literature

There is a class of seeds which at maturity are not subjected to desiccation and are remarkably short-lived. The terms 'orthodox' and 'recalcitrant' were introduced by Roberts (1973) to describe the behaviour of seeds under storage. Orthodox seeds are long lived, tolerant to desiccation and freezing temperature, whereas recalcitrant seeds are short-lived and are killed if their moisture content is reduced below certain critical value (12-31%) and /or if the seeds are stored at chilling temperature. The major characteristic difference between orthodox and recalcitrant seeds lies in the physiology of their response to desiccation and germination. Hanson (1984) considered the orthodox seeds as 'desiccation tolerant' and recalcitrant seeds as 'desiccation sensitive'.

Seeds of the major plantation crops, fruit trees and timber species are large and recalcitrant. Important tropical plantation crops, which produce recalcitrant seeds include rubber (*Hevea braziliensis*), cocoa (*Theobroma cacao*), coconut (*Cocos nucifera*), tea (*Camellia sinensis*) etc. Tropical fruit

crops with recalcitrant seeds are mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), mangostein (*Garcinia mangostana*), durian (*Durio zibethinus*), lychee (*Litchi chinensis)* and rambutan (*Nephelium lappaceum*). Tropical timber species belonging to family *Dipterocarpaceae* and *Araucariaceae* produce recalcitrant seeds. Temperate representatives of recalcitrant seeds are oak (*Quercus robur*), chestnut (*Castanea sativa*) and horse chestnut (*Aesculus hippocastanum*) (Chin, 1988).

Recalcitrant seeds are characterised by many special features. They are large sized and average weight far exceeds that of orthodox seeds (except Anthurium) (Chin, 1988). Due to high moisture content (30-70%), the life span of recalcitrant seeds is very short which varies from few days to few months even when the seeds are maintained in moist conditions.

Recalcitrant seeds are shed from parent plant at high moisture content. Because of their large size, these seeds lose water at a slower rate than do orthodox seeds. Large seed size may actually contribute to recalcitrant behavior as there is problem of internal transport of water (Chin, 1988). The critical moisture content, which kills recalcitrant seeds varies from species to species, but it is a relatively high value which is usually within the range from 12% to 31% (Roberts, 1973). Because metabolism is continuous in recalcitrant seeds, they lose viability if the moisture content drops below a certain critical level before germination occurs (Lin and Chen 1995).

Farrant *et al.*, (1988) classified recalcitrant seed types based on their rate of germination in the absence of additional water and on account of water loss tolerated, into three classes such as less recalcitrant (Eg. *Quercus* sp, *Araucaria* sp, *Podocarpus* sp.), moderately recalcitrant (*Cocoa, Hevea*) and highly recalcitrant (*Avicennia marina, Barrengtonia, Syzygium*). Those authors considered *Coffea arabica* and *Citrus* species which were earlier classified 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.

Finch-Savage (1996) stated that the categories suggested by Farrant *et al.*, (1988) are arbitrary and there is a continuous range of desiccation tolerance across species from orthodox through intermediate to recalcitrant seeds. An excellent review on recalcitrant seeds (Pammenter and Berjak, 1999) includes several reports, which describe the loss of viability during storage and desiccation. According to those authors, desiccation of recalcitrant seeds is due to mechanical damage associated with reduction of moisture content leading to failure of coordinated regulation of metabolism. Since water is intimately associated with macromolecular structures and is required to maintain the integrity of such molecules, removal of water leads to damage of membrane integrity.

Unlike orthodox seeds, recalcitrant seeds lack pronounced maturation drying, as they are usually shed at high moisture content. Generally recalcitrant seeds germinate within a short period of time in wet conditions due to high moisture content and as their germination processes are already started even from the time of shedding. There is a general consensus that desiccation intolerance involves the initiation of deleterious effects of water removal and resultant impaired metabolism (Chin and Roberts, 1980).

Recalcitrant seeds are susceptible to desiccation injury. Chin *et al.*, (1981) observed that sun-dried *Hevea* seeds and seeds stored in 45°C for 3 days exhibited detrimental effect due to dehydration and high temperature. High moisture content maintenance at ambient temperature is necessary for the retention of viability in recalcitrant seeds. According to Chin *et al.*, (1984) seeds of *Euphoria longan* and *Nephelium maliensis* were killed at an intermediate moisture level of 18% and 25% respectively compared to initial moisture content 46% and 36%. On drying, *Artocarpus heterophyllus* seeds

with 53% moisture content were killed at high moisture level i.e. 43% even though the seeds were equipped with a thin impermeable seed coat (Chin *et al.,* 1984).

Farrant *et al.*, (1988) put forward the concept that in recalcitrant seeds, a development-germination continuum exists and this was based on the observation pertaining to desiccation sensitivity. In *Avicennia marina,* seeds are increasingly sensitive to desiccation with germination related events Farrant *et al.,* (1988). If the seeds are rapidly dried before germination, they can tolerate greater amount of water loss and thus survive at 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 occur soon after shedding.

Quick response to desiccation by the recalcitrant seeds of *Shorea roxburghii*, *Hopea odorata*, *Mangifera indica* and *Symphonia globulifera* was reported by Corbineau and Come (1988). Those authors stated that *Shorea* and *Hopea* seeds were killed at moisture content 17%, *Mangifera* at 30% and *Symphonia* at 37%.

Effects of moisture content on germination of *Hancornia speciosa* (Apocyanaceae) seeds was studied by Oliveira and Valio (1992). These seeds were shed at moisture content 45% and when the moisture content fell below 25%, viability was lost due to dehydration associated changes such as increased leakage of electrolytes and organic solutes.

Changes in germination and desiccation sensitivity were measured throughout the seed expansion phase of development in *Quercus robur* (Finch-Savage, 1992) and the results revealed that recalcitrant behaviour of *Q. robur* might be due to premature termination of development so that desiccation tolerance was never achieved.

Kovach and Bradford (1992) investigated the desiccation tolerance of wild rice (*Zizania palustris*) seeds in relation to moisture content and temperature. They reported that 50% loss of viability corresponds to 32% moisture content in wild rice seeds and viability and moisture content were dependent upon particular dehydration and rehydration conditions. Dehydrin-like proteins were detected in these seeds at maturity which impart desiccation tolerance to a limited extend. According to them, temperature sensitivity of desiccation tolerance in wild rice seeds represents a unique relationship among seed moisture content, temperature and viability.

Germinability, storage response and desiccation effects on *Avicennia marina* seeds during development were studied by Farrant *et al.*, (1993). They found that developing *A. marina* seeds were not able to tolerate any drying until 70 DFS (days after fruit set), by that time, 30% of the reserve accumulation was completed and seeds attained germination capacity. Axes from rapid dried seeds were found to be more desiccation tolerant than those of slow dried seeds.

The drying of excised embryonic axes of Lychee (*Litchi chinensis*), longan (*Euphoria longan*) and Jackfruit (*Artocarpus heterophyllus*) seeds was investigated by Fu *et al.*, (1993). The moisture content of excised Jack fruit embryonic axes could safely reach 16% when dried with silica gel. When these were incubated in MS medium containing Arginine and NAA for 20 days, they differentiated to seedlings. There was no significant difference between the two different levels of air pressure during silica gel dehydration in the survival of excised axes of longan and lychee seeds. Excised embryonic axes of longan seeds could be dried to relatively low moisture content (13%) for maintaining viability, lower than that for lychee seeds. In the experiment on lychee and longan axes, they observed that the reduction of moisture content inhibited the growth of plumule and suggested that desiccation damage was more serious in the plumule.

Studies on desiccation tolerance in relation to the physical characteristics and chemical composition of the embryos of 7 species of *Inga* revealed that in all species, the viability was lost when the embryos were dehydrated to 20 to 30% moisture content (Pritchard *et al.*, 1995).

Desiccation sensitivity of recalcitrant seeds is dependent on moisture content, age/developmental stage of the seed and also related to inherent characters. Chandel *et al.*, (1995) pointed out that embryonic axes of partially mature and mature Jackfruit seeds could be desiccated to lower moisture level of 14% and 7% respectively whereas fully mature axes of tea seeds were desiccation tolerant. Those authors observed that Cocoa embryonic axes could not survive desiccation below 52% moisture content. According to Lin and Chen (1995) desiccation intolerance is an inherent character of recalcitrant seeds starting from early stage of seed development. Seed developmental studies in *Machilus kusanoi* by Chien and Lin (1997) revealed that mature recalcitrant seeds might not have better tolerance to desiccation than less mature seeds.

Wild nutmeg (*Virola surinamensis*) seeds were highly intolerant to desiccation and these seeds became nonviable within 24 hours in ambient conditions (Cunha *et al.*, 1995). Those authors reported that in these seeds viability was lost when the seeds were desiccated to 12% moisture content.

Bonner (1996 b) proposed a need of a protocol to test the recalcitrance. He studied the response of recalcitrant seeds of *Quercus nigra* by drying to 27°C and 40°C and suggested that fast to moderate drying at 27°C for 10 days would be suitable for a test for recalcitrance in *Quercus* seeds. According to those authors, the calculated critical moisture content for *Q. nigra* acorns at 27°C comes in the range of 10-12%.

By a comparative study of desiccation tolerance and viability in seeds from on-plant and detached capsules of foxglove (*Digitalis purpurea),*

Hay *et al.,* (1997) reported that in seeds from both detached and on-plant capsules, desiccation tolerance was acquired after attaining germinability, but the onset of desiccation tolerance was advanced in seeds from detached capsules compared to on-plant seeds. The failure of immature seeds to tolerate desiccation may be because they have not reached a critical level of storage reserves for there to be sufficient strong water binding sites in order to stabilize macromolecules.

The relationship between desiccation and moisture content of *Guarea guidonia* seeds was studied by Connor and Bonner (1998). Viability was reduced by more than 50% after 2-3 days of desiccation and a large drop in germination was observed before the axis moisture content fell below 38% and the critical moisture content was estimated between 15-20%. According to them, the intact seed moisture content is the fastest and easiest way of estimating viability in *G. guidonia* seeds.

Li and Sun (1999) studied the desiccation sensitivity of Cocoa (*Theobroma cacao*) embryonic axes and found that mature and immature axes of cocoa seeds tolerated desiccation under a rapid-drying regime to critical water contents of 1.0 and 1.07g g^{-1} dw respectively. The cotyledon tissues were more desiccation tolerant than axes, with a low critical water content of 0.24g g^{-1} dw.

The effect of drying rates on desiccation tolerance of embryonic axes of *Theobroma cacao* was investigated by Liang and Sun (2000). Rapid drying at low relative humidity and slow drying at high relative humidity were more harmful to Cocoa axis, because electrolyte leakage was increased along with the decrease in viability. The maximum desiccation tolerance was achieved by the axes at optimum drying rate (-0.2 h^{-1} corresponding to relative humidity between 88% and 91%). These studies confirmed that the physiological basis of the optimal drying rate is related to both mechanical

stress during desiccation and the duration of desiccation with which deleterious reactions may occur. The critical water content of embryonic axes of Cocoa was higher when axes were dried under low relative humidity or very high relative humidity.

An investigation on the effect of desiccation on fresh mature Sal seeds showed that seeds lost viability when stored for 6 days at ambient conditions (Chaitanya *et al.*, 2000 a). Desiccation of seeds from 42.2% to 36.8% leads to rapid loss of viability in these seeds. These seeds became nonviable at 6 days of storage. The moisture content as well as the rate of desiccation in embryonic axes (54.9%) and cotyledon (34.4%) varied during storage.

Anilkumar *et al.*, (2000) studied the viability of *Syzygium aromaticum* during storage. When the moisture content was reduced from 48.9% to 33.5%, the viability was completely lost within 48 hours of desiccation.

When *Avicennia marina* seeds were desiccated to 47% moisture content, the viability was completely lost (Greggains *et al.*, 2001). According to those authors over the range of moisture content between 65%-47%, drying was not associated with any significant change in dry weight or total protein content in the seed tissues.

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% or with 7.6-9.5% moisture content for *F. sylvatica* and *F. crenata* respectively.

Seeds of *Avicennia alba,* shed at a high moisture content (61%) were sensitive to desiccation (Le Tam *et al.*, 2004). Those authors noticed that none of these seeds survived below 35% moisture content.

Faria *et al.*, (2004) studied the water relations and loss of viability during desiccation of *Inga vera* seeds, and found that although the critical moisture content (1.84, 1.25 and 1.07g H_2O (g dry matter)$^{-1}$) decreased with development, the lethal moisture content was same (around 0.4g H_2O (g dry matter)$^{-1}$) for all developmental stages studied i.e. 6WAF, 9WAF, and 10WAF. For mature seeds, the three dehydration conditions tested resulted in decrease in the moisture content from 1.38g H_2O (g dry matter)$^{-1}$ (initial moisture content) to 0.39g H_2O (g dry matter)$^{-1}$ (critical moisture content) after 27, 51 and 106 hours in fast, intermediate and slow drying respectively.

The influence of rehydration technique on the response of recalcitrant seed embryos to desiccation was studied in *Artocarpus heterophyllus, Podocarpus henkelii* and *Ekebergia capensis* by Peran *et al.,* (2004). The excised embryonic axes were rapidly dried to safe moisture contents and reimbibed either rapidly by plunging directly into water or slowly, by placing the material on damp filter paper or exposing to a saturated atmosphere for several hours. In all cases, direct re-imbibition in water resulted in higher survival than either of the slow rehydration techniques.

Malik *et al.*, (2005) studied the desiccation-freezing sensitivity and longevity of freshly harvested seeds of *Garcinia indica, G. cambogia* and *G. xanthochymus* seeds with high moisture contents of 38.4%, 48.2% and

43.3% respectively. There was a substantial loss in germinability of seeds when desiccated to 27-32% moisture content. The critical moisture content value for *G. indica* was 19%, for *G. cambogia* 21% and for *G. xanthochymus* 27%. There was high desiccation sensitivity of the seeds in these three species where the *G. xanthochymus* was found to be relatively more desiccation sensitive.

Desiccation of recalcitrant seeds, resulting in different levels of tissue moisture content may lead to various changes in cytological, biochemical and physiological processes. Chin *et al.*, (1981), carried out the electron microscopic studies in *Hevea braziliensis and* revealed some of the ultrastructural changes associated with loss of viability due to desiccation. According to those authors, formation of reduced indistinct nucleus, damage to cell wall, disintegration of endoplasmic reticulum and tonoplast, were found to be characterized by desiccation.

The subcellular examination of desiccation sensitivity in Cocoa seeds using the SEM and TEM has been carried out (Ruhl, 1996). The primary site of desiccation damage in Cocoa seeds was cotyledon. The relatively short period of drying enabled the cotyledon to induce secondary damages in the radicle. The elevated exudation rate during imbibition as a result of drying pointed to the irreversible membrane damage. The ultramicroscopic examination revealed that in the cortical parenchyma cells of radicle, the double membrane enveloping the amyloplasts broke up exactly in the critical moisture range. This phenomenon was preceded by reduction in the number of ribosomes in the endoplasmic reticulum enveloping amyloplasts. The peculiar feature observed in the dried cortical parenchyma was the occurrence of large number of vesicles in the vicinity of plasmalemma at the critical moisture content. In the Cocoa axis cells, desiccation resulted in the decline of contrastability of nucleus and chromatin and finally their disappearance at critical moisture content. Groot *et al.*, (1996) studied the seed storage

behavior in relation to cell cycle progression and observed that partial hydration treatments like humidification and hydropriming increased the tolerance of the seeds to stress of deterioration. Osmopriming in -1 MPA PEG (Poly ethylene glycol) decreased the activity of seeds to cope up with the ageing stress. Those authors related this to the observed progression in the cell cycle that had occurred during the osmopriming. Prior to redrying, a considerable portion of the nuclei of the osmoprimed seeds had progressed towards the G_2 phase which had not occurred in humidification and hydropriming. Those authors suggested that in addition to DNA repair, other repair and replacement mechanisms of membranes, proteins and RNA might be influenced by the progression through cell cycle. Those authors recommended the investigations on cell cycle activity in recalcitrant seeds, which can indicate if and when the cells in recalcitrant seeds enter a stage of quiescence and may give an idea about maturity status of the seeds.

Kozeko and Troyan (2000) studied the relationship between the mitotic activity and moisture content of recalcitrant seeds of *Acer saccharinum* during maturation, post maturation drying and germination. They observed a decrease in mitotic activity and an increase in percentage of cells in the G_1 phase of cell cycle concomitant with a decline in moisture content during maturation of these seeds. Those authors concluded that in mature *Acer* seeds, the preservation of cell division capacity and increased mitotic activity might be essential for rapid germination immediately after shedding.

Changes in nucleic acid and RNAse activity have been monitored in the desiccating recalcitrant Sal seeds during storage (Chaitanya *et al.*, 2001). When freshly harvested undried seeds with 42.2% moisture content were desiccated to 15.8% (non-viable seeds) they showed 22% loss in DNA content. During the same period of desiccation, the loss in RNA content was relatively rapid. Those authors suggested that the dehydration induced decline

of nucleic acids preceded and lead to loss of germination in Sal seeds during storage. Similar pattern of RNAse activity was observed in the embryonic axis and the cotyledons of desiccating Sal seeds. Those authors concluded that as the overall process of germination is dependent on transcription and translation, the net loss of DNA and RNA contents due to reduced synthesis or enhanced catabolism or both could result in the reduced capacity of germination in Sal seeds when dehydrated below the critical moisture content.

Faria *et al.*, (2004) studied the relation between desiccation injury and cell cycle events in seeds of *Inga vera* subsp. *affinis* (Mimosoideae). According to them, these seeds were shed with about 81% of their cells in the root apex arrested at G_1 phase, with nuclei containing 2C DNA and suggested that this arrest of cell cycle in G_1 was not directly linked to the seed water status. During development of seeds, they observed that 2C and 4C DNA contents were constant and suggested that there were two blocks in the cell cycle one at G_2 which prevents the progress of cells with 4C nuclei through mitosis and another at G_1 which keeps the cells at pre-synthetic phase. They further stated that the abundant cortical microtubules (MTs) in cells of fresh axes of *I. vera* might be an indication that, at shedding, those cells were metabolically active. The different orientation of microtubules in cells of root (transverse) and shoot (no predominant orientation) could be related to their differential growth rate and patterning upon germination with root growth occurring before shoot elongation. According to those authors, there is a strong correlation between disappearance of MTs and loss of viability during drying of *I. vera* seeds and upon rehydration, severely damaged cells were no longer able to rebuild the microtubular cytoskeleton.

According to Chin *et al.*, (1981) even the brief exposure to adverse condition (sun-drying and storage at 45°C) reflected in the decline in germination percentage and vigour in *Hevea* seeds. Nautiyal and Purohit

(1985b) reported that one early event associated with loss of viability in Sal seeds is loss of membrane integrity. Very rapid loss of moisture content in seed coat lead to increased porosity of seed coat which ultimately allowed substances to leach out.

Hancornia speciosa (Apocyanaceae) seeds lost viability when the moisture content was decreased below 25% (Oliviera and Valio, 1992). According to those authors, germination and leachate of seeds were negatively correlated. Higher germination was obtained in seeds with high moisture content and low efflux of electrolytes and organic substances. Reduced germination and increased efflux of electrolytes and organic substances were due to dehydration of seeds.

Desiccation tolerance in relation to the physical characteristics and chemical composition of the embryos of 7 species of *Inga* was carried out by Pritchard *et al.*, (1995). Desiccation under three regimes (15°C, 26°C and 36°C) resulted in reduction of germination when the embryonic axis and cotyledon moisture content fell below 55 to 50% and 45 to 40% respectively. In all species, the viability was lost when the embryos were dehydrated to 20 to 30% moisture content.

The seeds of wild nutmeg (*Virola surinamensis*) lost viability completely when the moisture content was decreased to 12% from initial value of 26.5 % (Cunha *et al.*, 1995). They stated that the long period needed for wild nutmeg seeds to complete its germination in nature and laboratory conditions could be charged to the relative impermeability of the seed coat to water or oxygen and/or to the presence of secondary metabolites in the endosperm.

Pritchard (1996) suggested that germination rate might provide the first indication of desiccation stress in recalcitrant seeds and he stated that there is

a strong relation between germination and vigour in both orthodox and recalcitrant seeds.

In *Guarea guidonia* seeds, the viability was reduced to 50% in 2-3 days of desiccation, when moisture content of intact seeds was 24% and that of embryonic axes above 38% (Connor and Bonner 1998).

Tompsett and Pritchard (1998), suggested that in *Aesculus hippocastanum* seeds, at least four physiological processes (maturation desiccation, onset of desiccation tolerance, longevity and germination) could proceed between particular axis water potential limits, the overall range extending down to about -3MPa and the water potential at which these physiological processes occurred appear to be highly dependent on the post harvest history of seeds.

Differential Scanning Colorimetry was used to investigate the water relations of desiccation sensitivity in embryonic axes and cotyledons of *Quercus robur* seeds (Pritchard and Manger, 1998). Desiccated seeds as well as axes isolated from desiccating fruits and grown *in vitro* exhibited a similar pattern of viability loss with decreasing moisture content to that observed for seed germination. According to them, 25% of excised axes grew after rapid desiccation to 17% moisture content, which was within the 'bound' water fraction of the axis as determined by the DSC and from the sorption isotherm. They suggested that the desiccation stress in recalcitrant seed axes manifest at two levels i.e. a time-dependent, potentially reversible stress relating to the removal of vicinal water and an irreversible injury resulting from the loss of bound water.

Seeds of four Sahelian and Sudanian tree species *Cordyla pinnata* (Caesalpiniaceae), *Boscia senegalensis* (Capparidaceae), *Butyrospermum parkii* (Sapotaceae) and *Saba senegalensis* (Apocyanaceae) lost viability

rapidly when seed moisture contents were below 30% for *Cordyla pinnata* (Caesalpiniaceae) and 22-25% for the other three species (Danthu *et al.*, 2000).

Correlation between moisture content and viability loss has been investigated in plants such as Sal (*Shorea robusta)* seeds which became nonviable within 6 days after harvest, when the moisture content decreased from 42.1% to 26.5% (Chaitanya *et al.*, 2000 a) and *Avicennia marina* seeds lost viability when their moisture content decreased from 65% to 60% (Greggains *et al.*, 2001). These seeds lost viability completely when the moisture content was 47%. Chaitanya *et al.*, (2001) further suggested that the desiccation sensitive Sal seeds exhibited rapid loss of viability when the moisture content decreased from 42.2% to 26.8%.

Sal (*Shorea robusta*) seeds exhibited 80% loss of viability in seeds desiccated from 42.1% moisture content to 22.3% moisture content after 144 hours of shedding (HAS) (Varghese *et al.*, 2004) and fresh seeds of *Avicennia alba* germinated easily and completely within 10 days at 28-30°C, viability was reduced considerably by desiccation, only 2% seeds survived desiccation to 35% moisture content (Le Tam *et al.*, 2004).

Faria *et al.*, (2004) incubated the mature embryos of *Inga vera* at eight temperatures ranging from 5 to 40°C to determine best temperature for germination. They noticed 100% germination of these seeds at temperatures ranging from 15 to 40°C. Desiccation of embryos beyond 1.0 g H_2O (g dry matter)$^{-1}$ resulted in reduction of germination.

The desiccation-freezing sensitivity and longevity of *Garcinia indica, G. cambogia* and *G. xanthochymus* seeds was studied (Malik *et al.*, 2005). Freshly harvested seeds of *G. indica, G. cambogia* and *G. xanthochymus* were shed at high moisture contents of 38.4%, 48.2% and 43.3% respectively. The

loss of germinability occurred when seeds were desiccated to between 15 to 18% moisture content for all the species studied.

Recalcitrant seeds exhibit a wide range of characters towards temperature fluctuations (King and Roberts, 1979). Seeds of *Theobroma cacao* do not tolerate temperature of 15°C (Hor *et al.*, 1984). Seeds of *Symphonia globulifera* cannot tolerate temperature of 10°C (Corbineau and Come, 1988). Freshly harvested recalcitrant seeds with high moisture content were also killed by chilling injury which was reported by Chin *et al.* (1984). The wild nutmeg seeds showed intolerance to cold storage at temperatures of 5°C and 20°C (Cunha *et al.*, 1995).

Freezing damage in moist seeds is presumably associated with the formation of ice crystals, and usually occurs with moisture contents above14-20% (Roberts, 1973). Hor *et al.*, (1984) showed that there was a sharp reduction in storability of Cocoa seeds at 15°C compared to 17°C, indicating that they are very sensitive to slight temperature changes around a critical value.

Kovach and Bradford (1992) reported that wild rice (*Zizania palustris*) seeds are intolerant to desiccation at low temperature. This is because of the fact that dehydrin like proteins are not apparently sufficient in these seeds.

The subcellular examination of chilling sensitivity in Cocoa seeds using the SEM and TEM was carried out by Ruhl (1996). After chilling of Cocoa seeds, no ultrastructural changes in the cortical parenchyma cells of radicle is observed. But the storage parenchyma cells of cotyledons underwent serious ultrastructural changes during chilling. The lipid bodies were completely fused with one another (confluence of lipid bodies) and crystallized.

Seed storage behavior of four tree species *Boscia senegalensis* (Capparidaceae), *Butyrospermum parkii* (Sapotaceae), *Cordyla pinnata* (Caesalpiniaceae), and *Saba senegalensis* (Apocyanaceae) was carried out by Danthu *et al.*, (2000). When these seeds were stored at -5°C, *B. senegalensis* seeds died within 15 days, *B. parkii* and *C. pinnata* seeds survived for 5 days and *S. senegalensis* remained viable for two months.

According to Sacande *et al.*, (2000), longevity of Neem seeds was considerably improved and the sensitivity to chilling/subzero temperatures was reduced when axis and cotyledons were dehydrated to moisture contents of approximately 0.05g H_2O g^{-1} dw. The storage of *Syzygium aromaticum* seeds in polythene bags at freezing temperature (0°C) was found to be fatal while at chilling temperature (10°C) they remained viable for a month (Anilkumar *et al.*, 2000).

Varghese *et al.*, (2004) observed that dried and undried Sal (*Shorea robusta*) seeds were killed when exposed to ultralow (liquid nitrogen) temperature (-196°C), subfreezing (-20°C) and freezing temperature (0°C) within a day of storage. Seeds of *Avicennia alba* showed rapid loss of viability at 10°C with all moisture contents possibly as a result of chilling injury (Le Tam *et al.*, 2004).

According to Faria *et al.*, (2004), the high germination attained at 10°C (85%-90%) by fresh or slightly dried *Inga vera* embryos indicated that this species is quite resistant to low temperatures at least to 10°C but none of the seeds germinated at 5°C showing the chilling injury of these seeds.

The chilling temperature of 5°C was unfavourable for storage of *Garcinia indica, G. cambogia* and *G. xanthochymus* since these seeds lost germinability within 10-15 days of storage (Malik *et al.*, 2005). Storage of these seeds at freezing temperature of -20°C was proved to be lethal as no

survival observed after 5 days of storage in all the three species. Liquid nitrogen exposure of seeds desiccated to moisture content of 27% and lower in both *G. indica,* and *G. xanthochymus* and 31% and lower in *G. cambogia* was detrimental.

Chin *et al.*, (1981) determined critical lethal moisture level of *Hevea braziliensis* and studied the relationship between rate of loss of viability and changes in cell ultrastructure during storage. According to them, *Hevea* seeds can be successfully stored at cool temperature of 7-10°C along with use of moist sterilized saw dust to maintain high moisture level. Later, Chin *et al.*, (1984) suggested that seeds of *Theobroma cacao* could be stored effectively if they are air dried at a much lower temperature of 20°C using a fan.

Significant increase in moisture content during storage of seeds was reported in acorns of *Quercus* species. (Clatterbuck and Bonner, 1985). Those authors correlated significant increase of moisture content in acorns of *Quercus* species during storage to important biochemical changes during this period. They noticed significant decrease in lipid content during storage. According to them, breakdown of lipid content (20-35% to 4-7%) to carbohydrates probably by lipolysis, resultant oxidation and glyoxylate pathway allowed further tissue hydration and enhanced the moisture content. They noticed that soluble and insoluble carbohydrate contents were increased significantly during storage.

Nautiyal and Purohit (1985 a) reported a rapid loss of moisture content of Sal seeds from 50% to 2% resulting in loss of viability within 12 days when stored under natural condition and concomitant rapid increase in concentration of components in leachate within 12 days of maturation. According to Nautiyal and Purohit (1985 b) seeds could be stored in room open condition only for 10 days and after about 8-10 days of storage, the seeds crossed critical moisture level as a result of which repair and turnover

system failed to operate due to damage of cellular membranes and seed viability was completely lost. They opined that the basic reason for membrane deterioration is the desiccation of seeds below critical moisture content level (25%).

Nautiyal *et al.,* (1985) noticed progressive decline in number of proteins in recalcitrant seeds of *Shorea robusta* during loss of viability under storage. It was due to denaturation of protein with higher mobility during ageing process of seeds and this denaturation of proteins might lead to the structural changes in membranes and thus increased their permeability.

Studies on *Hopea parviflora, Mangifera indica* and *Shorea roxburghii* revealed that wet storage of these seeds was difficult as germination was induced by low temperature (Corbineau and Come, 1988). In *Symphonia globulifera*, storage at 15°C was possible for about one year because of slow growth of root and stem and long term storage of these seeds is possible only when measures are taken to prevent germination or growth without chilling or dehydration injury. Those authors suggested two possibilities (1) use of solution of suitable osmotic potential and (2) using cryoprotectants to enable seeds or seedlings to withstand lower storage temperature.

Seeds of *Hancornia speciosa* (Apocynaceae) could be effectively stored for nine weeks without losing viability in polythene bags irrespective of storage temperatures as the stored seeds maintained high moisture contents (Oliviera and Valio, 1992). After nine weeks of storage, the germination percentage was decreased considerably even when the high moisture content was maintained.

It is well known that recalcitrant seeds ultimately lose viability if maintained in a hydrated state because hydrated recalcitrant seeds are metabolically active and undergo germination-associated changes under

storage (Farrant *et al.,* 1992). Changes like extensive vacuolation and increased cell size occurring during germination require additional water as reported by Pammenter *et al.*, (1994). According to those authors, during storage, recalcitrant seeds are exposed to severe water stress. Harmful events associated with water stress for considerable duration lead to death of tissues. They interpreted that the damage that occurs on prolonged storage is unlikely to be associated with an inability to form glasses or prevent membrane-lipid phase changes as absolute water contents are higher than those at which these mechanisms are very important. They considered the most likely process leading to death of water stressed tissue is breakdown of co-ordination of metabolisms leading to uncontrolled free radical mediated oxidative damage.

In recalcitrant seeds of *Aesculus hippocastanum* (Tompsett and Pritchard, 1993) the storage tissue serve as a water reservoir for axes and water moves from cotyledons to axes during germination. Similarly viability of *Quercus robur* seeds was controlled by the water content of cotyledon rather than that of axes (Finch-Savage, 1992). Rapid loss of moisture content in dry storage was reported in most recalcitrant seeds (Hor *et al.,* 1984; Nautiyal and Purohit, 1985 a; Corbineau and Come, 1988; Finch-Savage, 1992; Chandel *et al.,* 1995; Chien and Lin 1997).

According to Pammenter *et al.*, (1994) during storage, recalcitrant seeds are exposed to severe water stress. Harmful events associated with water stress for considerable duration lead to death. The viability in *Quercus robur* seeds were controlled by the water content of cotyledon rather than that of axes.

Magill *et al.*, (1994) studied the physiological and biochemical aspects of seed storage and suggested that drying of Papaya seeds to 5% moisture content was accompanied by large decrease in intensity of free radical signal in EPR spectra. They concluded that free radical signal might reflect the post

treatment (chilling and wet-freezing or desiccation) metabolic status of seeds immediately before EPR measurements.

Lin and Chen (1995) reported that seeds of *Machilus thunbergii* can be stored wet for ten months at 4°C before any decrease of viability. In this plant, mature seeds entered shallow dormancy at maturity. Those authors attributed the continuous metabolism also as characteristic of recalcitrant seeds with observation of moderate energy charge and moderate rate of respiration in mature seeds of *Machilus thunbergii.* Even without desiccation, seeds of *Machilus kusanoi* showed short life span when stored under wet and cold conditions i.e. at 5°C (Chien and Lin, 1997). Those authors compared the high energy change in both maturing and mature seeds of *Machilus kusanoi* and concluded that metabolic activities such as high respiration rate and higher energy change of mature seeds occurring continuously in recalcitrant seeds account for the low storage potential of these seeds.

According to Pritchard (1996), methods for the determination of qualities of seeds with recalcitrant characteristics fall into three basic categories: biochemical, biophysical and physiological. The information provided by each approach can be complementary yielding details of possible early biochemical and biophysical events that occur during desiccation stress resulting in membrane instability/injury which ultimately leads to physiological incompetence (i.e. failure to germinate).

Bonner (1996 a) suggested certain solutions to the storage problem in commercial seed supply of recalcitrant and intermediate seeds. According to him, the extent of commercial trade of tropical recalcitrant species is very small and the primary problems that prevent widespread trade of all recalcitrant seeds are both physiological and administrative. He opined that the most interesting challenges are to determine the physiological basis for sensitivity to desiccation and sensitivity to chilling.

According to Hong and Ellis (1996), long-term seed storage serves as a safe and relatively inexpensive method of plant genetic resources conservation. For long term storage of recalcitrant seeds, measures are to be taken to prevent the chilling injury as well as the desiccation injury in order to maintain their viability. Moist storage or wet storage has been practiced to prevent the desiccation injury (Copeland and McDonald, 1995). Storage studies on *Syzygium* species revealed that *Syzygium aromaticum* seeds can be stored beyond one month at 0°C, where as seeds of *S. cumini* remained viable up to 2 years at 10°C (Anilkumar, 1998).

Farnsworth (2000) reviewed the physiology, morphology and ecology of viviparous and recalcitrant seeds. Embryos of recalcitrant and viviparous species cannot tolerate the maturation drying which is a pre-requisite for dormancy. Such desiccation intolerance creates challenges for storing and preserving such embryos. He opined that integrative data from ecological, genetic and physiological studies are needed to elucidate evolutionary origins and maintenance of reproductive strategies.

According to Wood *et al.*, (2000), Papaya seeds enter dormancy when dried to moisture content of about 4.5 to 11.5%. Dormancy can be induced in these seeds by the relative humidities of 20-14% and is probably imposed when water at weak binding sites is removed. They suggested that the specificity and reversibility of desiccation induced dormancy by heat shock cause the possibility of precise signal transduction pathway controlling germination response in Papaya seeds.

Cocoa seed axes can be easily stored for more than two months without significant loss of viability and vigour (Liang and Sun, 2000) and Lychee (*Litchi chinensis*) seeds could be stored for 15months when kept at 10°C in polythene bags without peat moss (Duarte *et al.*, 2001). According to Anilkumar *et al.*, (2000) depulped *Syzygium aromaticum* seeds could be

stored for nine months if they are stored in polythene bag at 30°C and they recommended this method for germplasm repository.

Danthu *et al.*, (2000) suggested that the four Sahelian and Sudanian tree species, *Boscia senegalensis* (Capparidaceae), *Butyrospermum parkii* (Sapotaceae), *Cordyla pinnata* (Caesalpiniaceae) and *Saba senegalensis* (Apocyanaceae) can be stored at 15°C without losing viability for 2 months, 3 months, 3 months and 4 months respectively.

The effects of two drying rates on desiccation tolerance of embryonic axes of jackfruit (*Artocarpus heterophyllus*) seeds was carried out by Smith *et al.*, (2001). Rapid drying conferred greater tolerance to drying with 100% viability at approximately 0.4g g^{-1} moisture content.

Recalcitrant behaviour of temperate forest tree seeds was studied by Connor and Sowa (2002). They studied the hydrated storage of live oak (*Quercus virginiana*) and Durand oak (*Quercus durandii*) at two temperatures 4°C and -2°C for 1 year and tested monthly. Seeds of red buckeye (*Aesculus pavia*) were stored similarly and analysed after 3 months. The seeds of cherrybark oak (*Quercus pagoda)* and water oak (*Quercus nigra)* were tested yearly. They observed that Durand oak, live oak and red buckeye seeds stored at -2°C maintained higher viability for a long time without sprouting. But in water oak and cherry bark oak, acorns lost viability after 2 years at -2°C due to the dehydration treatment before storage. They suggested that measures are to be taken to prevent dehydration from the collection of seeds onwards.

Varghese *et al.*, (2004) studied the effect of differential rates of drying on viability and storability in recalcitrant Sal (*Shorea robusta*) seeds. They found that continued dehydration from 42.1% to 19.5% moisture content in natural and 9.8% moisture content in rapid drying resulted in absolute loss of viability. They observed that rapid drying resulted in higher viability when

desiccated to lower moisture contents. According to them, the removal of bound water probably lead to deleterious subcellular changes and desiccation induced oxidative stress ultimately leading to loss of viability.

Le Tam *et al.*, (2004) investigated the seed storage of *Avicennia alba* and concluded that storage temperature of 17°C was most suitable for survival among three temperature regimes. When these seeds were mixed with sand at 10% moisture content at 17°C, 75% survived for four months, but at 28-30°C only 70% survived. So those authors recommended the former storage condition for short-term storage of *A. alba* seeds in mangrove reforestation programmes.

Storage studies on fresh embryos of *Inga vera* at different temperatures such as 5, 10 and 20°C revealed that after 10 days of storage, germination of embryos stored at these temperatures decreased from 100% to 50, 30 and 0% respectively (Faria *et al.*, 2004). After 18 days, only embryos stored at 5°C showed 5% germination.

Desiccation-freezing sensitivity and longevity studies on seed storage behaviour were investigated in *Garcinia indica, G. cambogia* and *G. xanthochymus* (Malik *et al.*, 2005). They found that freshly harvested seeds of all species stored at ambient temperatures retained viability for about 30 days and the seed longevity in all three species could be extended to 45 days by storage at 15°C.

For effective long term storage of recalcitrant seeds, new techniques like cryogenic storage of embryos is practiced. Earlier Roberts *et al.*, (1984) suggested that storage in liquid nitrogen (-196°C) is the most promising method. But this technique requires drying treatment prior to freezing as seeds can survive -196°C only at low moisture contents. The recalcitrant seeds are unable to tolerate desiccation and cannot be stored at sub-zero temperature

because ice nucleation leads to cell death. So techniques are being developed for cryopreservation of embryonic axes. (Pritchard, 1995). Chandel *et al.*, (1995) successfully cryopreseved partially and fully mature embryonic axes of jackfruit seeds with survival of 30% and 25% respectively. According to them, long-term conservation of germplasm of the Jackfruit seeds was possible through cryopreservation of excised embryonic axes. Those authors reported that in tea seeds, embryonic axes desiccated to 13% moisture levels could be successfully cryopreserved with survival percentage of 95%.

Neem seeds have been successfully cryopreserved at liquid nitrogen temperature when they were first dried to 0.09 g H_2O g^{-1} dw for the entire seed and 0.23g H_2O g^{-1}dw for isolated embryonic axes (Berjak and Dumet, 1996).

Krishnapillay and Engelmann (1996) suggested alternative methods like slow growth and cryopreservation for the storage of recalcitrant and intermediate seeds. According to them, the use of *in vitro* culture techniques is of great interest for collection, storage and exchange because it provides opportunity for very high multiplication rates. The classical cryopreservation procedures comprise a pretreatment with cryoprotectant followed by slow controlled freezing. The new cryopreservation techniques proposed by Krishnapillay and Engelmann (1996) are encapsulation-dehydration, vitrification, desiccation and pre-growth desiccation.

Several approaches have been pursued in the biochemical analysis of recalcitrant seeds to elucidate the mechanism of desiccation, storage and germination (Clatterbuck and Bonner 1985, Nautiyal and Purohit, 1985 a, Steadman *et al.*, 1996).

Clatterbuck and Bonner (1985) studied the utilization of seed reserves in four species of *Quercus* i.e. *Q. alba, Q. nigra, Q. shumardii and Q. falcata*

var. *pagodaefolia* during storage. They noticed a slow accumulation of starch in early stages of storage followed by a peak prior to emergence of radicle and then a decrease with the elongation of radicle. This pattern indicated the breakdown of starch for supplying simpler carbohydrates for the radicle growth. Breakdown of lipids to carbohydrates allowed further tissue hydration leading to high moisture content. Those authors proposed a hypothesis that storage life of *Quercus* species of red oak group viz. *Quercus nigra, Q. falcate* var. *pagodaefolia* and *Q. shumardii,* might be extended by blocking lipid metabolism with an inhibitor of lipases.

Studies on physiological and biochemical aspects of ageing in the seeds of *Shorea robusta* (Nautiyal and Purohit, 1985 a) revealed a decline in soluble carbohydrates, starch, protein and acid phosphatase activity. These changes were accompanied by increase in phenolic contents and gradual increase in sugar contents in seed leachate showing continuous loss of membrane integrity. According to those authors, the breakdown of lipid resulted in production of free radicals that inactivated enzymes, proteins and nucleic acids and ultimately lead to disruption of cell membrane which result in loss of differentially permeable nature. They also postulated that increased content of laminin in embryo could act as protein denaturants.

Soluble carbohydrates have been proposed to play an important role in conferring desiccation tolerance and storability in seeds. Chandel *et al.*, (1995) noted an increase in soluble carbohydrates with desiccation of tea and Cocoa axes and suggested that after desiccation and freezing, degradation of starch granule was common in recalcitrant species and it might have caused by conversion of starch into soluble sugars. Lin and Chen (1995) suggested that in *Machilus thunbergii* the induced sucrose and proline and decreased ABA at desiccation indicated that some maturation specific metabolic activities are quickly activated or induced and may be a common

phenomenon of recalcitrant seeds and orthodox seeds. The nature of mature *Machilus* seeds with a moderate energy charge at room temperature indicated continuous metabolic activities characteristic of recalcitrant seeds.

Desiccation tolerance in relation to the physical characteristics and chemical composition of the embryo of 7 species of *Inga* was carried out by Pritchard *et al.*, (1995). Those authors suggested that in all species, the viability was lost when the embryos were dehydrated to 20 to 30% moisture content. Frequently encountered desiccation intolerance in *Inga* embryos might be associated with critically low levels of specific soluble carbohydrates in their tissues.

A profound study on soluble sugar content of 13 genera belonged to 10 different families consisting of orthodox and recalcitrant seeds (Steadman *et al.*, 1996) revealed that accumulation of specific soluble carbohydrates might be used as diagnostic marker for seed storage category. They concluded that total quantity of sugars in whole embryos did not bear any relation to seed storage category. Oligosaccharide: sucrose ratio <0.083 was exhibited by the recalcitrant seeds. Those authors suggested that such low ratio observed in orthodox seeds prior to acquisition of desiccation tolerance and this may reflect physiologically immature nature of recalcitrant seeds when naturally shed from parent plant.

According to Bonner (1996 b), seeds with food reserves that are primarily carbohydrates, get desiccated quite rapidly and are much more likely to have split pericarps than species with fats as primary food reserves

Electrophoretic study on protein profile during loss of viability in Sal seeds (Nautiyal *et al.*, 1985) indicated a lower number of soluble proteins in nonviable seeds than viable seeds. According to them the presence of broad, diffused bands in nonviable seeds as compared to sharp clearly defined peaks

of proteins in viable seeds indicated deterioration of proteins in nonviable seeds. Those authors also noticed that proteins with higher mobility got denatured during ageing process and the denaturation of proteins may lead to structural changes in membranes and thereby increasing their permeability and porosity.

Proteins that have homology with dehydrins have been identified immunologically in recalcitrant seeds of English oak (*Quercus robur*), European chestnut (*Castanea sativa*), horse chestnut (*Aesculus hippocastanum*), sycamore (*Acer platanoides*) and silver maple (*Acer saccharinum*) and orthodox seeds of Norway maple (*Acer platanoides*) (Finch-Savage *et al.*, 1994). They found that in *Q. robur* the amount of dehydrin protein increased during seed development, and LEA mRNA was induced by limited desiccation and by abscissic acid. Those authors concluded that the presence of dehydrin proteins was not sufficient to confer desiccation tolerance in recalcitrant seeds and their presence or absence could not be used as criteria for identification of recalcitrant seeds.

By protein profile analysis of Jackfruit seeds during developmental stages, the maturation of immunoglobulin-A binding proteins was studied (Kabir and Daar, 1995). Those authors noticed that early developing seed contained several protein bands distributed over a molecular weight range of 10-67 kDa. The mature seed proteins consisted of powerful hemagglutinating activities, which were not detected in early developing seeds. By Western blotting they found that the early developing seed contained a novel 10 kDa IgA binding protein that was not present in mature seeds.

Chaitanya *et al.*, (2000 a), studied the changes in total protein and protease activity in dehydrating recalcitrant Sal (*Shorea robusta)* seeds. They noticed a gradual decline in total protein content due to corresponding increase in protease activity preceded loss of viability. After 3 days of

desiccation, the embryonic axes and cotyledon exhibited 43.7% and 52.6% loss of protein compared to the fresh seeds. They found that fresh seeds were without any protease activity but protease activity was detected after 12 hours of storage and increased rapidly up to loss of seed viability (6 days after harvest) and then declined sharply in cotyledon and embryonic axis. Enhanced protease activity in embryonic axis and cotyledons was correlated with decline in total protein during desiccation-induced loss of viability in Sal seeds. The same authors (Chaitanya *et al.,* 2001) made an attempt to elucidate the role of nucleic acid metabolism during desiccation induced loss of viability in recalcitrant Sal seeds. They observed that during storage, dehydration of Sal seeds resulted in sharp increase in RNAse activity, which was totally absent in fresh undried seeds and suggested that desiccation-induced deterioration in Sal seeds could be due to release of RNAse with other hydrolytic enzymes from lysosomes.

Water sorption isotherms of Neem seeds showed that at similar relative humidity (RH), the water content was consistently higher in axes than in cotyledons mainly due to the elevated lipid content (51%) in the cotyledons (Sacande *et al.*, 2000).

Recalcitrant behaviour during hydrated storage of temperate forest trees *Quercus virginiana*, *Q. durandii*, *Q. nigra, Q. pagoda* and *Aesculus pavia* was studied by Connor and Sowa (2002). FT-IR (Fourier transform infra red spectro-metry) studies have shown that *Q. pagoda* acorns subjected to severe desiccation exhibited irreversible changes in membrane lipid and protein secondary structure. Those authors interpreted this change as the most sensitive indicator of viability loss of seeds.

According to Hendry *et al.*, (1992) and Finch-Savage *et al.*, (1993, 1994) recalcitrant seeds do appear to possess antioxidant mechanisms and these protective mechanisms may become impaired under conditions of water

stress. Generation of super oxide anions (a free radical) accompanying membrane damage has been reported in the highly recalcitrant seeds of *Shorea robusta* (Chaitanya and Naithani, 1994). The metabolism induced damage associated with desiccation of recalcitrant seeds has been reviewed by Come and Corbineau (1996) and suggested that free radical generation as a consequence of uncontrolled metabolism may be a major injurious factor during relatively slow dehydration of recalcitrant seeds undergoing a spectrum of lethal lesions as already opined by Smith and Berjak (1995).

Axes and cotyledons of recalcitrant *Castanea sativa* seeds exhibited contrasting response of respiration to drying in relation to desiccation sensitivity (Leprince *et al.*, 1999). Axes of *C. sativa* were less sensitive to drying than cotyledons. They suggested that in mitochondria of *C. sativa* cotyledons, electrons might have leaked out of transport chains at or before cytochrome b and c promoting the formation of Reactive Oxygen Species (ROS). Formation of ROS and sustained damage to mitochondria lead to metabolic dysfunction and oxidative stress. The unabated respiration in drying *C. sativa* cotyledons might lead to loss of membrane integrity.

A comparative study of ascorbate system in recalcitrant and orthodox seeds was done by Tommasi *et al.*, (1999). The study materials included the recalcitrant seeds *Ginkgo biloba, Quercus cerris, Aesculus hippocastanum* and *Cycas revoluta* and orthodox seeds were *Vicia faba, Avena sativa* and *Pinus picea.* Those authors found that the recalcitrant seeds contained a large amount of ascorbic acid and maintained high ascorbate peroxidase activity. But the orthodox seeds were completely devoid of ascorbic acid and ascorbate peroxidase activity. According to those authors, slow dehydration affected the ascorbate peroxidase activity in the recalcitrant seeds leading to complete loss of germinability. They noticed that recalcitrant seeds were characterized by the presence of low activity of enzyme dehydroascorbate reductase and high

activity of ascorbate free radical reductase. But in the case of orthodox seeds, high activity of enzyme dehydroascorbate reductase and low activity of ascorbate free radical reductase were observed. They related the high activity of ascorbate free radical reductase to the high metabolic activity of recalcitrant seeds. The presence of high dehydroascorbate reductase activity in orthodox seeds is found to be essential at the beginning of germination because it is the only way to provide embryo cells with ascorbate. In recalcitrant seeds the ascorbate biosynthesis through galactono dehydrogenase is always functional and dehydroascorbate reduction plays a secondary role in the maintenance of ascorbate pool in cell.

Chaitanya *et al.*, (2000 b) studied the ascorbic acid metabolism in ageing recalcitrant Sal (*Shorea robusta)* seeds and reported that ascorbate levels were significantly higher initially in the embryonic axes and cotyledons of fresh Sal seeds. Ascorbate levels declined sharply in desiccated seed tissues. They suggested that relatively higher amounts of ascorbate might effectively suppress the desiccation induced oxidative stress leading to loss of viability in Sal seeds.

Rapid decrease in the activities of superoxide dismutase and peroxidases during desiccation in mature and immature Cocoa axis were found to be corresponded to desiccation sensitivity (Li and Sun, 1999). They suggested that the enzymic protection against free radical attack plays an important role in desiccation sensitivity of recalcitrant seeds during desiccation. They correlated the decrease in enzymic protection with associated lipid peroxidation in Cocoa seed tissue. Lipid peroxidation produces highly reactive free radical intermediates that can damage membrane proteins and nucleic acids. According to those authors, desiccation sensitivity of recalcitrant Cocoa axes did not appear to be due to lack of sugar

related protective mechanisms during desiccation because axes had a sucrose to oligosaccharide mass ratio of 4.5:1 which was similar to orthodox seeds.

According to Liang and Sun (2000), under low relative humidity, the water potential of Cocoa seed axes changed very rapidly which may result in uneven rapid volumetric changes that induce great damage within well organized seed tissues. Under very slow drying conditions, seed axes may be damaged by various deleterious processes that take place during the period of prolonged dehydration, ranging from disruption of metabolic regulation to failure of antioxidant systems.

In *Avicennia marina* seeds, Greggains *et al.,* (2001) noticed an increase in amount of tocopherol and activity of superoxide dismutase (SOD) in the plumule as free radicals accumulated which was followed by decline in viability during desiccation. They suggested that propagules of *A. marina* experienced oxidative stress since lipid peroxidation was increased in advance of viability loss.

Eventhough germination is considered as an index or marker or indicator of viability of seeds in general and desiccated seeds in particular, metabolic aspects of desiccated recalcitrant seeds during germination also are not investigated properly. However Jisha Mathew (2006) made a preliminary study on germination of desiccated mango seeds of different developmental stages and suggested that starch depletion and a concomitant increase in sugars are observed during germination of both control and desiccated seeds. The germination associated metabolic changes are presumed to be observed in fresh recalcitrant seeds which are metabolically active at the time of shedding, and development and germination takes place as a continuation as suggested by Farrant *et al.*, (1988).

Seed reserve composition and mobilization during germination and initial seedling development in *Euphorbia heterophylla* was carried out by

Suda and Giorgini (2000). Lipid was the main reserve in these seeds and its degradation began immediately after imbibition. Total sugar remained unchanged up to 72 hours and then declined. Protein degradation occurred between 36 and 72 hours which was confirmed by histochemical studies which showed the abundance of protein bodies in the beginning followed by their fusion and disappearance until 72 hours. However, those authors concluded that dry weight, lipid, total protein and amino acids were decreased over 72 hours during germination at higher rates under light compared to dark.

Pritchard *et al.*, (1999) investigated the kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds and stated that stratification at 6°C prior to germination at warmer temperatures increased the proportion of seeds that germinated and the increased sensitivity to chilling with warmer temperatures during the period of seed filling. According to those authors, freshly harvested seeds of *Aesculus hippocastanum* were capable of germination to a certain extent at high temperature (30°C and 36°C) and to full capacity at low temperature (2 to 6°C) but very low germination at intermediate temperature (16 to 26°C). They also suggested that the thermal history of the seeds on the parent tree influences their subsequent responses to dormancy breaking treatments.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON JACKFRUIT SEEDS (*Artocarpus heterophyllus Lam.*) DURING STORAGE AND GERMINATION

Thesis
submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT

2007

Materials and methods

Jackfruits (*Artocarpus heterophyllus* Lam.) for the present study were collected from a specific (marked) tree growing at Chathannur Village in Kollam District during November to February of 2004-'05 and 2005-'06. Such a particular tree was selected because this tree flowers earlier than the Jackfruit trees of Calicut University Campus and hence mature fruits become available during November to February as and when the rainy season erases. Since Jackfruit seeds are recalcitrant and desiccation is an important aspect of this investigation 'seed moisture content' should not be varied much during the collection of ripened fruits. The firm flesh variety (Anonymous, 2000) of Jackfruit was selected for the present investigation. Inflorescences were tagged on the day of anthesis to record the number of days taken for development and designated as days after anthesis (DAA). Fruits ripened on the mother plant (120 DAA) were collected manually and brought to the laboratory. Fruits were cut open and seeds were collected without damage. Seeds were depulped to remove perianth and aril.

Washing

Depulped seeds were soon washed thoroughly in running water followed by distilled water to remove any trace of perianth or aril. Washed seeds were wiped with clean towel.

Surface sterilization

Seeds were surface sterilised by wiping with a clean towel wetted with 80% ethyl alcohol and kept for surface drying for 15 minutes at room temperature.

DESICCATION STUDIES

Seeds of ripened fruits (120 DAA) only were used for desiccation, storage and germination studies. Surface sterilised seeds (approximately 500) collected from 3-4 of ripened fruits were kept immediately after sterilisation for desiccation in open trays at room temperature (30±3°C) and designated as **Room-Open**.

Sampling

Desiccated seeds were sampled for moisture content determination and viability studies at an interval of 4, 8, 12, 13, 14, 15 and 16 days after desiccation and for biochemical studies at an interval of 4, 8, 12, 14 and 16 days. Fresh seeds immediately after collection served as the control (0 sample)

STORAGE STUDIES

Surface sterilized seeds of ripened fruits (120 DAA) were kept for storage under different conditions. One part of the collected seeds (approximately 300 numbers) were kept in sealed polythene bags providing sufficient space for air, and kept at room temperature designated as (**Room-**

Polythene). Another lot consisting of about 300 seeds were kept in sealed polythene bags in the lowest tray of fridge (**Refrigerator** 4-8°C). Seeds of both types of storage were sampled for germination, moisture content determination and biochemical studies at an interval of 10 days each upto 130 days (until the seeds become non-viable).

Viability

Ten seeds each in duplicate of control, desiccated and stored seeds were sampled as described above and kept for germination in the Petri plates lined with moist Whatman No. 1 filter paper (between paper). The number of germinated seeds was noted daily and the germination percentage was calculated as given below

$$\text{Germination percentage} = \frac{\text{No of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seed Vigour Index

Ten seeds in duplicate of control, desiccated and stored seeds were kept for germination as described above and daily count of sprouted seeds were taken. The seed vigour index (SVI) was calculated from the daily count data (Copeland and McDonald, 1995) using the formula given below.

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

Moisture content determination

Ten seeds in duplicate of control, desiccated and stored seeds were taken and fresh weights were determined using Shimadsu Ax 120 Electronic balance. Then the weighed seeds were kept in hot air oven at 100°C for one hour and then at 60°C. Drying and weighing were repeated until concordant

values were obtained. Moisture content percentage was calculated as explained by ISTA (1985).

$$MC\ \% = \frac{\text{Fresh weight of seeds -- Dry weight of seeds}}{\text{Fresh weight of seeds}} \times 100$$

BIOCHEMICAL STUDIES

Biochemical analysis of metabolites and amylase assay was carried out using control, desiccated and stored seeds. The seeds were sampled at different intervals as described earlier. Four seeds each in duplicate were decoated, separated into embryonic axis and cotyledons, chopped into small pieces and pooled. Samples for biochemical studies and dry weight determination were taken from this pooled tissue. Random sampling procedure was followed for each estimation. Separate samples were taken for dry weight determination, biochemical estimation of starch, sugars, total and soluble protein and amylase assay.

Dry weight determination of tissues

A known amount of fresh pooled tissue was taken and weighed tissue was kept in hot air oven at 100°C for one hour and then at 60°C until constant weight was obtained.

Analysis of Starch
Extraction

The method of Pucher *et al.,* (1948) described by Whelan (1955) was used to determine the starch in seed samples. Two hundred milligram of tissue in duplicate was weighed and homogenized in a glass mortar and pestle by adding 30% perchloric acid. The homogenate was centrifuged for five minutes and the supernatant was collected. The residue was re-extracted six times with 30% perchloric acid to ensure the complete extraction of starch.

The supernatants after each centrifugation were pooled and the volume of the combined extract was noted. A known volume of the combined extract was pipetted and an equal volume of the freshly prepared iodine potassium iodide reagent was added and thoroughly mixed. After 10 minutes, it was centrifuged for 10 minutes and the supernatant was decanted. The precipitate was washed with alcoholic sodium chloride solution to remove the excess iodine potassium iodide reagent. After centrifugation, the blue precipitate obtained was treated with alcoholic sodium hydroxide solution till blue colour was completely discharged. It was then centrifuged and washed again with alcoholic sodium chloride solution to remove liberated iodine. The precipitate was dissolved in a known quantity of 10% (v/v) sulphuric acid by heating in a boiling water bath, cooled and centrifuged for 10 minutes.

Estimation

Estimation of starch was done according to Montgomery (1957). Suitable aliquot was taken and its volume was made up to 1 ml using double distilled water. To this 0.1ml 80% (w/v) phenol was added and shaken well. Five milliliter of concentrated sulphuric 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 procured from Merck Chemical Company was used as standard.

Analysis of Sugars

Extraction

Five hundred milligram tissue was homogenised in 80% ethyl alcohol in a glass mortar and pestle and refluxed for two hours. The homogenate was centrifuged and the supernatant was collected. The residue was again extracted with 80% ethyl alcohol and after each centrifugation the supernatants were combined. The combined extract was evaporated to dryness

over a boiling water bath and eluted in 4 ml double distilled water and cleared by centrifugation.

HPLC of Sugars

Samples extracted as described above were used for HPLC studies. It was conducted at the Laboratory for Polymer Analysis, Sree Chithra Institute for Medical Science and Technology, Thiruvananthapuram. HPLC system consisting of Waters u Bobdapak $-NH_2$ column, Waters 600 pump, 7725 Rheodyne, 7725 injector and Waters 2414 Refractive Index detector with sensitivity - 4 were used for the analysis. The mobile phase used was Acetonitrile/Water-70/30. The flow rate was 1ml per minute. The injection volume used was 20 µl. for standards and samples.

Glucose, fructose, rhamnose, maltose, sucrose, galactose and raffinose procured from Merck Chemical Company were used as the standards. From the chromatogram of standards and samples, comparison was made and from the peaks and area of each sample, the amounts of individual sugars were calculated.

Amylase assay

Enzyme extraction

Eight hundred milligram tissue each of control, desiccated and stored seeds were used for the enzyme assay. Individual samples were homogenized in 8ml, chilled 0.2 M Phosphate buffer (pH-7.5), using a pre-chilled mortar and pestle kept in ice bath. A known aliquot of the homogenate was taken for the estimation of total protein. The homogenate was centrifuged in refrigerated centrifuge (Plastocraft model ROTA R4R V/FM) at 10,000 rpm for 10 minutes. The supernatant was used as enzyme source for amylase assay. A known aliquot of the supernatant was precipitated with 10% TCA for soluble protein estimation.

Enzyme Assay

Dinitrosalicylic acid method as explained by Bernfeld (1955) was followed to estimate amylase activity.

Preparation of Dinitrosalicylic acid

One gram of 3, 5-Dinitrosalicylic acid was dissolved in 20 ml of 2N NaOH and 50 ml of distilled water and stirred slowly to avoid capturing of carbon dioxide. Thirty grams of Rochelle salt (sodium potassium tartrate) was added, dissolved and made up to 100ml. The solution was kept in amber coloured bottle.

Assay : Following optimal conditions of the assay system were standardized.

pH optimum : The optimum pH for enzyme activity was determined by incubating enzyme assay system consisting of 0.5ml buffer of each pH, 0.2ml of enzyme and 0.3ml of substrate (soluble starch) for 30 minutes at 37°C in buffers of pH ranging from 4-8 at an interval of 0.4 pH. The enzyme action was ceased by the addition of 1ml of dinitrosalycilic acid reagent at the 30^{th} minute. The tubes containing the reaction mixture were kept in boiling water bath for 5 minutes and then cooled. It was made up to 10ml by adding double distilled water. The optical density of solution containing the reduction product was measured using Genesis 20 (Bausch and Lomb) Spectrophotometer at 540nm. Maltose procured from Merck Chemical Company was used as the standard. The buffer pH in which the enzyme showed highest activity was taken as pH optimum.

Temperature optimum: The temperature optimum of enzyme activity was determined by incubating the assay system for 30 minutes at temperature ranging from 20°C to 40°C at an interval of 5°C with substrate and buffer

having optimum pH (5.3 and 8.0). The temperature at which the enzyme showed highest activity was considered as temperature optimum.

Enzyme proportionality: The enzyme proportionality range for enzyme activity was determined by incubating the assay system for 30 minutes at optimum temperature (37°C) with optimum pH (5.3 and 8.0), 0.3ml of 2% (w/v) soluble starch and 10% (w/v) enzyme extract ranging from 0.05ml to 0.4ml at an interval of 0.05ml.

Substrate optimum: The substrate saturation for enzyme activity was determined by incubating the assay system for 30 minutes at optimum temperature (37°C) with optimum pH (5.3 and 8.0), 0.1ml of 10% enzyme extract and different quantities of 2% (w/v) soluble starch ranging from 0.1ml to 0.4ml.

As per standardised optimal conditions, the assay system contained one hundred microlitre (0.1ml) of homogenate, 0.6ml of 0.1M sodium acetate buffer (pH-5.3) or 0.1M sodium phosphate buffer (pH-8.0) of optimum pH and 0.3ml of 2% substrate (soluble starch procured from Merck Chemical Company) was incubated for 30 minutes at optimum temperature (37°C) and the product formed was estimated according to Bernfeld (1955) using dinitrosalicylic acid. Unit activity and specific activity of the enzyme were calculated.

Unit activity: Unit activity, defined as mg maltose formed during 30 minutes at 37°C per g tissue (dry weight) was calculated.

Specific activity: Specific activity is units of enzyme activity per mg of protein. The amount of protein in enzyme solution was determined by Lowry's (1951) method. The specific activity of amylase was calculated by dividing unit activity by mg protein present in the tissue.

Confirmatory test for α and β amylase:

α-amylase: Confirmatory test for α-amylase was carried out using the method of Kneen *et al.*, (1943). The enzyme preparation was held at 70°C for 30 minutes in the presence of 0.2% (w/v) calcium acetate. The suspension was cooled, centrifuged in cold at 10,000 rpm for 10 minutes and the supernatant was used for the assay. α-amylase withstand heat treatment in the presence of calcium acetate and β-amylase is destroyed.

β-amylase : To the enzyme preparation, cold 0.1N HCl was added slowly with stirring until pH lowers to 3.3. The suspension was held at 4-5°C for 18 hours and centrifuged in cold at 10,000 rpm for 10 minutes. The pH of the suspension was raised to 4.6 by cautious addition of 0.1N NaOH. The resulting suspension was again centrifuged in cold at 10,000 rpm for 10 minutes. The supernatant was used for the assay. β-amylase withstand the treatment.

Analysis of Total and Soluble Proteins

Extraction

The method of Lowry *et al.*, (1951) was followed to estimate the total and soluble protein.

Aliquot of homogenate and supernatant taken during the enzyme assay was used for the estimation of total and soluble protein respectively. A known aliquot was pipetted and mixed with equal volume of 10% (w/v) trichloracetic acid and kept for flocculation for one hour in an ice bath. The protein precipitate was collected by centrifugation for 10 minutes and the supernatant was decanted off. The residue was washed with 2% (w/v) trichloracetic acid, followed by washing twice with 15% perchloric acid to remove the starch. The residue was washed with 80% acetone followed by anhydrous acetone and centrifuged. The precipitate obtained after centrifugation was digested in

known volume of 0.1N NaOH and kept in boiling water bath for 10 minutes and centrifuged.

Estimation

Known aliquots were taken from the supernatant and made up to 1ml. To this, 5ml of alkaline copper reagent was added and shaken well. After 10 minutes, 0.5 ml of 1N Folin-Ciocalteau reagent was added, immediately shaken well and kept for 30 minutes. The optical density was read at 700nm using Genesis 20 Spectrophotometer. Bovine Serum Albumin (BSA) fraction V powder procured from Merck Chemical Company was used as the standard.

Electrophoretic Study of Protein Profile (SDS PAGE) :

Protein profile of the seed during desiccation was studied by SDS PAGE. One gram of the seed sample was homogenized in pre chilled glass mortar and pestle in Tris-HCl buffer of pH-7.2. The homogenate was centrifuged at 10,000 rpm for 10 minutes using Plastocraft - model ROTA R4R V/FM refrigerated centrifuge and the supernatant was collected. The protein sample was dissociated into its polypeptide subunits by adding equal volume of gel loading buffer containing 10% SDS, β-Mercaptoethanol, Glycerol, 0.1% Bromophenol blue and Tris HCl (pH-6.8). The mixture was heated for 2 minutes (Laemmli, 1970) in boiling water bath and immediately kept in freezer. The sub units were separated electrophoretically using GENEI electrophoresis unit in SDS-PAGE slab gel having 10% separating gel and 4% stacking gel. The gel after electrophoresis was stained with Coomassie brilliant blue and the bands were compared with known Bovine Serum Albumin (BSA) fraction V powder procured from Merck Chemical Company.

Composition of the separating gel (10%)

30% Acrylamide / 0.8% Bisacrylamide	3.33ml
4X Resolving Gel buffer	2.5ml
10% SDS	100 µl.
Double distilled water	4.00ml
% (w/v) Ammonium Per Sulphate	50 µl.
TEMED	5 µl.

Composition of the stacking gel (4%)

30% Acrylamide/ 0.8% Bisacrylamide	670 µl.
4X Stacking Gel buffer	1.25ml
10% SDS	25 µl.
Double distilled water	3.00ml
10% (w/v) Ammonium Per Sulphate	50 µl.
TEMED	2.5 µl.

The sample was electrophoresed using Tris-Glycine running buffer of pH 8.3.

HISTOCHEMICAL STUDIES

Axes and small cotyledons of control and desiccated seeds were fixed for histochemical studies.

Tissue preparation

Samples were fixed in FAA, dehydrated through alcohol-TBA series, infiltrated and embedded in paraffin wax (Johansen, 1940). Using a rotary microtome (LEICA, model 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 starch and proteins.

Localisation of Starch

Localisation of starch was done according to Berlyn and Miksche (1976) using periodic acid-Schiff's reagent as well as Safranin-Iodine potassium iodide solution.

Periodic acid-Schiff's reagent (PAS) staining

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

Safranin - Iodine potassium iodide staining

The hydrated sections were dipped in dilute solution of safranin and washed immediately to remove excess stain. Iodine potassium iodide solution was added dropwise using filler followed by thorough washing in running water. Slides were immediately dried over the slide warming table and the sections were cleared in xylene and mounted in DPX.

Localisation of Total Protein

For the localisation of total protein, sections were stained with mercuric bromophenol blue according to Mazia *et al.*, (1953) as explained by Berlyn and Miksche (1976). The hydrated sections were placed in bromophenol blue stain for 15 minutes and then in 0.5% acetic acid for 20 minutes. The sections were then placed in water. The sections were dehydrated through alcohol-TBA series, cleared in xylene and were mounted in DPX.

Scanning Electron Microscopic study of starch grains

Sample preparation

Control seeds were soaked in water, crushed and mixed well mechanically and filtered gently. Starch grains with water was kept undisturbed to settle down. The supernatant was drained off and the residue was washed three times with 0.2M Phosphate buffer (pH-7.2). Sufficient time was given to settle down and the residue was washed with distilled water repeatedly. The supernatant was completely drained and the residue dried in hot air oven at 40°C. Starch powder was collected after complete drying.

SEM study

Starch powder smeared after wetting with distilled water and covered with two sided cellophane tape adhesive. Aluminium stub with starch grain powder dried in hot air oven at 40°C slowly. After complete drying, gold ion sputtering was done and observed in the S–2400 Scanning Electron Microscope (Hitachi) available at Sree Chithra Institute of Medical Science and Technology, Thiruvananthapuram. Starch grain surface was scanned and exposed at 2000x, 3000x, 4000x and 5000x magnification.

GERMINATION AND RESERVE MOBILIZATION STUDIES

Control (fresh) seeds only were used for the germination and reserve mobilization studies. Sixty fresh seeds in duplicate were kept for germination in Petri plates (between paper) in darkness. Samples were collected at the interval of 2, 5, 10, 20, 30, 40 and 50 days after germination / seedling growth. Four seeds / seedlings each collected on sampling days were decoated, separated the cotyledons and axis. For dry weight determination of seedling parts, the samples were kept in hot air oven at 100°C for one hour and then at 60°C till weight became constant.

During initial days, axis and cotyledons were sampled separately and used for dry weight estimation and biochemical studies. After the elongation of radicle i.e. 20^{th} day onwards, the pooled cotyledon tissue alone was used for these studies. The biochemical estimation of starch, sugars, total protein, soluble protein and amylase assay were conducted as described earlier.

Histochemical localization of starch and proteins of cotyledons were done to localize these reserves during germination. Staining was done as per the methods described under desiccation studies. Observations of stained sections were done and photomicrographs were taken using Nikon microscope (ECLIPSE E 400) and Nikon Digital Camera (DXM 1200F) attached with digital image analyser.

Statistical analysis

All experiments starting from sampling were repeated minimum of 6 times using seeds of fruits collected 4-5 times each during two consecutive years and mean value was taken and the standard deviation / standard error was calculated. Test of significance was done following Fischer's 't' test.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON JACKFRUIT SEEDS (*Artocarpus heterophyllus Lam.*) DURING STORAGE AND GERMINATION

Thesis
submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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Results

1. DESICCATION

1.1. Biochemical Studies

1.1.1. Moisture Content of Seeds

Fresh (control) seeds contained 50.19% moisture content. During desiccation there was a gradual reduction in moisture content of seeds (Table 1, Fig.1). A gradual but negligible reduction was observed upto 10^{th} day. On 10^{th} day of desiccation, there was a 20% reduction in initial moisture content. A sharp decline in moisture content was observed between 10^{th} and 12^{th} day of desiccation. But on the 13^{th} day there was 40% reduction of the initial moisture content. From 15^{th} day onwards, the moisture content was reduced to half of the initial moisture content.

1.1.2. Viability

The control (0 sample) seeds showed 100% viability and upto 10^{th} day of desiccation, these seeds maintained cent percent viability (Table 1, Fig.1). But on 12^{th} day the viability was reduced to 80% and only 46% of seeds remained viable on 13^{th} day. Afterwards, these seeds were considered

Table: 1. Relationship between moisture content and viability of Jackfruit seeds during desiccation.

Days of Desiccation	MC %	Germination %
0	50.19±1.59	100
4	47.99±1.84	100
8	43.15±1.46	100
10	41.15±1.73	100
12	33.58±2.10	78.00±3.46
13	31.81±2.47	46.00±2.12
14	30.21±0.89	41.60±3.24
15	27.26±1.11	17.56±2.10
16	25.70±1.15	8.00±1.80

Table: 2. Seed Vigour Index of Jackfruit seeds during desiccation and storage under different conditions.

Days of Storage	Seed Vigour Index		
	Storage conditions		
	Room-Open (Desiccation)	Room- Polythene	Refrigerator
0	2.59±0.21	2.59±0.21	2.59±0.21
8	1.02±0.16	ND	ND
12	0.48±0.19	ND	ND
14	0.24±0.13	ND	ND
16	0.06±0.18	ND	ND
20	ND	2.60±0.17	2.57±0.23
30	ND	2.56±0.15	2.58±0.18
40	ND	2.48±0.20	2.51±0.13
50	ND	2.39±0.18	2.42±0.21
60	ND	2.33±0.12	2.40±0.16
70	ND	1.93±0.10	1.20±0.23
80	ND	1.94±0.09	1.13±0.21
90	ND	1.90±0.16	0.92±0.12
100	ND	1.55±0.14	0.84±0.11
110	ND	0.91±0.12	0.60±0.14
120	ND	0.62±0.14	0.41±0.17

ND - Not done because desiccated seeds lost viability on the 16th day. Seeds stored in Room-polythene and Refrigerator retained viability upto 120 days and hence sampled only on 10 days intervals.

57.A

Table: 4. Distribution of starch and protein in Jackfruit seeds during desiccation mg g^{-1} dry tissue.

Days of desiccation	Starch	Protein	
		Total	Soluble
0	730.30±20.48	104.92±3.78	101.03±2.18
4	653.71±13.24	121.30±4.73	95.16±5.14
8	599.06±11.96	88.08±2.85	90.26±2.34
12	564.12±10.36	126.13±4.26	93.65±3.11
14	469.33±8.73	71.22±1.82	76.57±1.32
16	452.34±12.54	63.79±3.68	84.49±2.85

Table: 5. Distribution of sugars in Jackfruit seeds during desiccation mg g^{-1} dry tissue.

Sugars	Days of Desiccation				
	0	8	12	14	16
Rhamnose	6.73	5.45	8.97	24.07	23.56
Glucose	7.02	3.84	21.15	56.16	41.42
Fructose	7.66	4.35	23.72	62.46	46.17
Sucrose	10.22	2.30	5.77	5.24	29.88
Maltose	7.67	2.05	5.45	6.02	34.22
Raffinose	3.83	0.67	13.46	10.88	8.15
Unknown-I	6.39	-	-	-	-
Unknown-II	2.04	2.92	5.59	2.41	2.82
Unknown-III	2.06	-	-	1.68	-
Total	**53.61**	**21.58**	**84.11**	**168.92**	**186.22**

Table: 6. Amylase activity in Jackfruit seeds during desiccation mg g^{-1} dry tissue.

Days of desiccation	Unit Activity		Specific Activity	
	pH – 5.3	pH – 8.0	pH – 5.3	pH – 8.0
0	53.28±4.73	45.83±4.09	0.53 ± 0.01	0.45 ±0.04
4	67.63±3.67	56.90±4.05	0.71 ±0.04	0.60 ±0.04
8	91.07±2.78	64.09±3.48	1.01 ± 0.03	0.71 ±0.03
12	103.81±3.79	84.32±5.13	1.11 ±0.04	0.91 ±0.02
14	77.79±4.44	76.72±2.39	1.02 ±0.06	0.99 ±0.03
16	76.59±3.90	72.52±2.15	0.91 ±0.04	0.86 ±0.03

Figure: 1. Effect of desiccation on moisture content and viability percentage in Jackfruit seeds.

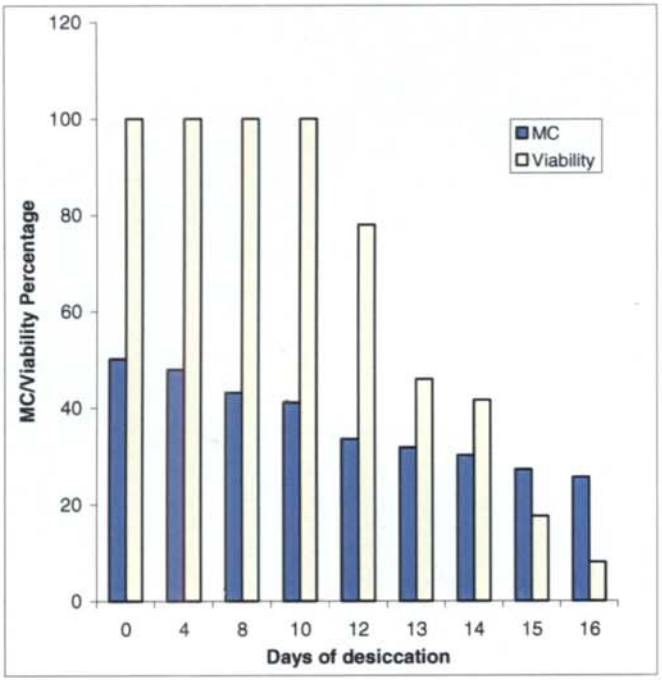


Figure: 2. Seed vigour index of Jackfruit seeds during desiccation and storage under different conditions.

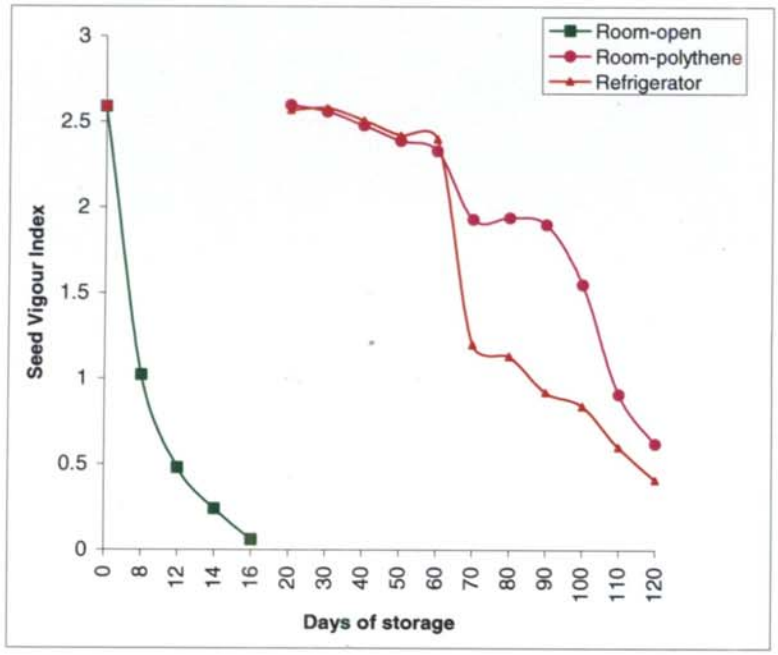


Table: 3. Tissue dry weight percentage of Jackfruit seeds during desiccation and storage under different conditions.

Days of storage	Tissue dry weight Percentage		
	Storage conditions		
	Room-Open (Desiccation)	Room- Polythene	Refrigerator
0	50.07±1.11	50.07+±1.11	50.07±1.11
4	54.58±0.89	ND	ND
8	58.59±2.45	ND	ND
10	ND	53.69+±0.90	54.44±0.93
12	62.40±3.19	ND	ND
14	69.84±2.30	ND	ND
16	73.60±0.98	ND	ND
20	ND	53.26±1.06	54.22±1.80
30	ND	51.30±1.52	53.31±1.12
40	ND	51.34±0.84	55.13±0.92
50	ND	52.50±1.38	56.43±0.99
60	ND	53.80±3.38	55.00±0.61
70	ND	56.60±2.35	56.67±0.81
80	ND	57.83±0.83	57.33±1.38
90	ND	59.50± 2.22	57.75±2.07
100	ND	58.50±2.19	62.60±2.65
110	ND	59.00±0.28	59.66±3.13
120	ND	58.33±1.76	60.50±0.96

ND - Not done because desiccated seeds lost viability on the 16th day. Seeds stored in Room-polythene and Refrigerator retained viability upto 120 days and hence sampled only on 10 days intervals.

nonviable, seed technologically, because the germination percentage was only 46. Jack seeds lost the viability completely within sixteen days of desiccation.

There was a correlation with the moisture content percentage and viability. With reduction in moisture content, there was a concomitant decline in viability. After critical moisture content (33%), the seeds became nonviable. From the Table 1, it was clear that in Jackfruit seeds, on the 12^{th} day, about 80% of seeds were viable when the moisture content was 33.58% (Fig.1). On the 13^{th} day of desiccation, the moisture content and the viability were sharply declined (non viable seeds). In room open condition the Jackfruit seeds remained viable for only 12 days.

1.1.3. Seed Vigour Index

The seed vigour index of control seeds was 2.59. During desiccation, the seed vigour was found to decrease. When the seeds were desiccated for 8 days, the seed vigour was decreased to less than half as that of control (Table 2, Fig. 2). In the seeds desiccated for 12 days, the seed vigour was decreased to about half compared to that of 8^{th} day sample. This was again reduced to about half in the seeds desiccated for 14 days. The seed vigour index of samples desiccated for 16 days was only 0.06.

1.1.4. Tissue dry weight percentage

The tissue dry weight percentage of control seeds was 50.07. There was a gradual increase in dry weight of tissue during the entire period of desiccation (Table 3). About 5% increase in dry weight was shown by seeds desiccated for 4 days. This trend was continued upto 12^{th} day. A significant ($P<0.05$) increase in tissue dry weight was obtained when these seeds were desiccated for 14 days. After 14 days, only 5% increase was observed.

1.1.5. Starch

Control seeds showed 730mg g^{-1} dry tissue starch content (Table 4, Fig.4a). Starch content of seeds decreased throughout the desiccation period. During the initial 4 days of desiccation, the starch content was decreased by about 10% of initial value. About 25% reduction in starch content was observed in seeds desiccated for 12 days. A significant ($P<0.01$) reduction was observed on 14^{th} day and there after the reduction in starch content was gradual.

1.1.6. Sugars

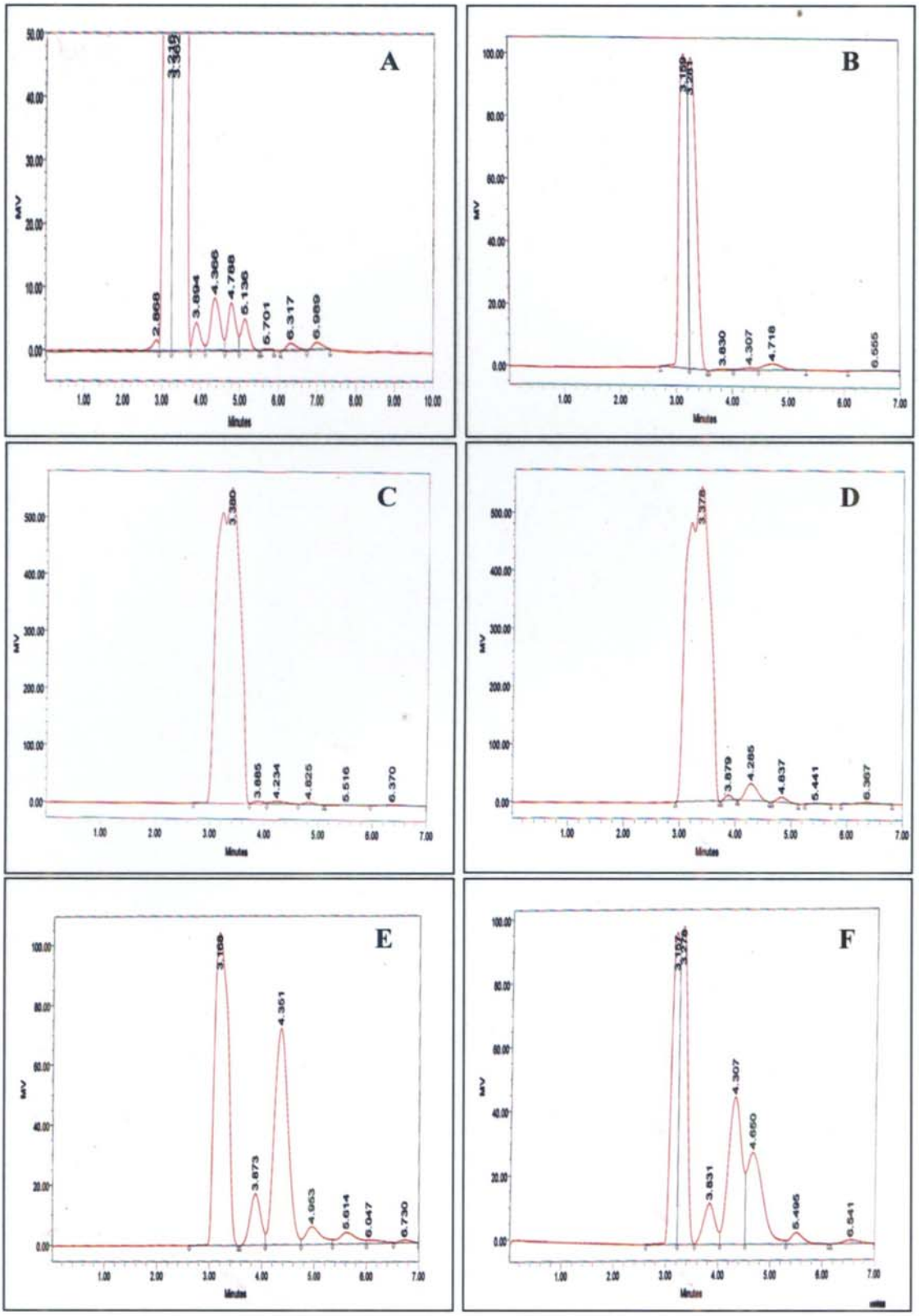
HPLC study on sugars (Fig. 3) showed that in the cotyledon of control seeds, monosaccharides-rhamnose, glucose and fructose, disaccharides-sucrose and maltose, trisaccharide-raffinose and three unidentified sugars (calculated as glucose equivalents) were present (Table 5, Fig. 4b). Sucrose was the dominant sugar in control seeds. Glucose, fructose, rhamnose and maltose occurred in almost equal quantities. The total sugar content was about 5.4 percentage.

The seeds desiccated for eight days, showed a rapid decrease in all the sugars (Fig. 4b), two unknown sugars (designated as I and III) disappeared (Table 5). Rhamnose and fructose were the dominant sugars in this sample. Total sugars were reduced to less than half of the control seeds.

In seeds desiccated for twelve days, there was a rapid increase in total sugar content. Glucose and fructose were increased to more than seven times compared to that of seeds desiccated for eight days (Table 5, Fig. 4b). But sucrose and maltose were doubled compared to that of 8^{th} day sample. Raffinose registered an increase showing three times quantity compared to the control seeds. Maximum quantity of raffinose was present in this sample.

57-B

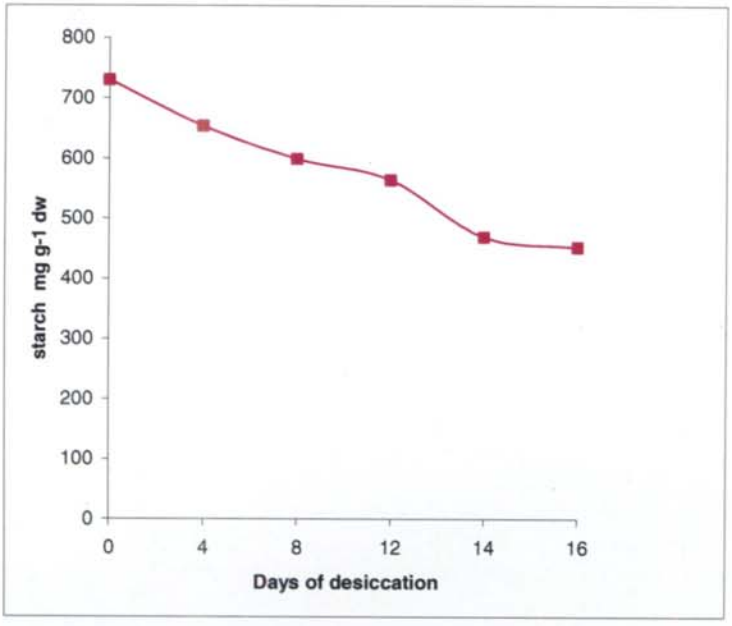
Figure: 3. HPLC sugar profile of Jackfruit seeds during desiccation.



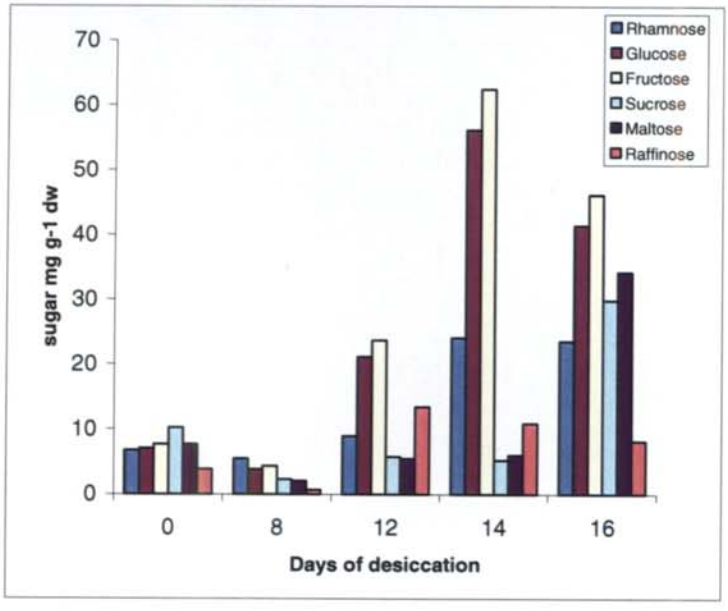
A- Control cotyledon
B - Control axis
C - 8th day
D- 12 th day
E - 14 th day
F - 16 th day

Figure: 4. Distribution of starch and sugar contents during desiccation of Jackfruit seeds.

a) Starch



b) Sugars



Maximum content of rhamnose, glucose and fructose were present in seeds desiccated for 14 days which was more than two fold compared to the 12^{th} day sample. Sucrose and maltose were almost similar to that of the 12^{th} day sample but were less than that of control seeds. The raffinose was slightly reduced compared to the earlier sample. Here in addition to six known sugars, two unknown sugars (II and III) were present. The total sugar content was doubled in comparison with that of the seeds desiccated for 12 days.

In the seeds desiccated for 16 days, glucose and fructose were reduced in comparison to that of 14^{th} day sample whereas rhamnose maintained the same quantity as that of the 12^{th} day sample. Highest quantities of sucrose and maltose were present in these seeds. Raffinose was slightly reduced in this sample. The seeds desiccated for 16 days registered the highest total sugar content compared to all other samples.

1.1.7. Amylase Assay

pH optimum for Amylase activity

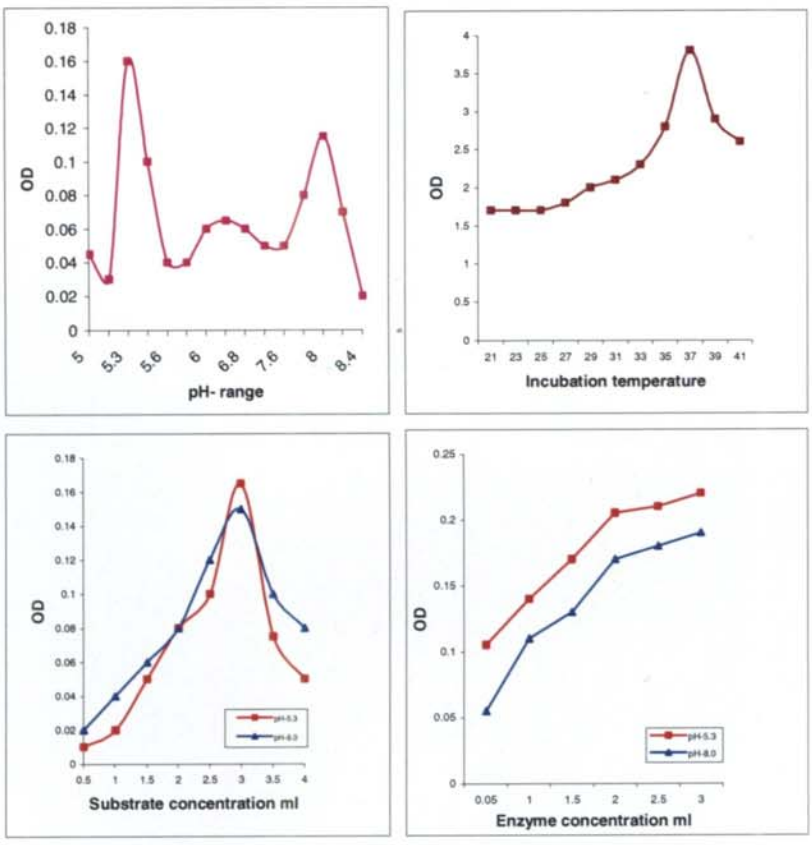
The enzyme extract of control and the desiccated seeds assayed at different pH ranging from 4 to 8 at an interval of pH 0.4 (Fig. 5a). Two peaks were obtained and maximum activity was observed at pH-5.3 (sodium acetate buffer) and 8.0 (sodium phosphate buffer).

Temperature optimum for Amylase activity

When assay was conducted at the optimum pH at different temperatures ranging from 20°C to 40°C, maximum activity was shown at 37°C (Fig. 5b). Hence the optimum temperature for the amylase activity was taken as 37°C.

Figure: 5. a) pH optimum for amylase activity

b) Temperature optimum for amylase activity
c) Enzyme volume for optimum amylase activity
d) Substrate saturation for amylase activity



Enzyme proportionality of Amylase activity

The assay was conducted at both optimum pH (5.3 and 8.0) and optimum temperature (37°C) using different volumes of enzyme extract (10% w/v) ranging from 0.05 to 0.4ml. The assay system showed optimum activity at 0.1ml enzyme extract. Hence the optimum enzyme concentration for the amylase assay was confirmed as 0.1ml of 10% (w/v) enzyme extract (Fig. 5c).

Substrate optimum for Amylase activity

When the assay was carried out at both optimum pHs (5.3 and 8.0), optimum temperature (37°C) and optimum enzyme content (0.1ml) and with different quantities of substrate (2% soluble starch) ranging from 0.1 to 0.5 ml, the assay system showed optimum activity with 0.3ml substrate (Fig. 5d).

Confirmatory Test

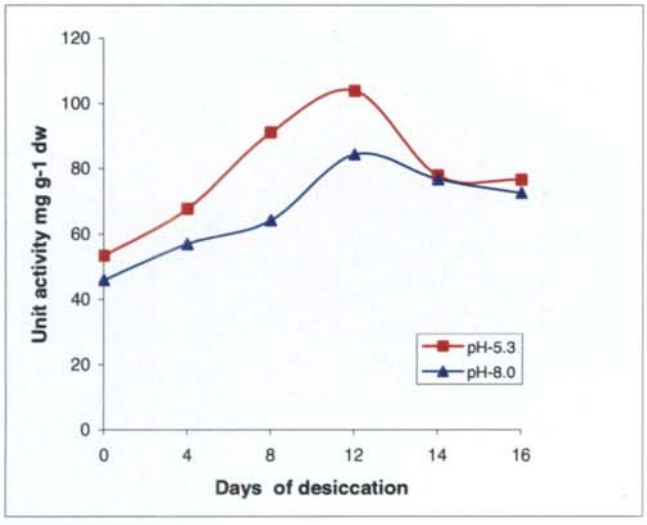
The presence of α-and β-amylases were confirmed in all samples (control, desiccated, stored and germinated seeds).

1.1.7.1. Unit Activity of Amylase

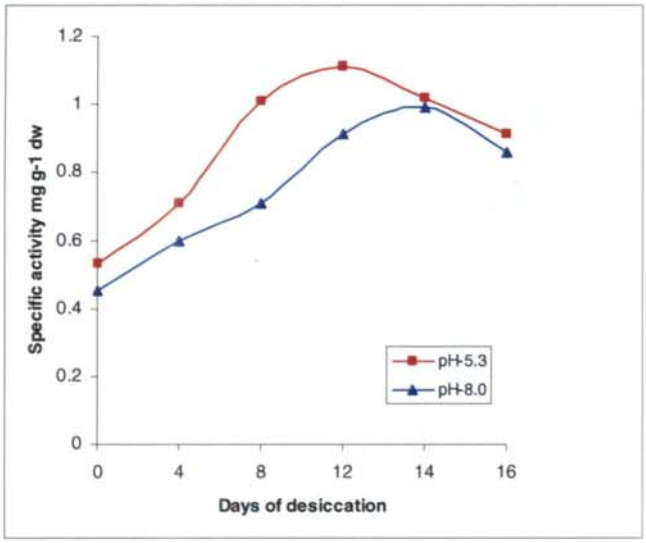
The amylase activity showed two pH peaks i.e., at pH 5.3 (Acetate buffer) and pH 8.0 (Phosphate buffer) representing β-and α-amylases respectively. The control seeds showed 53.28 and 45.83 unit activity at pH-5.3 and pH-8.0 respectively (Table-6, Fig-6a). The unit activity of both α- and β-amylases showed a gradual increase on 4^{th} day. A significant ($P<0.01$) increase was observed in the unit activity of amylase on 8^{th} day. Maximum unit activity was shown on the 12^{th} day of desiccation at both the pHs. From 12^{th} day onwards amylase was very active but was slightly declined on 14^{th} day and maintained a still higher value compared to the control seeds.

Figure: 6. Pattern of amylase activity during desiccation in Jackfruit seeds

a) Unit activity



b) Specific activity



1.1.7.2. Specific Activity of Amylase

The specific activity of control seeds at pH-5.3 and 8.0 were 0.53 and 0.45 respectively. The specific activity showed the same trend as that of unit activity (Table 6, Fig. 6b) especially at pH-5.3 (β-amylase). During desiccation, the specific activity was increased, and the maximum activity was shown by seeds desiccated for 12 days. After 12^{th} day of desiccation, the specific activity was decreased significantly ($P<0.02$). Nonviable seeds were also showed specific activity higher than the control seeds. The specific activity of α-amylase showed a slight variation than the activity of β-amylases. The maximum activity was shown by seeds desiccated for 14 days. Specific activity of α-amylase was increased insignificantly upto 14^{th} day of desiccation and then declined. Here also the nonviable seeds showed a higher specific activity than that of the control seeds

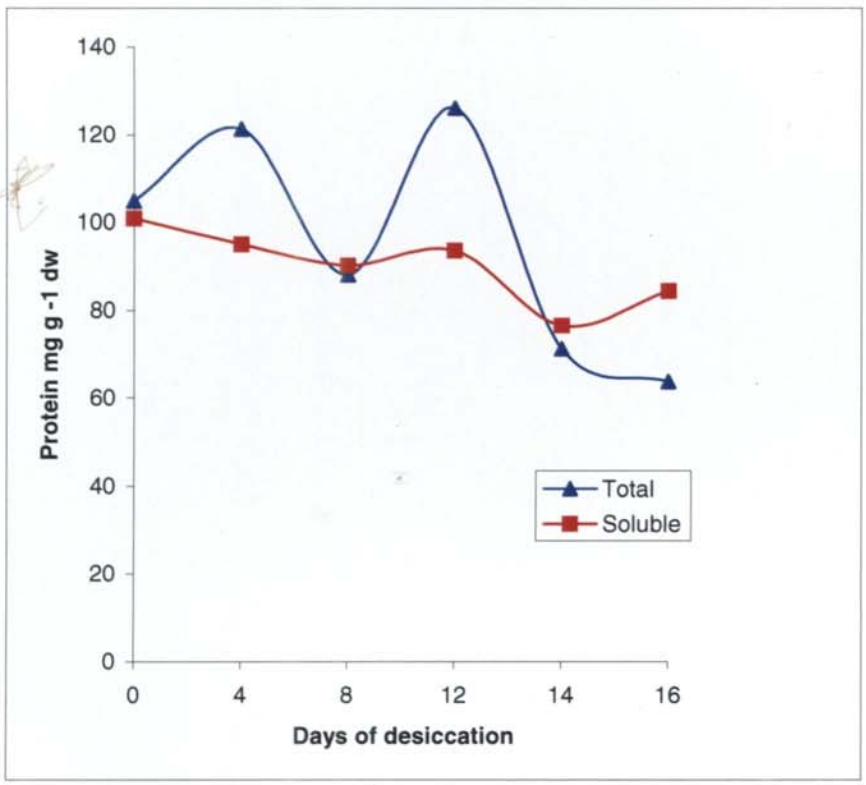
1.1.8. Total Protein

Total protein content in control Jackfruit seeds was 104.92 mg g^{-1}dry tissue (Table 4, Fig. 7). On the 4^{th} day of desiccation, the total protein content was increased insignificantly compared to the control seeds. But during the next 4 days, there was a sharp reduction in protein content i.e. about 30% less than that of seeds desiccated for 4 days. The maximum total protein content was shown by the seeds desiccated for 12 days. There was 40% reduction in protein content in the seeds desiccated for 14 days, later the reduction in total protein content was continued.

1.1.8.1. Soluble Protein

Control seeds showed the maximum soluble protein content (Table 4, Fig. 7) and during desiccation the soluble protein content declined gradually. The seeds desiccated for 4 to 12 days showed almost same soluble protein

Figure: 7. Distribution of protein content during desiccation of Jackfruit seeds.



content. After 12th day, soluble protein content was reduced significantly ($P<0.01$) showing a further increase on 16th day.

The control seeds showed almost similar content of total and soluble proteins. Although the total protein was increased upto 12th day, it showed a decrease during further days of desiccation, the soluble protein decreased gradually during desiccation but this reduction was not significant upto 12th day.

1.1.8.2. Protein Profile

The protein profile of control seeds revealed that there were 16 bands, five bands above standard BSA (66 kDa) and eleven bands below. The low molecular weight bands were prominent (Fig. 8). The seeds desiccated for 8 days showed almost same bands as that of the control but with two prominent low molecular weight bands. Some of the bands were disappeared in the 12th day desiccated seeds, four feeble bands were present above 66 kDa and nine bands below, one of which was prominent. More bands were found disappeared in the 14 days desiccated seed sample. In this sample, only seven bands were present below standard and three bands above. The disappearance of bands continued in 16th day sample in which only six feeble bands were present below and three very feeble bands above the standard. The high molecular weight protein bands were found to disappear more during desiccation.

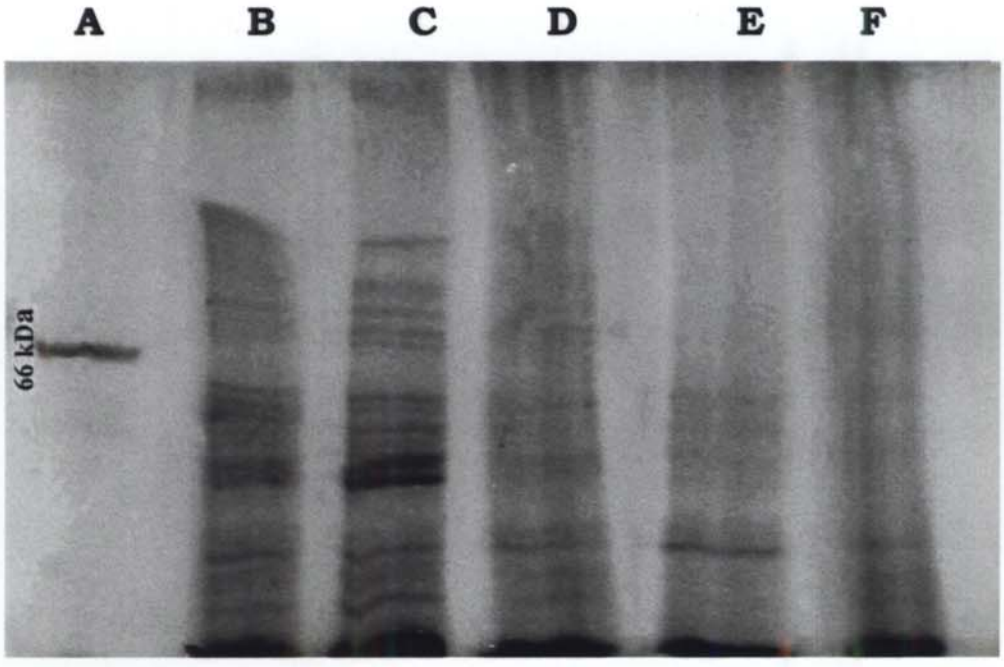
1.2. Histochemical Studies

1.2.1. Cotyledon

1.2.1.1. Localisation of Starch - PAS

Periodic acid-Schiff's (PAS) reagent specifically stains insoluble polysaccharides. The sections of small cotyledon stained with the PAS showed the magenta coloured moderately stained cell walls and densely

Figure: 8. SDS - PAGE protein profile of Jackfruit seeds during desiccation.



A - BSA
B - Control
C - 8days
D - 12 days
E - 14 days
F - 16 days

stained starch grains (Fig. 9, A, B, C). The cell lumen was almost filled with starch grains the number of which varied from cell to cell of different regions.

1.2.1.2. I_2kI-Safranin staining

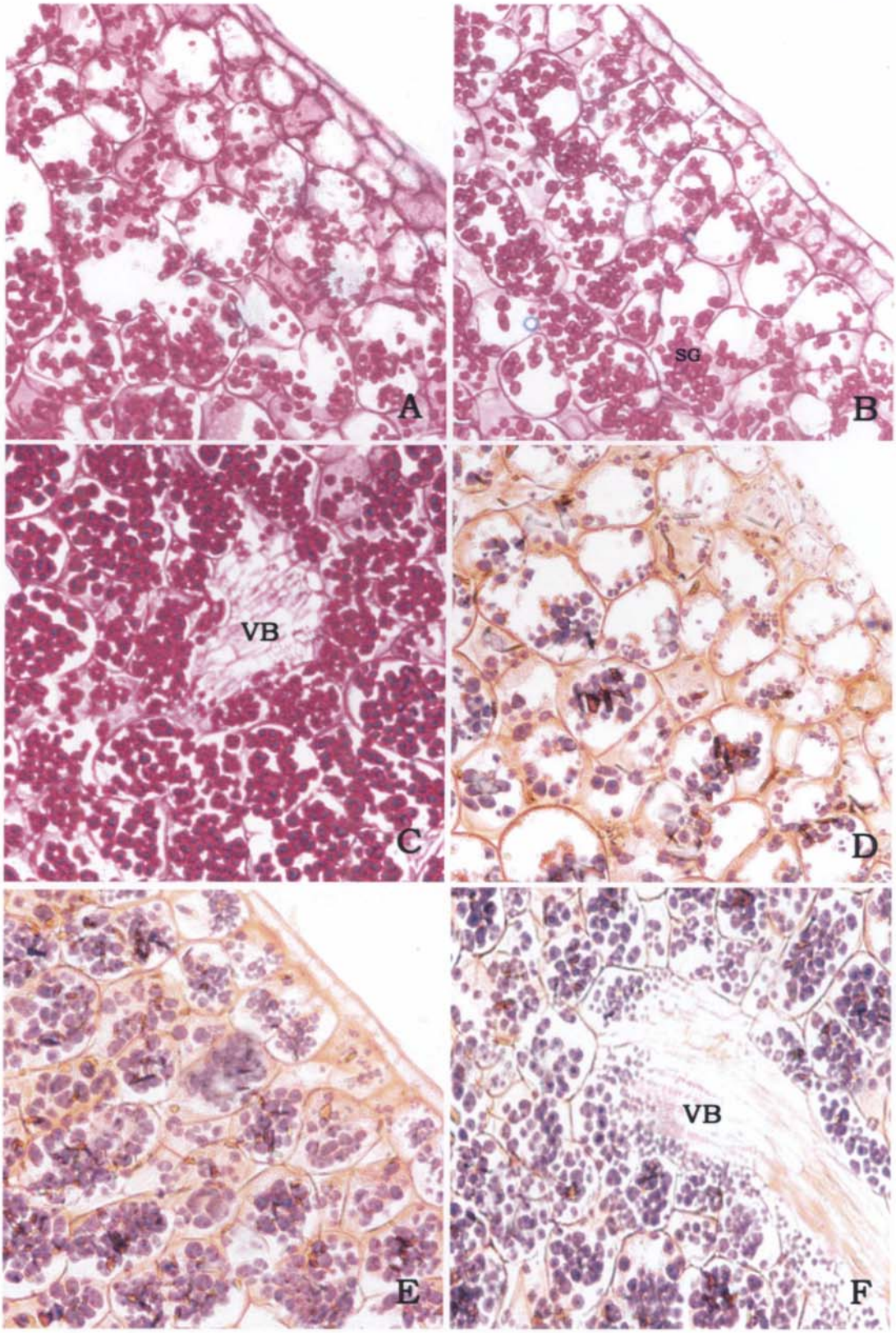
Iodine potassium iodide stains only the starch grains in deep violetish blue colour. The cell wall, cytoplasm and nucleus of the cells were stained orange red in colour with safranin (Fig. 9, D, E, F). In this staining the starch grains appear beautifully and the hilum was visible in the centre of simple grains and individual grains of compound grains.

In cotyledon of control seeds, epidermal cells on adaxial side were small, elongated and without any starch grains (Fig. 9, A, D). Cells of 1-2 hypodermal layers were also devoid of starch grains. Very small starch grains were present in the cells of hypodermis from third layer onwards (Fig. 9, A, D). Cells of three to four layers inner to hypodermis contained only 5-8 grains per cell. The starch grains showed a gradation of increase in size in the cells towards centre. Nucleus is visible in all cells of both adaxial and abaxial sides. Starch grains were found in close association with the nucleus.

On abaxial side, the epidermal cells were elongated and were devoid of starch grains (Fig. 9, B, E). Hypodermal cells contained about 10 to 14 starch grains with various degrees of aggregation. Cells of immediate hypodermal layer contained very smaller grains. Simple and compound grains of various sizes, shapes and aggregations (2-4) were present.

Cross section of cotyledon showed 21 to 34 vascular bundles. In the centre, cells were comparatively larger, compactly arranged and contained nucleus and about 25 to 40 starch grains per cell (Fig. 9, C, F). Here also simple and compound grains (aggregation of 2-10 grains) of different sizes and shapes were present. Parenchyma cells near vascular bundles contained smaller grains which also showed various degrees of aggregation.

Figure: 9. Distribution of starch grains in the cotyledon of control Jackfruit seeds.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

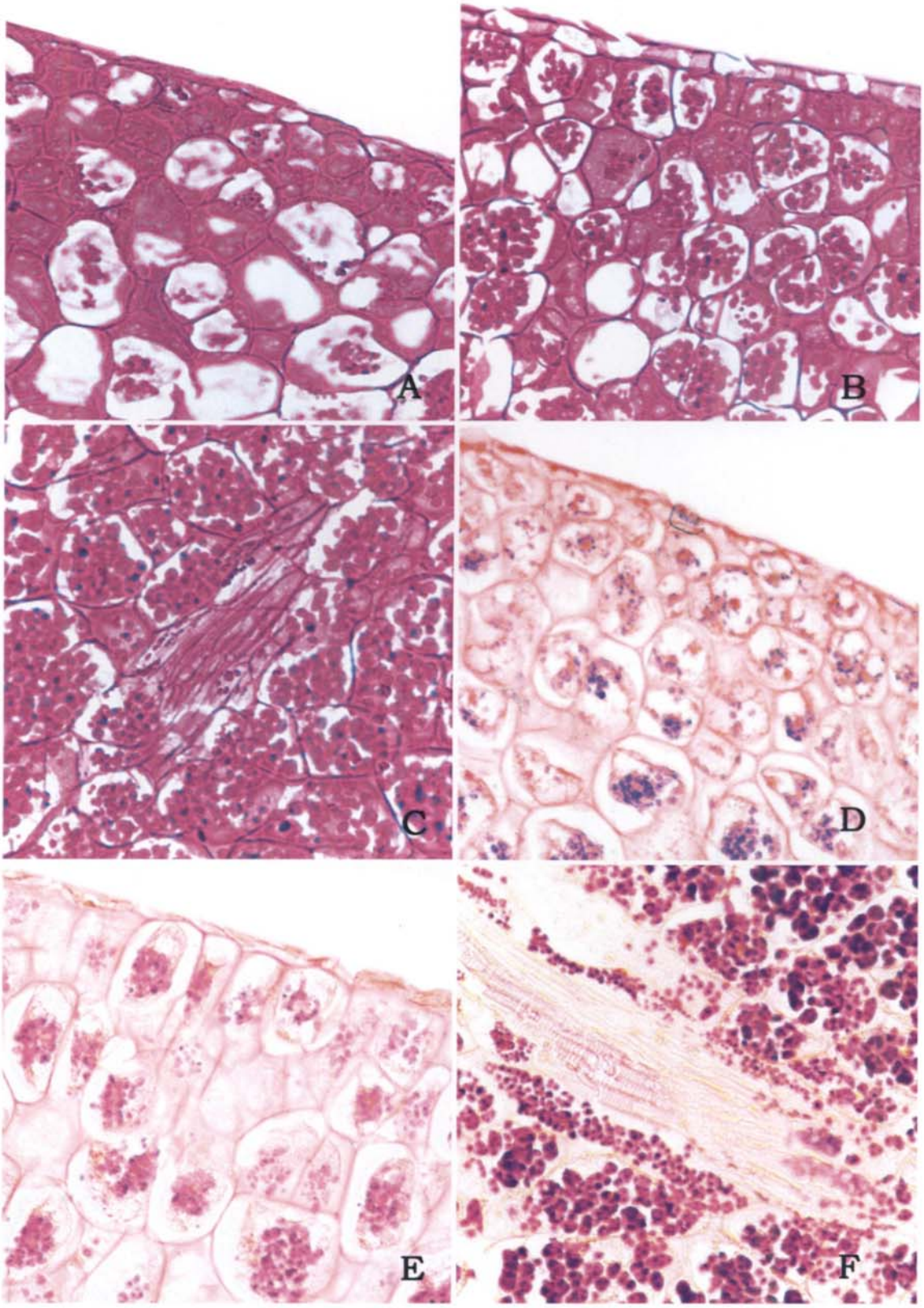
On the eighth day of desiccation, there were no significant changes in the starch grains. The cells near the adaxial side showed a slight difference from the control seeds. The small grains of these cells showed some sort of aggregation among themselves (Fig. 10, A, D). Cells near the abaxial side also did not show much difference from the control seeds. But slightly larger grains were observed and they showed aggregation between all grains within the cells (Fig. 10, A, E).The cells in the centre of cotyledon were almost similar to that of the control seeds (Fig. 10, C, F).

On the 12^{th} day of desiccation, the cells in adaxial side showed slight difference. Smaller grains in the cells inner to the epidermis, showed aggregation into a single mass around the nucleus (Fig. 11, A, D). There were no noticeable changes in cells on the abaxial side compared to the adaxial side. The starch grains and nucleus were present in the cells. The starch grains were closely aggregated around the nucleus (Fig. 11, B, E). The parenchyma cells in the centre also maintained the same characters of the control seeds.

The cotyledon of seeds desiccated for 14 days showed that the starch grains and nucleus of the cells near the adaxial side disappeared (Fig. 12, A, D). About 5-6 layers of cells inner to the epidermis were devoid of starch grains. The size of the grains in the cells towards the centre was gradually reduced.

The starch grains in the cells near the abaxial side showed an increase in size and also slight separation from each other than the previous sample (Fig. 12, B, E). The cells were intact with nucleus and there was no change in shape of the cells. In the centre region, there was a slight beginning of coalescence of grains but they maintained their identity (Fig. 12, C, F). There was a reduction in number of grains per cell. Here the grains in cells adjacent to the vascular bundles were smaller.

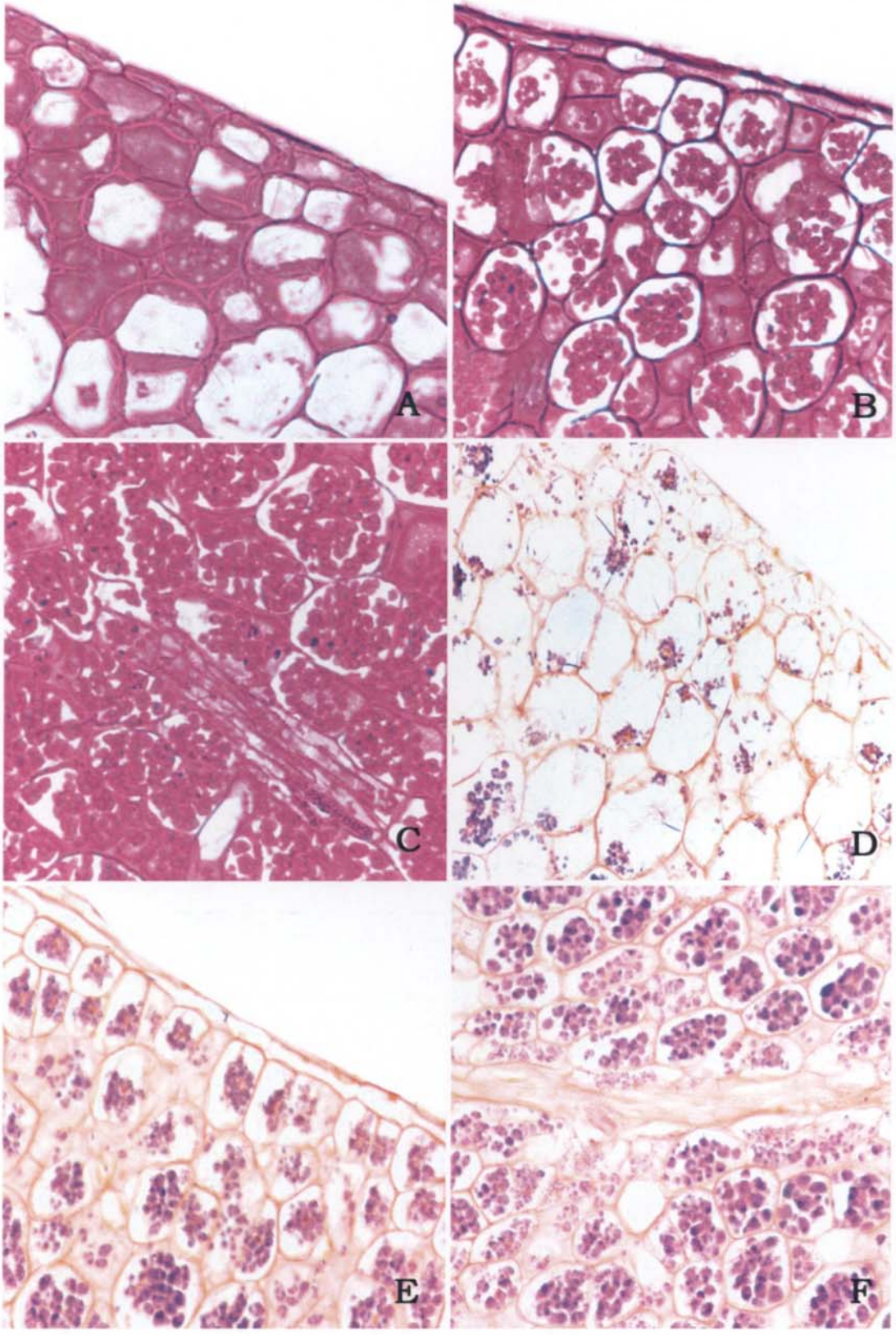
Figure: 10. Distribution of starch grains in the cotyledon of Jackfruit seeds desiccated for 8 days.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

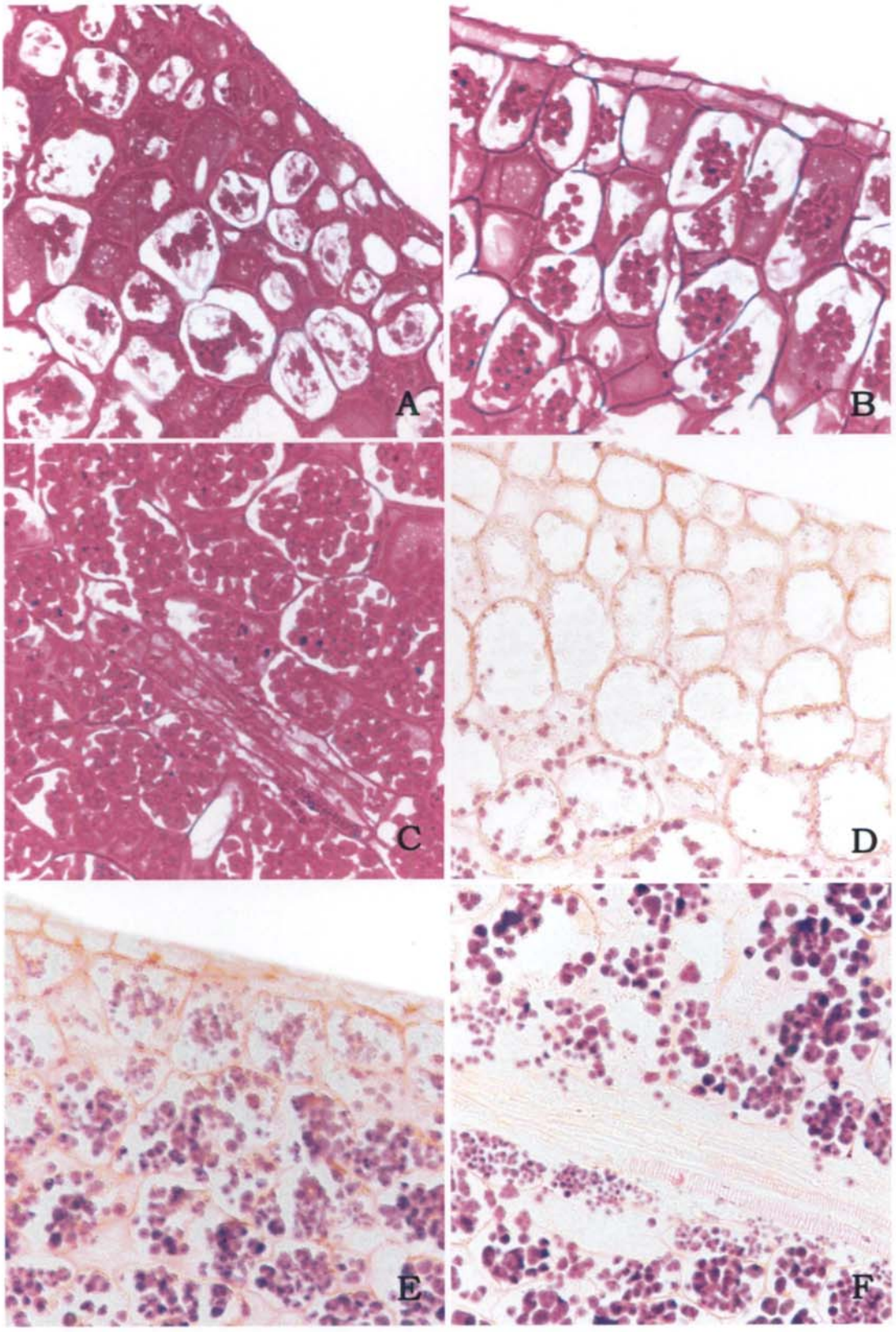
Figure: 11. Distribution of starch grains in the cotyledon of Jack fruit seeds desiccated for 12 days.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

Figure: 12. Distribution of starch grains in the cotyledon of Jackfruit seeds desiccated for 14 days.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

In the cotyledon of seeds desiccated for 16 days, the cells of 4-6 layers near the adaxial side showed almost disappearance of starch grains and nucleus (Fig. 13, A, D). Very few small grains which appeared distorted and clumped were present in some cells towards the centre. But the cells in the abaxial side showed the presence of almost same number of starch grains compared to that of 14^{th} day sample and the nucleus and the grains showed a sort of aggregation (Fig. 13, B, E). The cells in the central region were damaged resultantly changes in shape and loss of integrity of cell wall were occurred. Starch grains in the cells in the centre showed sign of disintegration (Fig. 13, C, F).

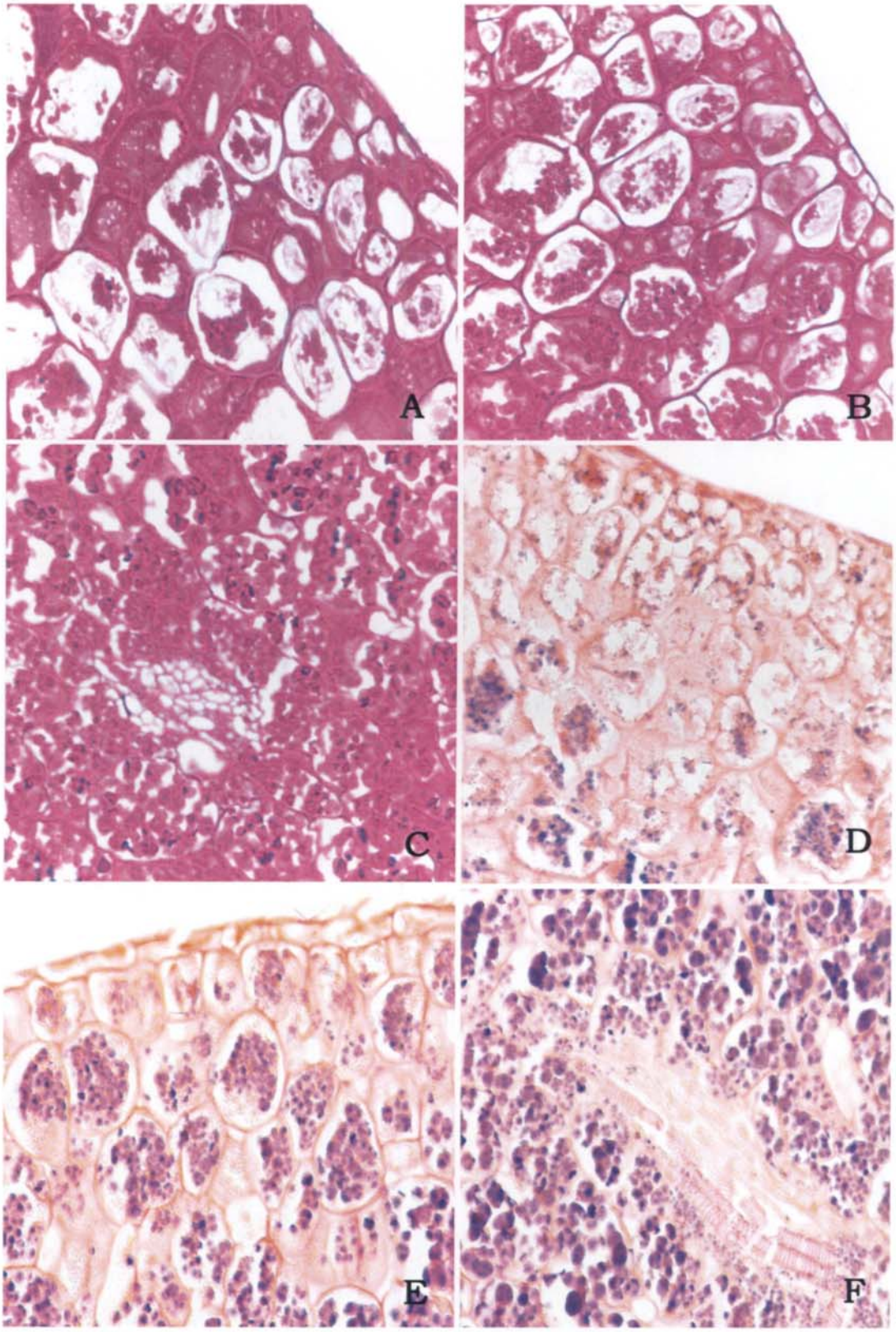
1.2.1.3. Localisation of Protein

Sections of cotyledon stained with mercuric bromophenol blue showed protein content as blue masses and starch grains remained unstained and appeared as hyaline bodies. The protein in cotyledon of control seeds appeared as blue scattered mass and the starch grains were hyaline (Fig. 14) The starch grains near the vascular bundles showed a reddish spot in the centre indicating the hilum (Fig. 14, C). The nucleus in the cells appeared red in colour. The protein contents in the adaxial and abaxial side of the cotyledon were almost alike (Fig. 14, A, B).

In the cotyledon of seeds desiccated for eight days, the cells of adaxial side were closely packed with protoplast containing the protein mass and hyaline starch grains filling the entire cell lumen. The nuclei were visible as red structures with the red colour spreading into the contents (Fig. 14, D). Cells in the inner layers showed the protoplast slightly receded from cell wall.

In the abaxial side, the epidermal cells were same as that of the control seeds (Fig. 14, E). But in all other cells, the protoplast containing the protein mass and hyaline starch grains were found receding from cell wall to occupy

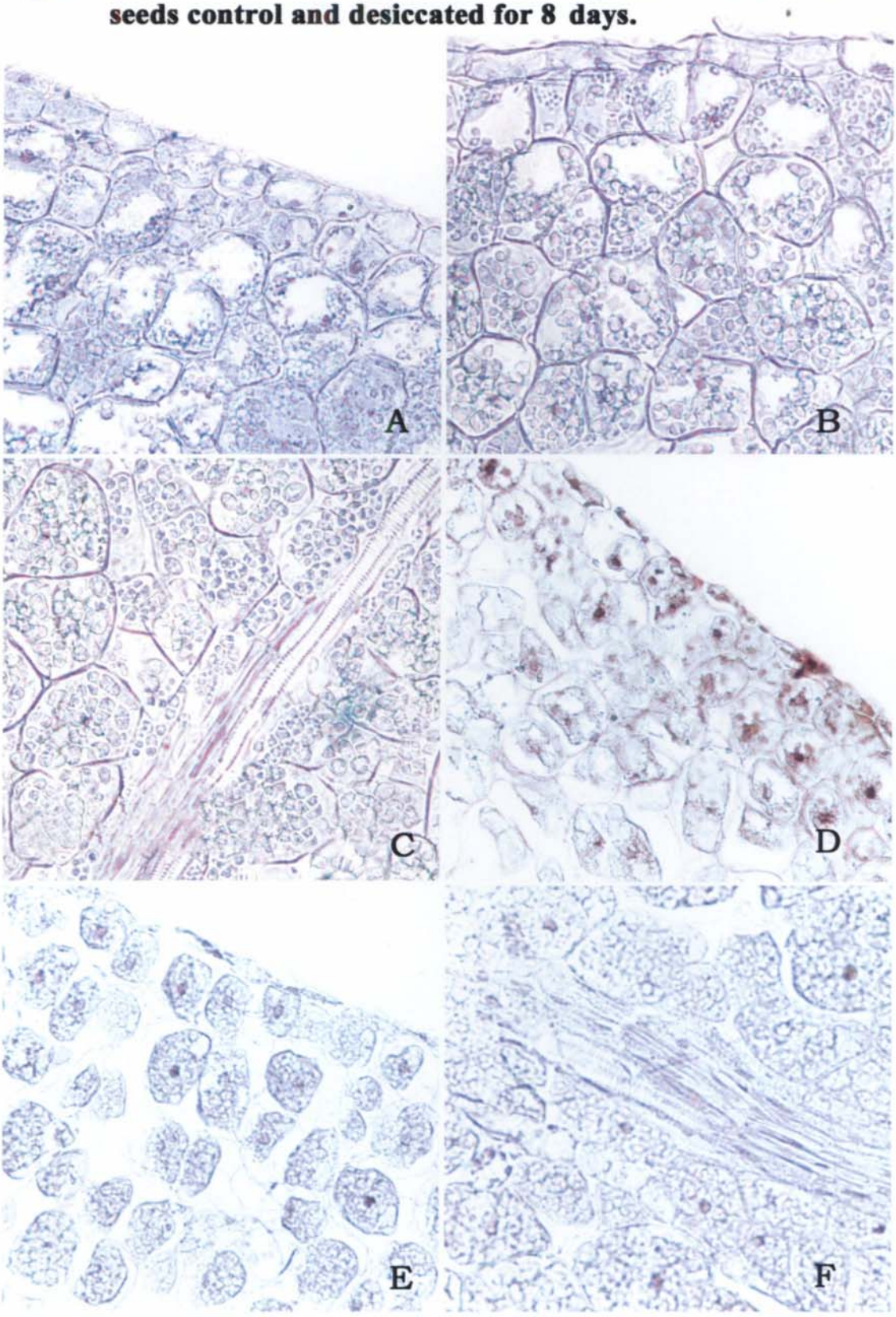
Figure: 13. Distribution of starch grains in the cotyledon of Jackfruit seeds desiccated for 16 days.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

Figure: 14. Distribution of protein in the cotyledon of Jackfruit seeds control and desiccated for 8 days.



A. Adaxial side
B. Abaxial side
C. Centre
} Control

D. Adaxial side
E. Abaxial side
F. Centre
} 8th day

the centre of the cell lumen. The nucleus was visible as in the cells of adaxial side. In the centre, cells were filled with the cell contents with blue protein mass and visible nucleus (Fig. 14, F).

In the cotyledon of seeds desiccated for 12 days, there was no significant difference from the8thday sample. The cells inner to the epidermis of adaxial side showed the beginning of the withdrawal of protoplasmic contents from the cell wall (Fig. 15, A) The cells in the abaxial side and the centre region showed the same characteristics of the 8th day sample (Fig. 15, B, C).

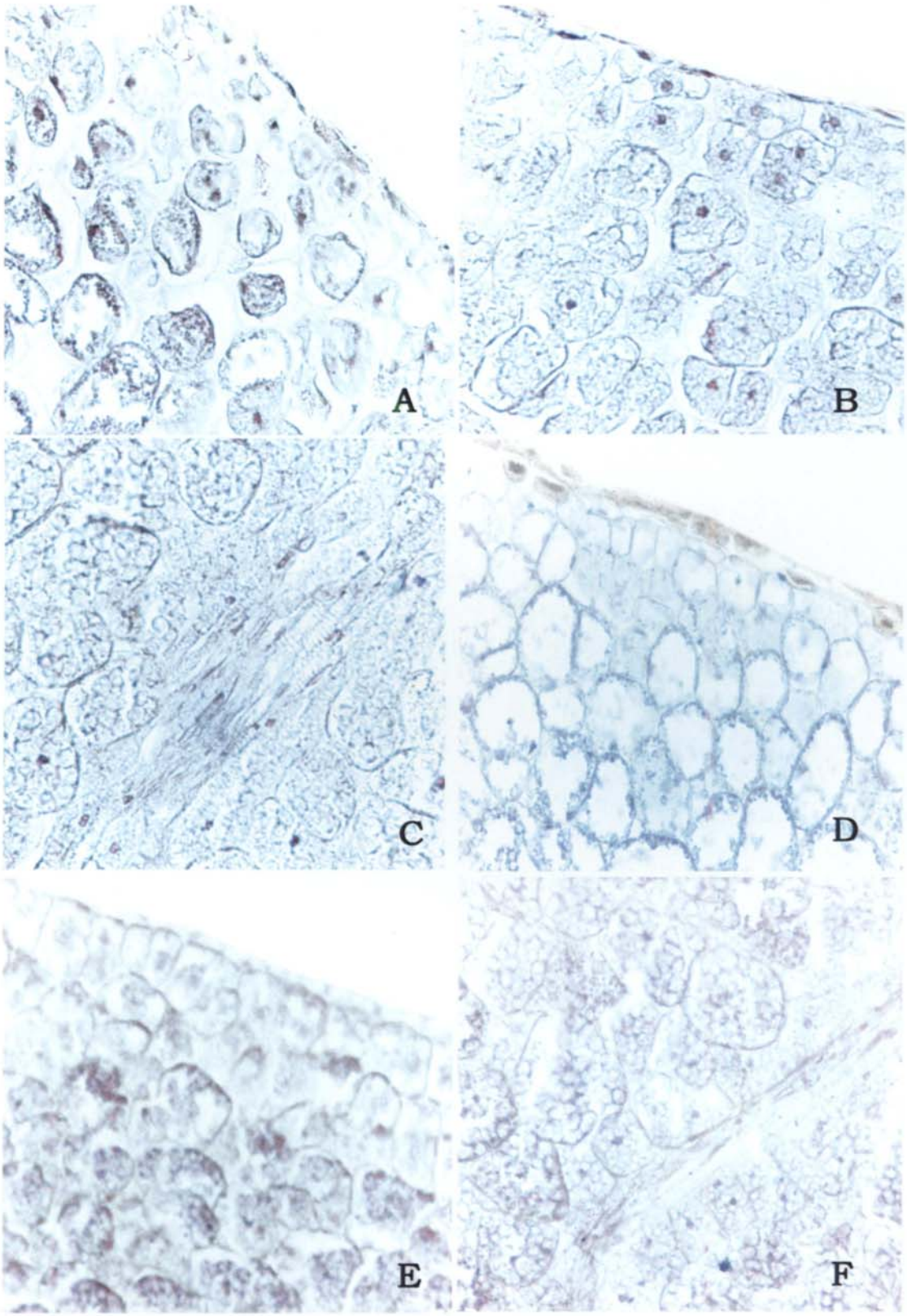
The seeds desiccated for 14 days showed changes in the cells near the adaxial and abaxial sides. The cells in the adaxial side showed more contraction of the protoplast from the cell wall (Fig. 15, D). The cells in the abaxial side and centre of cotyledon were similar to that of the 12th day sample (Fig. 15, E, F).

In the seeds desiccated for 16 days, the cotyledonary cells in the adaxial side showed a slight disappearance of the protein mass and the protoplast was distorted but the nucleus was visible (Fig. 16, A). In the cells near the abaxial side, the protein mass along with hyaline starch grains disappeared and the contents were concentrated towards the centre (Fig. 16, B). The cells in the centre also showed slight distortion with decrease in protein content (Fig. 16, C). The protoplast of these cells receded from the cell wall.

1.2.2. Histochemical Study of Embryonic Axis

Histochemical study of embryonic axis of control seeds revealed that axis is well protected within the tegmen on one side and a portion of cotyledon on other side (Fig. 17, A, B). Starch grains were present at the extreme tip of radicle. In the seeds desiccated for 8 days, the structure of axis

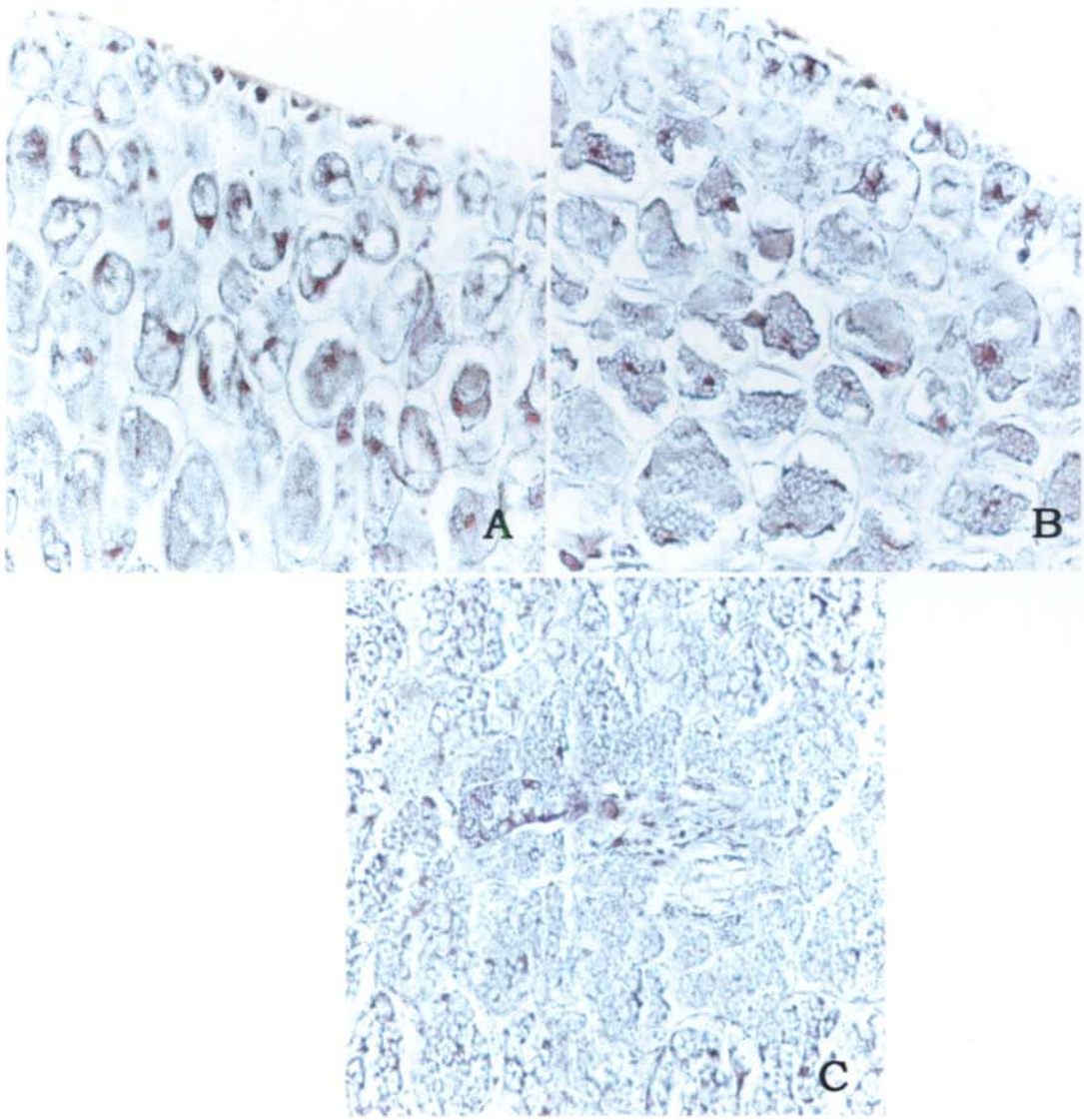
Figure: 15. Distribution of protein in the cotyledon of Jackfruit seeds desiccated for 12 and 14 days.



A. Adaxial side
B. Abaxial side
C. Centre
} 12th day

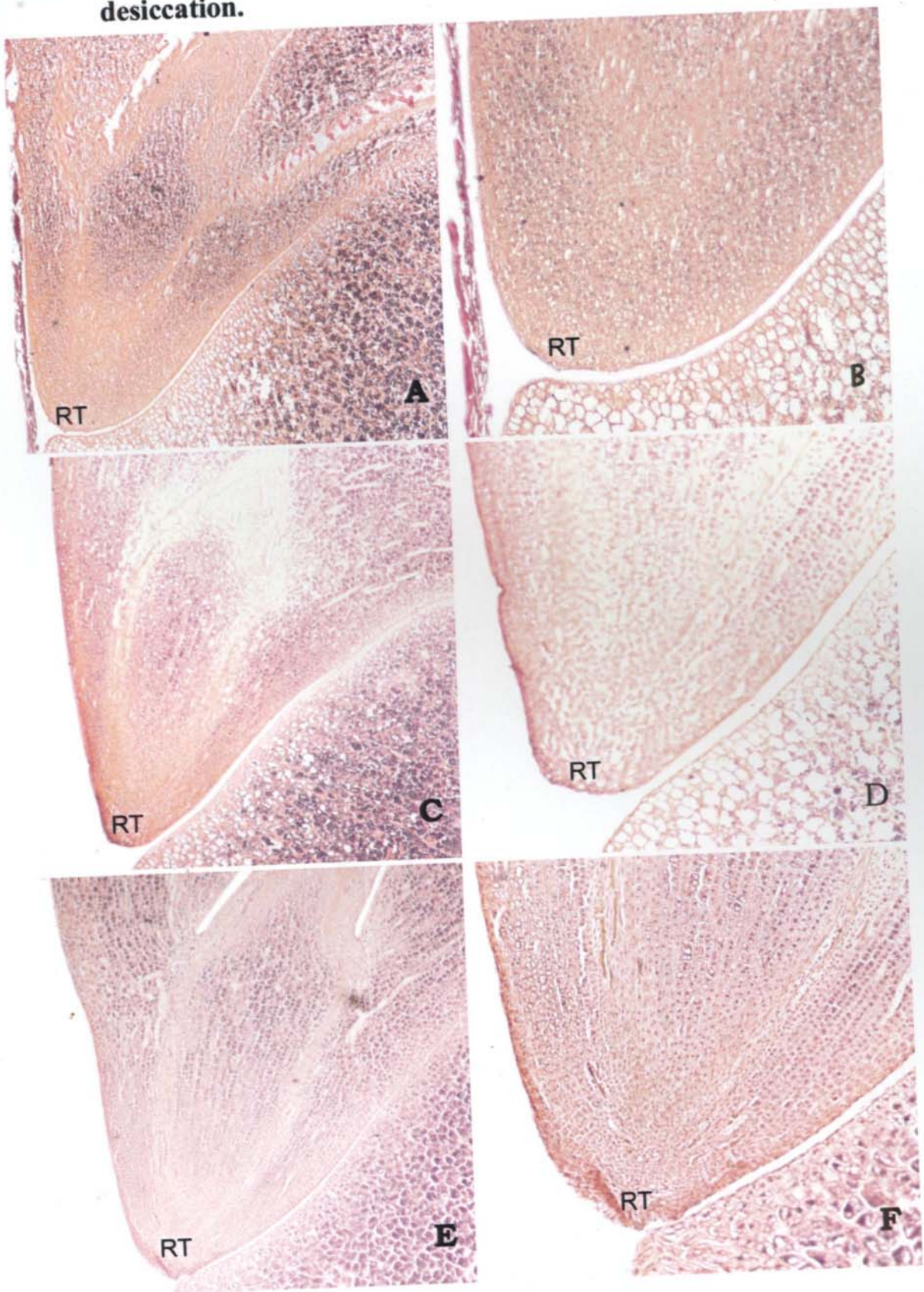
D. Adaxial side
E. Abaxial side
F. Centre
} 14thday

Figure: 16. Distribution of protein in the cotyledon of Jackfruit seeds desiccated for 16 days.



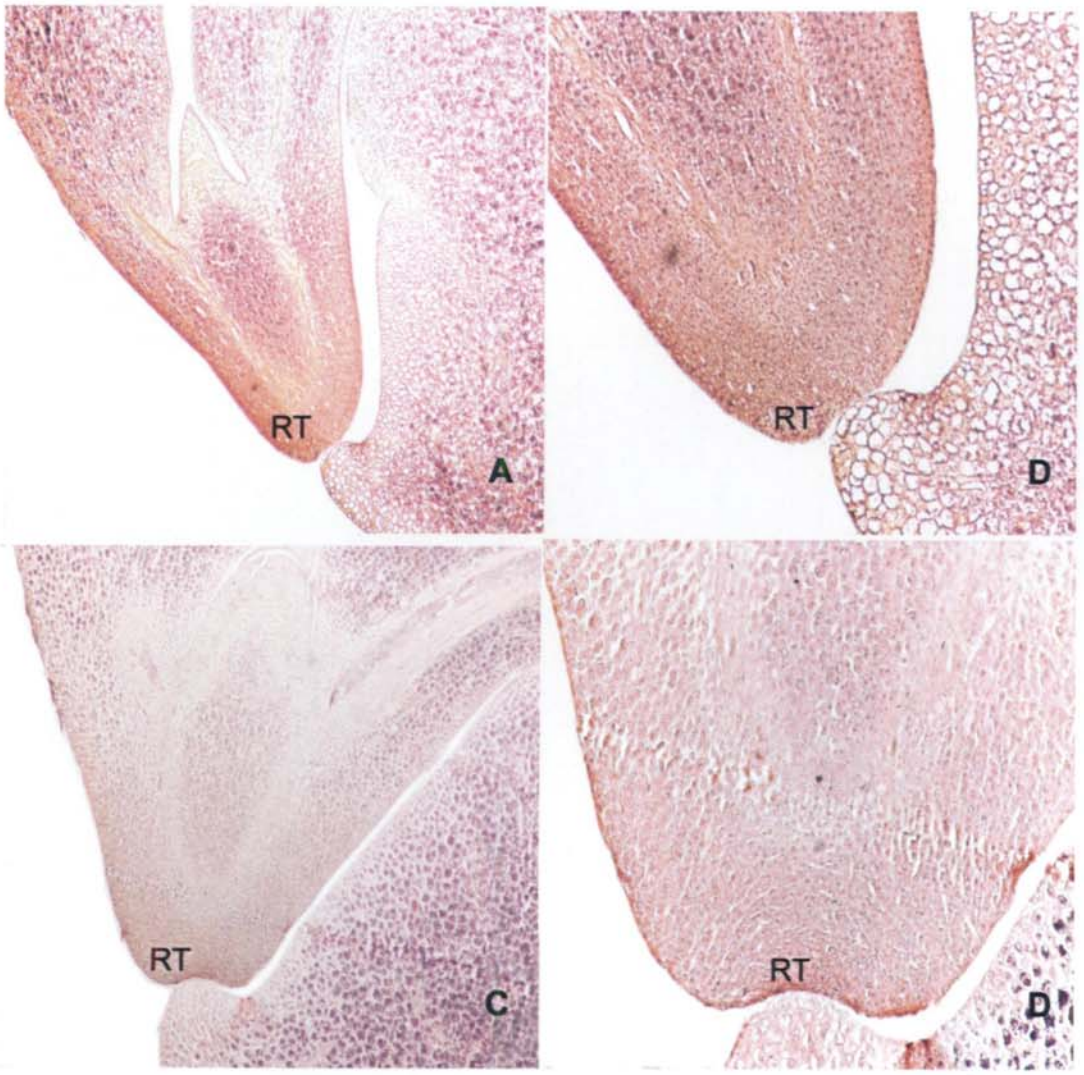
A. Adaxial side
B. Abaxial side
C. Centre

Figure: 17. Changes in embryonic axis of Jackfruit seeds during desiccation.



RT - Radicle tip
A,B - Control
C,D -8 days
E,F - 12 days

Figure: 18. Changes in embryonic axis of Jackfruit seeds during desiccation for 14 and 16 days.



RT - Radicle tip, A,B - 14 days, C,D - 16 days

was same as that of the control seeds. Here the number of starch grains in the apex showed a slight decrease (Fig. 17, C, D). On the 12th day of desiccation, the radicle showed the beginning of desiccation induced effects. Radicle tip showed slight distortion of cells (Fig. 17, E, F). The clarity of cell boundary at the extreme tip was almost disappeared. There was a significant decrease in number of starch grains at the radicle tip. The seeds were viable and showed 87% viability.

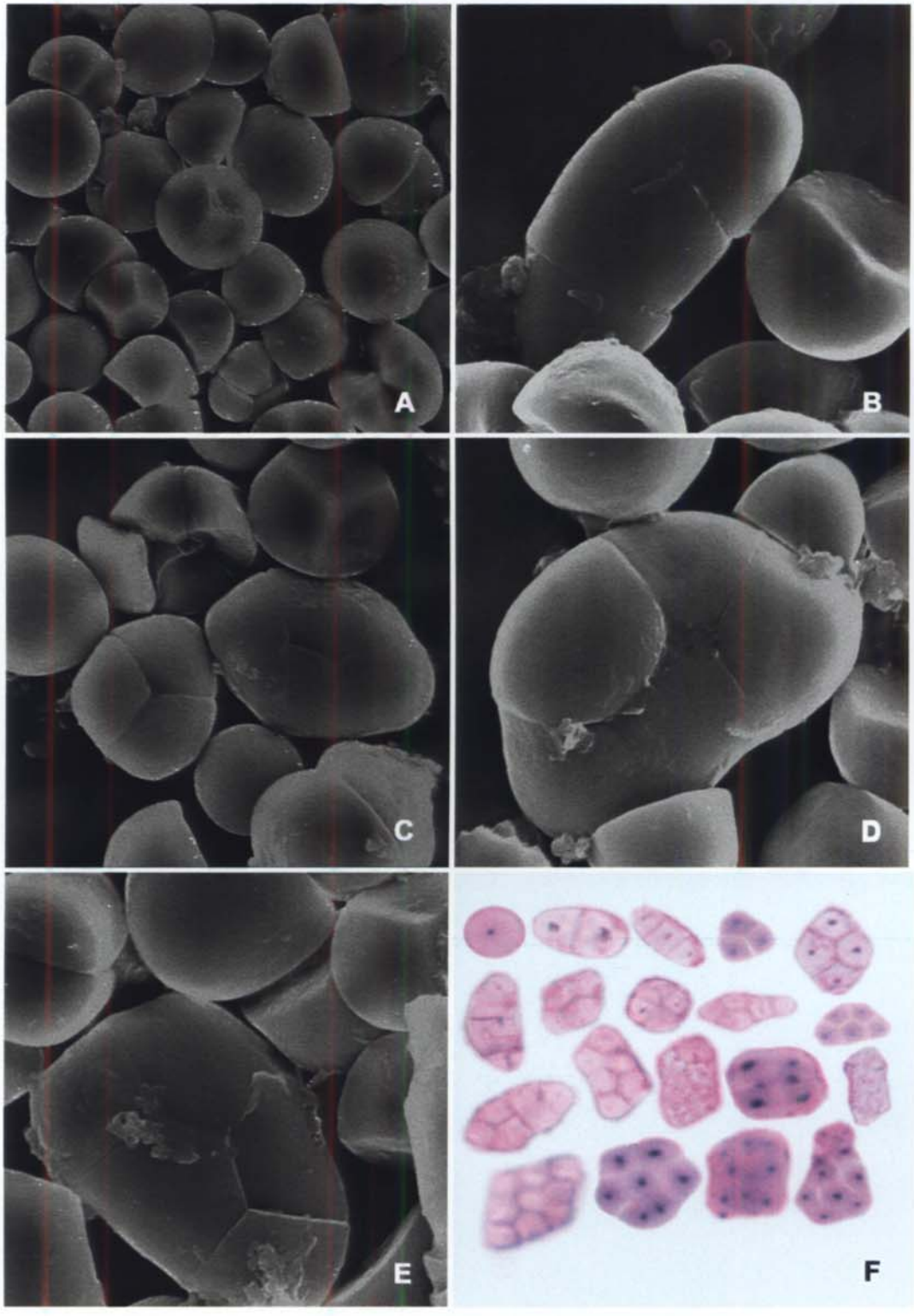
In the seeds desiccated for 14 days, radicle showed a clear desiccation induced morphological changes. At the extreme tip, beginning of invagination was observed (Fig. 18, A, B) and the starch grains almost disappeared. The portion of cotyledon near the radicle seems to touch the extreme tip of radicle at the region of invagination. These seeds were considered nonviable because they showed only 42% viability.

The seeds desiccated for 16 days showed more prominent desiccation induced histochemical characters. The radicle tip appeared like a bilobed structure due to the deep invagination. The portion of cotyledon was closely appressed to the invaginated region of radicle tip (Fig. 18, C, D). The cells of apical region were distorted and showed complete disappearance of starch grains. Seeds desiccated for 16 days were nonviable and the viability was only 8 percentage.

1.2.3. Scanning Electron Microscopic (SEM) Study of Starch Grains

A wide variation in size, shape and cluster formation was observed among the starch grains in Jackfruit seeds (Fig. 19 F). Simple and compound grains consisting of various numbers were present in the same cell. Simple grains of different sizes also were observed (Fig. 19 F). Number of grains in the compound grains varied from 2-11 with variation in their orientation /

Figure: 19. Scanning electron micrograph of starchgrains of Jackfruit seeds.



A - 200 X, B- 5000 X, C- 3000 X, D- 4000 X, E- 4000 X,
F- Starch grains of different shape, size , aggregation-
selected from different regions of cotyledon (40X)

clustering of individual grains. Hilum was visible in the centre of simple grains and individual grains of compound grains (Fig. 19 F).

SEM studies revealed only simple and compound grains consisting of 2, 3, 4 and 5 individual grains (Fig. 19, A, B, C. D).

2. STORAGE

2.1. Biochemical Studies

2.1.1. Moisture Content of Seeds

As mentioned earlier, the moisture content of fresh seeds (control) was 50.19%. In room-polythene condition, the seeds showed a slight increase in moisture content on 10^{th} day of storage (Table 7, Fig. 20). After 20 days of storage, moisture content showed only insignificant changes (reduction/increase) upto 60 days. The moisture content was increased insignificantly on 70^{th} day and remained almost same upto110^{th} day of storage. After that there was a slight decrease in moisture content.

In the case of seeds stored in refrigerator, only negligible increase in moisture content was observed during the initial days of storage and the seeds maintained this upto 40^{th} day. Thereafter, the moisture content was decreased and maintained the same upto 90^{th} day of storage. Then these seeds showed a gradual decline in moisture content on 100^{th} day and remained unchanged during the rest of the storage period.

The stored seeds in both the conditions maintained the same moisture content during the initial days of storage and showed the same trend upto 90 days. In room - polythene seeds, the moisture content was declined significantly after 110 days of storage, but in refrigerator stored seeds, this occurred after 90 days of storage.

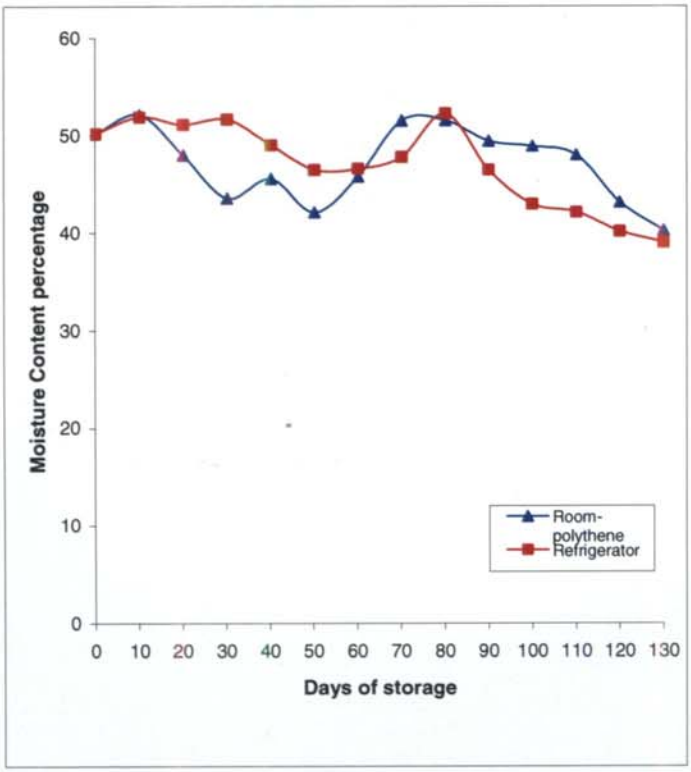
Table: 7. Moisture content and viability of Jackfruit seeds during storage under different conditions.

Days of storage	Room-Polythene		Refrigerator	
	MC %	Germination%	MC %	Germination%
0	50.19±1.59	100	50.19±1.59	100
10	52.15±1.32	100	51.92±1.98	100
20	47.99±2.17	100	51.13±2.16	100
30	43.56±1.39	100	51.65±1.43	100
40	45.55±3.01	100	49.05±1.23	100
50	42.12±1.53	100	46.50±2.49	100
60	45.80±2.19	100	46.59±1.36	100
70	51.53±1.92	100	47.80±3.10	100
80	51.55±2.14	100	52.27±1.82	100
90	49.43±2.36	100	46.46±2.96	100
100	48.90±1.05	100	42.97±1.34	80± 2.56
110	48.00±3.21	87.5±2.15	42.14±2.17	62.5±3.12
120	43.15±1.86	24±1.89	40.18±1.54	36.66±2.16
130	40.24±1.94	0	39.04±3.06	0

Table: 8. Distribution of starch in Jackfruit seeds during storage under different conditions mg g^{-1} dry tissue.

Days of storage	Room-Polythene	Refrigerator
0	730.30±20.48	730.30±20.48
10	722.66±22.12	710.40±14.33
20	709.07±22.12	688.42±19.27
30	672.14±27.09	687.37±16.61
40	673.52±20.98	680.49±12.04
50	671.87±14.82	676.49±14.01
60	646.56±22.31	634.81±7.31
70	620.89±32.72	614.30±31.76
80	607.73±24.52	606.16±13.01
90	601.73±17.67	598.79±21.85
100	575.35±30.35	482.63±22.81
110	462.02±21.83	456.90±22.10
120	460.89±17.65	455.25±17.49

Figure: 20. Effect of storage conditions on moisture content percentage in Jackfruit seeds under different conditions.



2.1.2. Viability

The control seeds showed 100% viability. Cent percent viability was shown by room-polythene seeds upto 100 days of storage (Table 7, Fig. 21). Then these seeds showed a significant decrease ($P< 0.01$) in germination percentage. After 110 days of storage, the germination percentage of seeds declined sharply. On 130^{th} day of storage, all seeds became nonviable.

In seeds stored in refrigerator, the viability was 100% upto 90^{th} day of storage. Thereafter, the germination percentage was decreased significantly ($P<0.01$). On 110^{th} day of refrigerated storage, only 62.5% seeds remained viable. None of the seed was viable on 130^{th} day of storage.

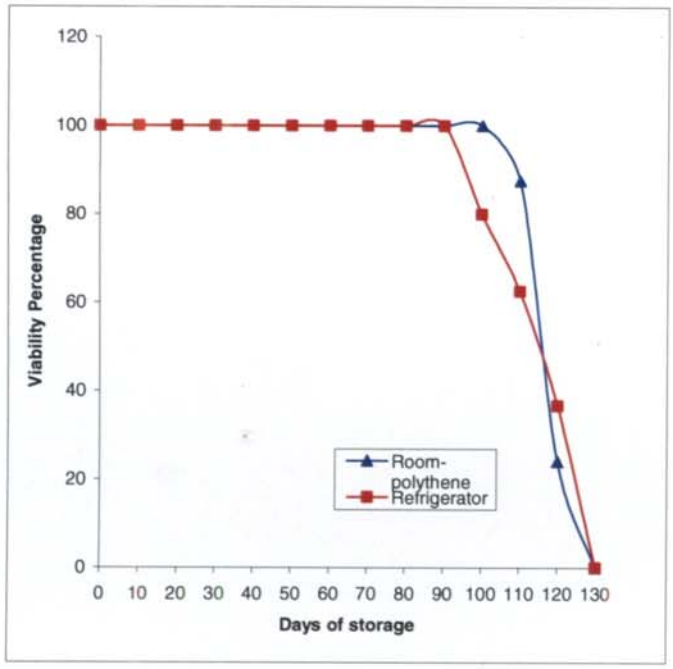
In the case of room-polythene storage, the seeds remained viable upto 110^{th} day and the corresponding moisture content was 48%. On 120^{th} day, when the moisture content decreased to 43.15%, the germination percentage was also decreased to just 24%. When the seeds were stored in refrigerator for 110 days, they retained 42.14% moisture content and the viability was only 62.5%.

2.1.3. Seed Vigour Index

Room polythene storage: There was no much difference in seed vigour upto 50 days in room-polythene storage. After 50 days, the seed vigour was found to decrease. It was reduced to half as that of control seeds on 100^{th} day of storage (Table 2, Fig. 2). This decrease was continued during further period of storage.

Refrigerator storage: Seed vigour of refrigerator stored seeds showed the same trend as that of room-polythene stored seeds upto 50 days. On 70^{th} day of storage, seed vigour was reduced to half as that of seed vigour of seeds

Figure: 21. Effect of storage conditions on viability percentage in Jackfruit seeds under different conditions.



stored for 60 days (Table 2, Fig. 2). Afterwards, there was a gradual decline of seed vigour during the entire period of refrigerator storage.

2.1.4. Tissue dry weight percentage

The tissue dry weight of control seeds was 50.07 percentage (Table 3). During initial days of room-polythene storage, the tissue dry weight was increased insignificantly upto 60 days of storage. After this, there was an insignificant increase in tissue dry weight and more or less same trend was maintained throughout the entire storage period.

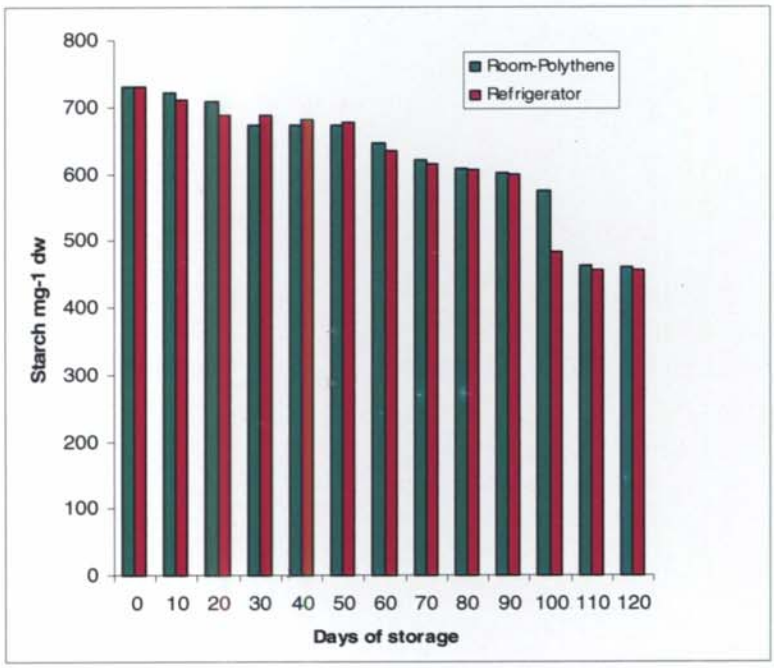
In the case of refrigerator storage, the increase in tissue dry weight during initial days of storage was slightly higher than that of room-polythene stored seeds. After 30 days of storage, there was a gradual increase in dry weight which was continued throughout the storage period. The maximum tissue dry weight was observed in seeds stored for 100days.

2.1.5. Starch

Jackfruit seeds are starch-rich. The control seeds showed maximum starch content. i.e. 730.30mg g^{-1} dry tissue (Table 8, Fig. 22). During storage, the starch content was continuously decreased upto 30 days and afterwards starch content remained unchanged upto 50 days. The starch content was decreased gradually from 60 days to 100days of storage. Thereafter, there was a significant decline in starch content upto 110 days which coincided with viability loss.

In the refrigerator storage, the seeds maintained almost same starch content showing negligible fluctuation upto 50 days. From 60 days onwards, the starch content was decreased. A sharp decline in starch content was noticed from 100 days of storage and the trend was continued during further storage period.

Figure: 22. Distribution of starch content during storage of Jackfruit seeds under different conditions.



2.1.6. Sugars

HPLC studies of sugars in seeds stored in room-polythene for 40 days showed that glucose and fructose were doubled the amount than that of control seeds (Table 9, Fig. 23, 24 a). Rhamnose was maintained almost the same amounts as that of the control seeds. In these seeds almost the same quantity of sucrose and maltose were present. Sucrose content was slightly decreased and the raffinose was the same as that of control seeds. These seeds showed the presence of only one unknown sugar (II). The total sugar content was almost similar to that of control seeds.

The seeds stored in room-polythene for 80 days, showed a slight increase in rhamnose and doubling of monosaccharides compared to that of control seeds. Sucrose and maltose remained almost unchanged. Raffinose was decreased to less than half of the 40^{th} day sample. Total sugar content was higher than the control and 40^{th} day sample. These seeds showed the presence of only one unknown sugar (II).

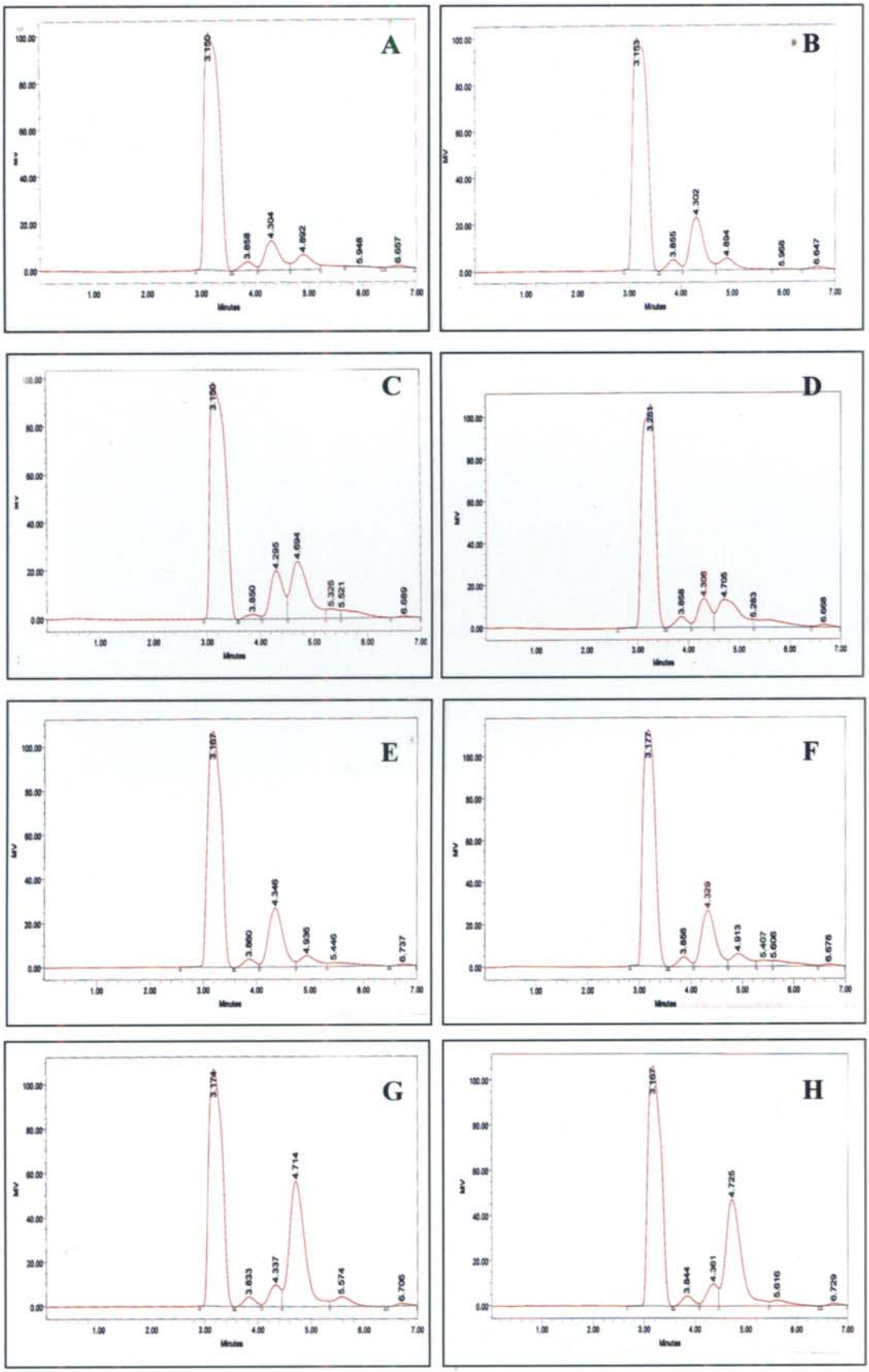
The seeds stored for 100 days showed an increase in total sugar content which was about 11.8%. Slight reduction was shown by rhamnose, glucose and fructose than the 80^{th} day sample. Sucrose and maltose exhibited a four fold increase resulting in the maximum quantity during storage in room-polythene condition. Raffinose showed a rapid increase which was about ten times higher than that of seeds stored for 80 days. Here the unknown II sugar disappeared but the unknown I and III were present.

Slight reduction of glucose and fructose was occurred in the seeds stored in room-polythene for 120 days. Raffinose registered its maximum amount in this sample. The dominant sugars in this sample were sucrose and maltose. Raffinose showed a rapid increase reaching the maximum amount during storage in room-polythene condition. This sample showed the presence

Table: 9. Distribution of sugars in Jackfruit seeds during storage under different conditions mg g^{-1} dry tissue.

Sugars	Room-Polythene					Refrigerator				
	Days of storage					Days of storage				
	0	40	80	100	120	0	40	80	100	120
Rhamnose	6.73	7.25	9.68	4.12	10.35	6.73	6.54	8.99	8.33	8.21
Glucose	7.02	12.66	24.77	17.13	12.34	7.02	24.40	27.57	7.17	7.57
Fructose	7.66	14.10	27.58	19.08	13.71	7.66	27.25	30.47	8.00	8.44
Sucrose	10.22	7.26	6.62	27.01	20.53	10.22	5.14	7.38	47.60	57.30
Maltose	7.67	8.30	7.01	30.79	23.41	7.67	6.04	8.34	54.21	65.33
Raffinose	3.83	3.94	1.25	12.99	19.88	3.83	8.95	11.61	1.98	14.80
Unknown-I	6.39	-	-	6.48	16.61	6.39	-	5.66	-	-
Unknown-II	2.04	1.66	2.40	-	2.89	2.04	-	1.98	-	-
Unknown-III	2.06	-	-	1.15	-	2.06	1.08	-	1.53	2.11
Total	**53.61**	**55.17**	**79.30**	**118.74**	**119.75**	**53.61**	**79.41**	**101.99**	**128.83**	**163.46**

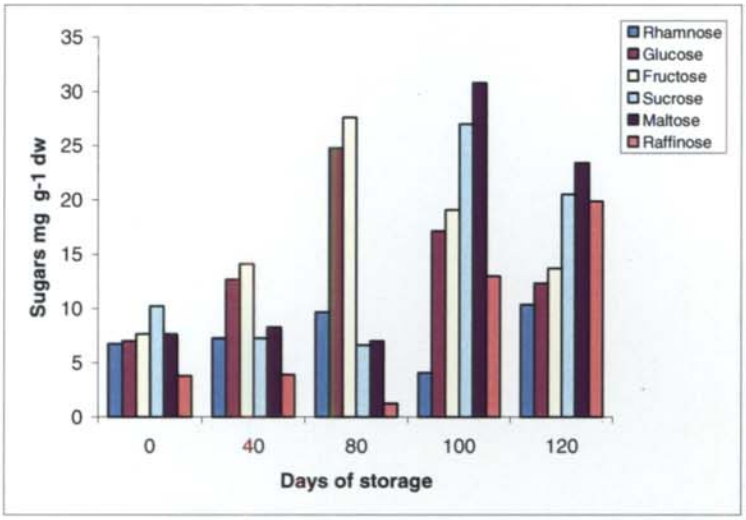
Figure: 23. HPLC sugar profile of Jackfruit seeds during storage.



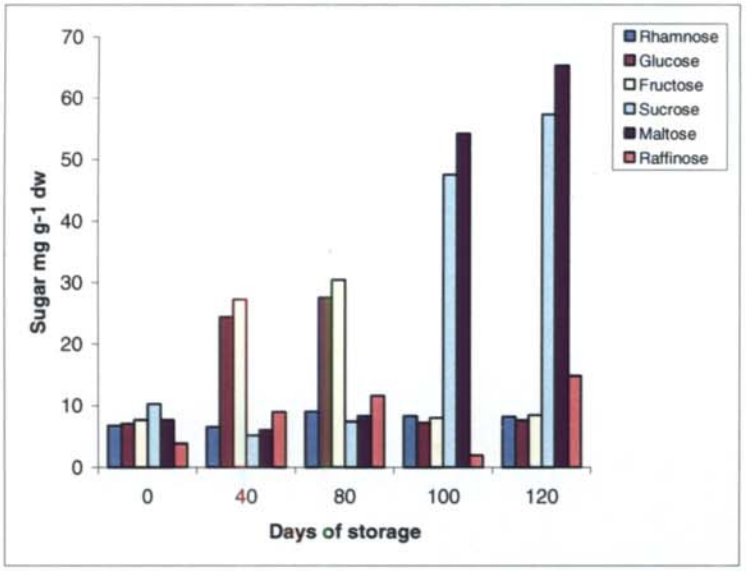
A, B, C, D - Seeds stored in room-polythene for 40, 80, 100, 120 days
E, F, G, H - Seeds stored in refrigerator for 40, 80, 100, 120 days

Figure: 24. Distribution of sugars during storage of Jackfruit seeds under different conditions.

a) Room-Polythene condition



b) Refrigerator



of two unknown sugars (I and II). Maximum quantity of total sugar content (about 11.9 %) was shown by the seeds in this storage condition.

The seeds stored in refrigerator for 40 days showed a significant increase in compared to the control. Rhamnose was same as that of the control seeds (Table 9, Fig. 23, 24b). Sucrose was reduced to about half of the control seeds, but maltose maintained almost the same level. Raffinose was doubled in comparison with that of control seeds. In addition to monosaccharides, disaccharides and raffinose, these seeds showed the presence of one unidentified sugar (III).

In seeds stored for 80 days, glucose and fructose were almost same as that of the 40^{th} day sample, but the sucrose and maltose showed a slight increase. Rhamnose was slightly increased and was the maximum in this storage condition. The quantities of raffinose and total sugar content were increased. Two unknown sugars (I and II) were present in this sample.

The seeds stored in refrigerator for 100 days showed a remarkable decrease (about one fourth) in the glucose and fructose contents compared to that of the seeds stored for 80 days. Rhamnose maintained the same amount as that of the earlier sample. But the sucrose and maltose registered a sharp increase which was about seven times compared to that of the 80^{th} day sample. Seeds stored in refrigerator for 100 days showed the minimum raffinose content registering a significant decline compared to the previous sample. These seeds showed the presence of only one unknown sugar (III).

In the seeds stored for 120 days, the monosaccharide contents maintained the same amount as that of the 100^{th} day sample and were almost similar to control seeds (Table 9, Fig. 23, 24b). Sucrose and maltose were increased to the maximum amount compared to all other samples during storage. Raffinose content was increased significantly to attain the maximum

value than all other samples. Only one unknown sugar (III) was present in these seeds. The total sugar content showed the maximum value in these seeds.

2.1.7. Amylase Assay

2.1.7.1. Unit Activity

Room-polythene storage

Amylase activity was obtained at pH-5.3 and pH-8.0 representing β- and α-amylases. The control seeds showed 53.28 unit of β-and 45.83 units of α-amylase. On 10^{th} day of room-polythene storage, the activity at pH-5.3 was increased. Thereafter, the unit activity was decreased upto 30^{th} day of storage (Table 10, Fig. 25a). Then the activity at pH-5.3 increased with a decrease on 60^{th} day. On 70^{th} day of room-polythene storage, the activity was again increased. Thereafter the activity was declined sharply upto 90 days of storage. The seeds of 100^{th} and 110^{th} days of storage showed the maximum β-amylase activity (pH-5.3).

The seeds stored in room-polythene showed an increase in unit activity at pH-8.0 during the initial days of storage (Table 10, Fig. 25 a) and maintained the same rate upto 50 days of storage. Then a 10% reduction was observed on 90 days of storage. Then a sharp increase in the activity of α-amylase was shown by seeds stored for 100days. These seeds showed the maximum unit activity of α-amylase on 100^{th} day of storage.

Refrigerator storage

The seeds stored in the refrigerator showed about 40% increase in amylase activity at pH-5.3 (β-amylase) on 10^{th} day of storage (Table 10, Fig. 26a). It was reduced to half on 20^{th} day and increased on 30^{th} day and again decreased upto 50 days. There after, the activity at pH-5.3 increased and

Table: 10. Unit activity of Amylases in Jackfruit seeds during storage under different conditions mg g^{-1} dry tissue.

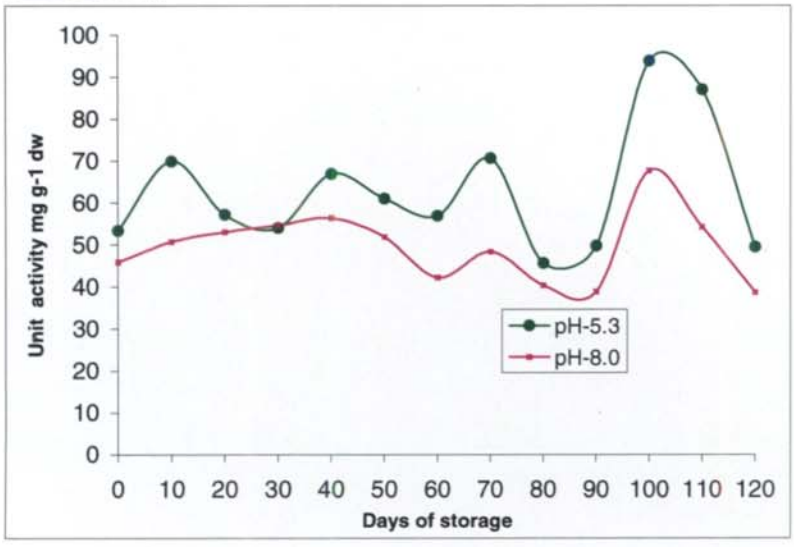
Days of storage	Room-Polythene		Refrigerator	
	pH – 5.3	pH – 8.0	pH – 5.3	pH – 8.0
0	53.28±4.73	45.83±4.09	53.28±4.73	45.83±4.09
10	69.86±4.99	50.70±2.08	81.74±3.60	70.65±4.19
20	57.14±3.85	52.99±2.85	41.00±2.44	37.67±2.80
30	54.01±3.88	54.63±2.40	59.80±4.20	48.26±2.51
40	66.84±3.47	56.36±4.40	42.91±3.92	42.66±2.09
50	60.99±3.24	51.83±1.60	43.57±1.90	44.42±2.18
60	56.86±2.67	42.18±3.36	56.56±3.58	38.72±3.27
70	70.58±4.86	48.35±2.61	48.40±3.23	43.22±3.37
80	45.58±1.99	40.32±3.22	48.19±2.91	41.61±1.48
90	49.77±2.42	38.85±3.37	46.35±3.08	35.11±1.82
100	93.74±3.93	67.60±3.64	45.75±2.33	29.62±1.13
110	86.98±5.03	54.23±3.07	27.65±1.93	33.30±1.81
120	49.43±3.15	38.66±3.41	27.27±1.62	29.30±2.99

Table: 11. Specific activity of Amylases in Jackfruit seeds during storage under different conditions mg g^{-1} dry tissue.

Days of storage	Room-Polythene		Refrigerator	
	pH – 5.3	pH – 8.0	pH – 5.3	pH – 8.0
0	0.53 ± 0.01	0.45± 0.04	0.53±0.01	0.45 ±0.04
10	0.87±0.06	0.63±0.03	1.12±0.05	0.47±0.06
20	0.69±0.05	0.64±0.03	0.50±0.03	0.46±0.03
30	0.63±0.04	0.64±0.03	0.76±0.05	0.62±0.03
40	0.90±0.05	0.76±0.06	0.50±0.04	0.49±0.02
50	0.57±0.03	0.49±0.02	0.52±0.02	0.53±0.03
60	0.60±0.03	0.44±0.04	0.68±0.03	0.46±0.04
70	0.92±0.06	0.63±0.03	0.51±0.03	0.46±0.04
80	0.47±0.02	0.41±0.03	0.31±.03	0.29±0.02
90	0.51±0.02	0.40±0.03	0.37±0.03	0.29±0.02
100	0.80±0.03	0.58±0.03	0.48±0.03	0.23±0.01
110	0.61±0.03	0.38±0.02	0.28±0.01	0.24±0.01
120	0.60±0.04	0.47±0.04	0.36±0.02	0.38±0.04

Figure: 25. Pattern of Amylase activity during storage of Jackfruit seeds under Room- Polythene condition.

a) Unitactivity



b) Specific activity

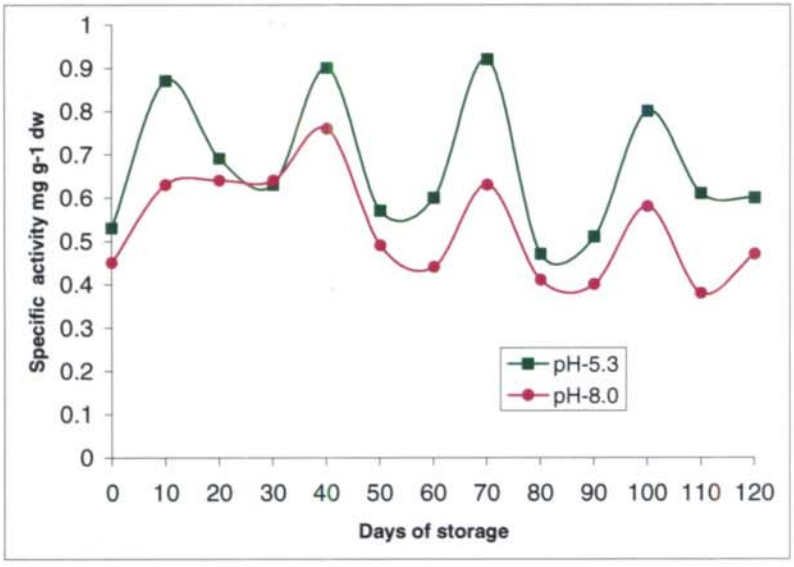
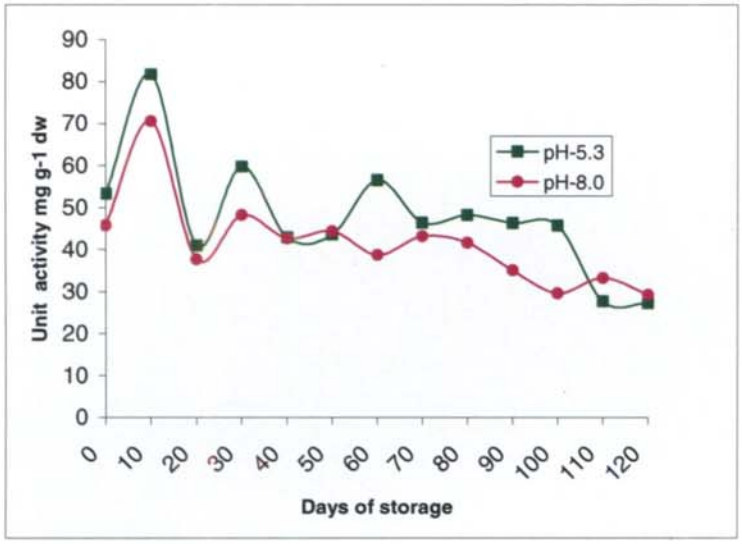
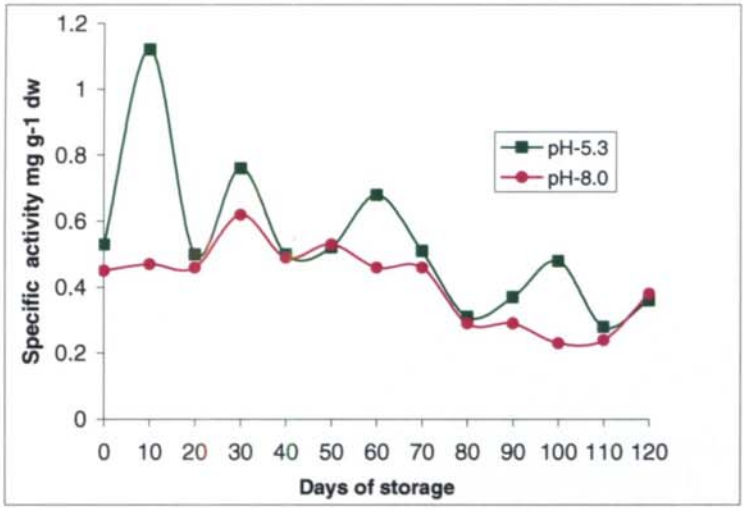


Figure: 26. Pattern of Amylase activity during storage of Jackfruit seeds under Refrigerator condition.

a) Unit activity



c) Specific activity



remained unchanged upto 100 days of storage and afterwards declined sharply.

The seeds stored in refrigerator showed about 50% increase of α-amylase activity during 10^{th} day of storage (Table 10, Fig. 26a). The maximum activity of this enzyme was registered by the seeds stored for 10 days and was reduced to half on 20^{th} day and afterwards continuous reduction was observed at this pH. The enzyme showed significant reduction ($P<0.02$) on 100^{th} day of storage and maintained the same on 120^{th} day after a slight increase on 110^{th} day.

2.1.7.2. Specific Activity

Room-polythene storage

The seeds stored in room-polythene registered about 50% increase in the specific activity of β-amylase during ten days of storage (Table 11, Fig. 25b) but during further storage, the specific activity was decreased upto 30 days and again increased. These seeds showed maximum activity at pH-5.3 (β-amylase) on 70^{th} day of storage. But the activity was decreased to half within the next 10 days and again increased upto 100 days of storage and afterwards further decline was observed.

Seeds stored in room-polythene showed a 50% increase in the specific activity of α-amylase during ten days of storage and maintained the same upto 30 days (Table 11, Fig. 25b). The specific activity was maximum on 40^{th} day and again decreased upto 60 days. The seeds stored for 70 days showed a significant increase ($P<0.01$) in specific activity and decreased during further storage, but showed an increase on 100^{th} day, then again declined significantly upto 120 days. The specific activity of α-amylase of room-polythene seeds was maximum on 40^{th} day and minimum on 110^{th} day of storage.

Refrigerator storage

The seeds stored in refrigerator showed sharp increase (more than two fold) of β-amylase specific activity (pH-5.3) during the ten days of storage, but on 20th day it was reduced to less than half (Table 11, Fig. 26b). A 50% increase was shown on 30th day and these seeds maintained the similar activity as that of control upto 50 days. During 60 days of refrigerated storage, the specific activity was increased significantly. During subsequent storage, the activity of β-amylase was decreased continuously and then increased by100 days of storage followed by a further decrease.

In the case of specific activity of α-amylase (pH-8.0), the refrigerator stored seeds maintained the same activity as that of the control seeds upto 20 days (Table 11, Fig. 26b). On 30th day, the activity was increased ($P<0.01$) and again declined and the same activity was retained upto 70 days of storage. Beyond that period, the activity was decreased on 80th day and the same activity was maintained upto 110 days and again an increase was observed. The refrigerator stored seeds showed maximum α-amylase activity on 30th day and minimum on 100th day.

2.1.8. Total Protein

The total protein content of the control seeds was 104.92 mg g $^{-1}$ dry tissue (Table 12, Fig. 27a). In room-polythene storage, a significant decline ($P<0.01$) was noticed on 10th day and almost same protein content was present in 20th day sample. Thereafter, the total protein content was increased and the value remained unchanged upto 70 days of storage. Then the total protein content increased gradually upto 110 days and then declined significantly ($P<0.01$). The maximum total protein content was shown by seeds stored for 110 days.

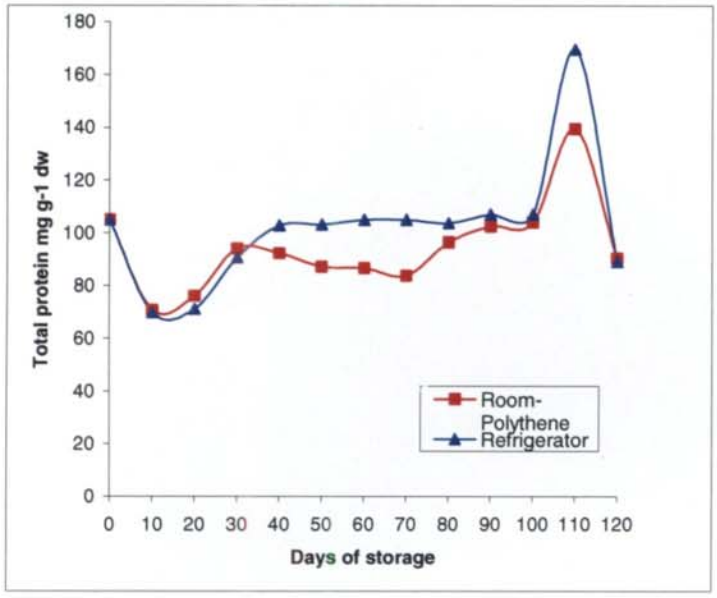
Table: 12. Protein content in Jackfruit seeds during storage under different conditions mg g^{-1} dry tissue.

Days of storage	Room-Polythene	Refrigerator
0	104.92±3.78 (101.03±2.18)	104.92±3.78 (101.03±2.18)
10	70.73±2.18 (80.13±2.75)	69.78±4.20 (72.96±4.61)
20	76.14±2.78 (83.14±1.80)	71.07±3.13 (81.21±3.13)
30	94.04± 2.55 (85.12±2.81)	90.62± 2.68 (78.46±2.27)
40	92.21±5.32 (74.41±1.69)	102.75± 6.76 (86.56±1.90)
50	87.18±6.57 (110.48±3.98)	103.18±5.07 (84.24±3.15)
60	86.65±4.07 (94.70±4.85)	104.90±4.50 (83.39±3.15)
70	83.71±4.13 (76.59±3.27)	104.96±4.01 (94.75±2.29)
80	96.47±2.45 (98.00±2.25)	103.70±4.05 (95.80±3.89)
90	102.54±4.84 (96.32±4.62)	106.96±4.87 (96.23±3.89)
100	103.96±4.17 (116.96±2.36)	106.98±3.07 (100.89±3.58)
110	139.49±4.42 (141.78±2.34)	169.63±5.80 (140.72±1.93
120	90.32±4.88 (82.85±0.94)	89.14±5.78 (76.62±3.22)

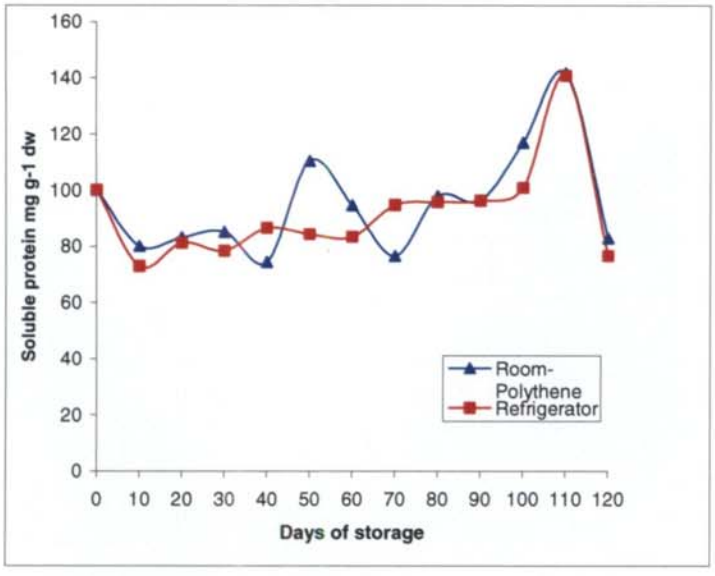
Values in parenthesis are soluble protein

Figure: 27. Distribution of protein content in Jackfruit seeds under different conditions.

a) Total protein



b) Soluble protein



Seeds stored in refrigerator showed almost same trend in protein content change upto 20th day of storage as that of seeds stored in room-polythene condition (Table 12, Fig. 27a). Then the total protein content was increased significantly ($P<0.02$) and thereafter the same total protein content was maintained as that of control seeds upto 100 days of storage. The total protein content was increased significantly ($P<0.01$) on 110th day and that was the maximum protein content present in this storage condition. In the samples of 120 days, there was a sharp decline in total protein content.

2.1.8.1. Soluble Protein

The soluble protein content of the control seeds was 101.03 mg g $^{-1}$ dry tissue (Table 12, Fig. 27b). A significant reduction ($P<0.01$) of soluble protein was shown by seeds stored in room-polythene on 10th day and upto 30 days of storage, these seeds maintained the same soluble protein content. But the soluble protein content was decreased ($P<0.01$) on 40 days of storage and again increased significantly on 50th day and afterwards a gradual decrease was observed upto 90th day. Then these seeds showed an increase ($P<0.01$) in soluble protein content and the maximum content was shown by seeds stored for 110 days. On 120th day, the soluble protein content was decreased significantly.

In seeds stored in refrigerator, there was a significant ($P<0.01$) reduction in soluble protein during the 10th day (Table 12, Fig. 27b). On 20th day, the soluble protein content was increased slightly and this increase was maintained upto 60 days of storage. Then the soluble protein content was increased significantly upto 70 days of refrigerated storage and the soluble protein content remained unchanged upto 100 days. The maximum soluble protein content was shown by seeds stored for 110 days. But during last 10 days of storage, the soluble protein content was decreased to about half of the previous sample.

3. RESERVE MOBILISATION

3.1. Biochemical Studies

3.1.1. Tissue dry weight percentage

The tissue dry weight percentage of the seeds upto 50 days of germination was given in Table 13. During early days of germination, the dry weight percentage of axis and cotyledons were determined separately, but from 20 days onwards the tissue dry weight of cotyledons alone was taken.

On the second day of germination, the cotyledon tissue dry weight showed a slight insignificant increase than that of control seeds (Table 13). During subsequent days of germination, the dry weight percentage was decreased gradually. Only 60% dry weight was retained during germination upto 50 days.

In the case of embryonic axis, the tissue dry weight remained the same upto 5^{th} day of germination (Table 13). Then it showed a sharp decline which was about one third of the initial dry weight of the axis.

3.1.2. Starch

The starch content showed a gradual reduction during the entire germination period studied (Table 14, Fig. 28). The cotyledons of control seeds showed maximum starch content i.e. 730 mg g^{-1}dry weight and that of axis was 497.74mg g^{-1} dry weight. After two days of germination, a significant reduction ($P<0.01$) of starch content was observed and more or less same amount of starch was present in the cotyledon upto 30 days of seedling growth. But afterwards starch content was reduced drastically during 40-50 days of seedling growth. On 40^{th} day of seedling growth, the starch content in cotyledon decreased to less than half of the control seeds. On 50^{th} day of seedling growth, starch content was reduced to less than half of the cotyledon of control seed.

Table: 13. Tissue dry weight percentage distribution of Jackfruit seeds during germination/seedling growth.

Days of germination	Tissue Dry Weight %	
	Cotyledon	Axis
0	50.07±1.11	16.51±1.91
2	53.25±1.56	14.50±1.45
5	48.66±2.06	16.55±0.57
10	45.92±1.77	6.31±1.58
20	39.12±1.87	ND
30	34.50±1.61	ND
40	33.40±2.38	ND
50	30.80±2.15	ND

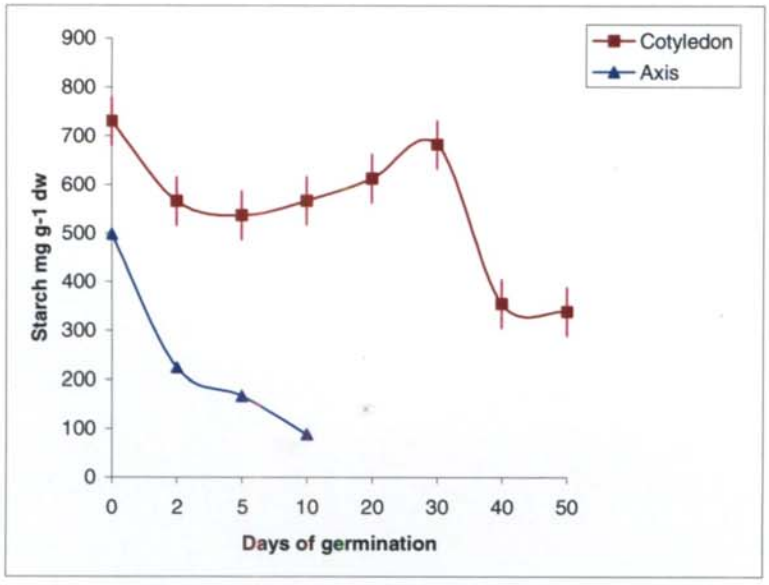
ND: Not done because after 10^{th} day of germination, the embryonic axis is highly heterogeneous tissue since plumule and radicle get differentiated.

Table: 14. Distribution of metabolites in Jackfruit seeds during germination/seedling growth mg g^{-1} dry tissue.

Days of Germin-ation	Tissue	Starch	Protein		Lipid
			Total	Soluble	
0	Cotyledon	730.30±20.48	104.92±3.78	101.03±2.18	0.0005±0.001
	Axis	497.74±32.64	179.92±10.11	51.82±3.36	ND
2	Cotyledon	565.74±27.16	96.66±3.24	80.23±1.97	0.0036±0.001
	Axis	224.09±14.37	159.58±6.62	87.92±7.24	ND
5	Cotyledon	536.25±14.65	127.83±4.27	97.74±4.56	0.010±0.001
	Axis	166.94±13.41	131.30±7.73	38.55±1.51	ND
10	Cotyledon	566.66±21.08	103.85±6.03	76.28±1.72	0.034±0.001
	Axis	88.45±3.48	103.96±10.62	35.97±8.24	ND
20	Cotyledon	612.85±29.40	87.29±6.80	79.60±5.83	0.016±0.001
30	Cotyledon	682.13±25.42	85.80±5.85	56.61±4.41	0.023±0.001
40	Cotyledon	355.12±33.17	82.54±5.03	78.89±6.02	0.015±0.001
50	Cotyledon	339.25±27.47	60.16±5.26	42.92±4.42	0.016±0.001

ND: Not done because lipid was estimated in cotyledon using 5g tissue.

Figure: 28. Distribution of starch in Jackfruit seeds during germination.



The starch content in embryonic axis showed a very sharp decline on the 2nd day of germination (Table 14, Fig. 28) showing only less than half of control seeds. On 5th day of germination also starch content of axis was reduced significantly. But on 10th day the starch content in axis was reduced to half of the starch content of 5th day and this value registered about 80% reduction from the starch content of control seeds.

3.1.3. Sugars

HPLC study on the sugars of control seeds showed that the embryonic axis contained only glucose, fructose, sucrose and maltose and these values were higher than that of cotyledon. Almost same amounts of glucose and fructose were present in the embryonic axis and these quantities were about three times higher than the cotyledons. In addition to glucose and fructose, cotyledon showed the presence of another monosaccharide, rhamnose. The sucrose and maltose showed about five times and eight times increase respectively. (Table 15, Fig. 29, 30a) The total sugar content of control axis was about 15.4% whereas cotyledons contained only 5.2%. Raffinose and three unidentified sugars were present in the cotyledons.

On 5th day of germination, seeds showed that the monosaccharide contents in axis were increased to about four times than that of the control seeds and increased amount of rhamnose was also present (Table 15, Fig. 30a). But the disaccharides in the axis were reduced to half when compared to control. Like control axis, raffinose and unknown sugars were absent in the axis of this sample.

The sugar content of cotyledons on 5th day of germination showed the same amount of total sugars as that of control samples. The glucose and fructose were increased to about double the amount as that of control seeds (Table 15, Fig. 30b). Rhamnose was only slightly increased in this sample.

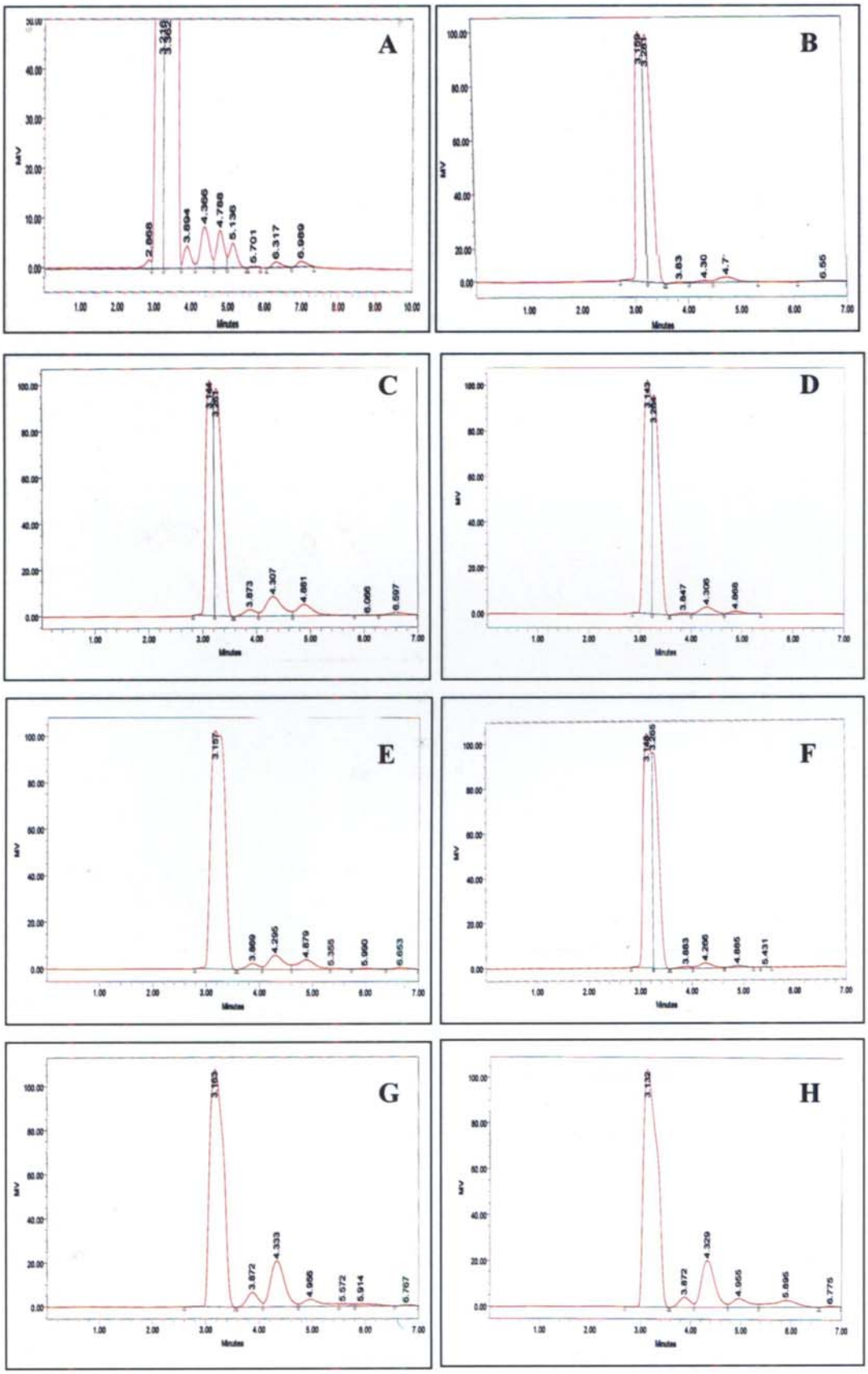
Table: 15. Distribution of sugars in Jackfruit seeds during germination / seedling growth mg g^{-1} dry tissue.

Sugars	Days of germination/ seedling growth							
	0		5		10		30	50
	Axis	Coty.	Axis	Coty.	Axis	Coty.	Cotyledon	Cotyledon
Rhamnose	-	6.73	42.08	7.96	110.51	7.69	20.15	15.68
Glucose	19.68	7.02	80.06	13.36	140.41	9.80	42.17	37.00
Fructose	22.71	7.66	90.60	14.90	158.48	10.89	47.10	41.58
Sucrose	52.99	10.22	24.16	8.32	39.62	7.86	9.45	8.76
Maltose	59.05	7.67	27.18	9.25	47.54	8.93	10.14	10.00
Raffinose	-	3.83	-	-	1.12	1.96	5.94	29.86
Unknown-I	-	6.39	-	-	-	-	7.25	-
Unknown-II	-	2.04	-	0.58	-	1.85	-	-
Unknown-III	-	2.06	-	3.90	-	2.20	1.62	1.36
Total	**154.43**	**53.61**	**264.08**	**58.17**	**497.76**	**51.16**	**143.82**	**144.21**

Table: 16. Amylase activity in Jackfruit seeds during germination/ seedling growth mg g^{-1} dry tissue.

Days of germi-nation	Tissue	Unit Activity		Specific Activity	
		pH- 5.3	pH-8.0	pH- 5.3	pH-8.0
0	Cotyledon	53.28±4.73	45.83±4.09	0.53±0.01	0.45±0.04
	Axis	198.87±19.74	138.98±12.59	3.84±0.38	3.39±0.24
2	Cotyledon	78.24±1.17	44.42±3.42	1.01±0.02	0.57±0.04
	Axis	361.51±25.38	310.82±17.59	4.11±0.62	3.53±0.43
5	Cotyledon	100.23±4.21	63.87±3.47	1.03±0.04	0.65±0.03
	Axis	152.50±8.22	85.92±8.94	3.95±0.21	2.23±0.35
10	Cotyledon	58.47±3.76	41.51±2.24	0.77±0.05	0.54±0.03
	Axis	123.02±10.51	25.01±2.96	3.42±0.04	0.69±0.01
20	Cotyledon	69.48±2.15	55.14±3.58	0.87±0.03	0.69±0.04
30	Cotyledon	74.60±3.22	43.80±2.46	1.16±0.06	0.77±0.04
40	Cotyledon	72.54±3.92	51.59±2.87	0.92±0.05	0.65±0.04
50	Cotyledon	78.80±2.63	41.75±4.03	1.84+0.06	0.97+0.09

Figure: 29. HPLC sugar profile of Jackfruit seeds during germination.

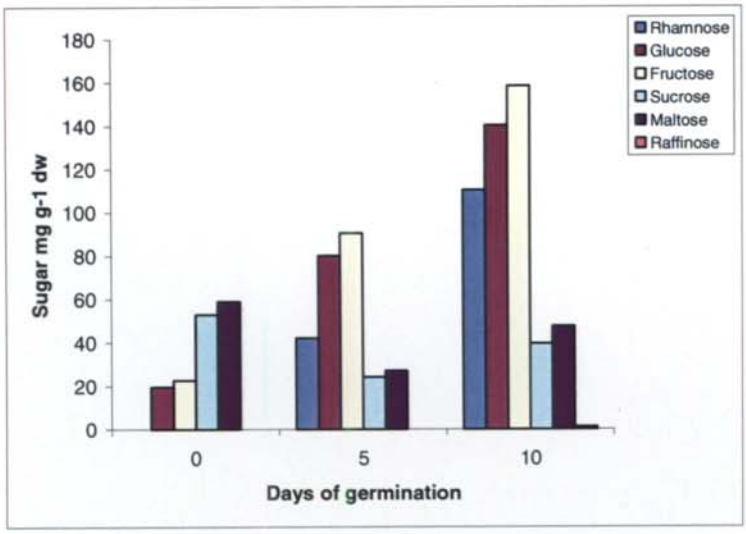


A, C, E, G,H - Cotyledon of control, 2nd, 10th , 30th, 50th day samples

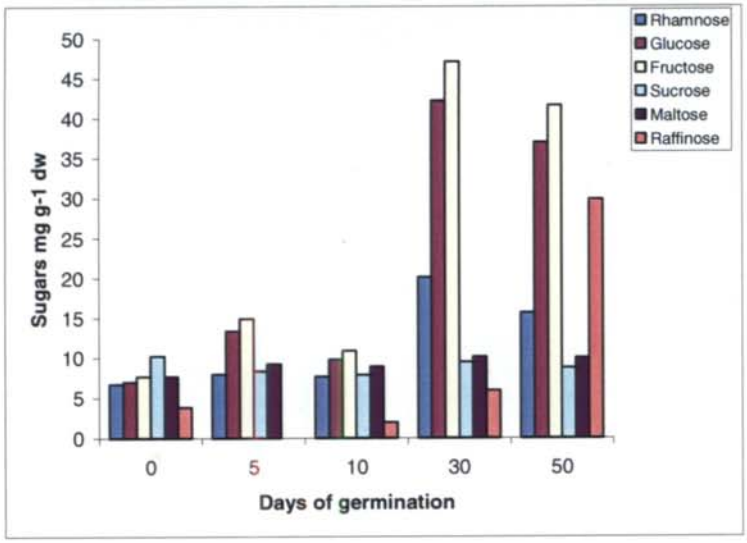
B, D, F - Axis of control, 2nd, 10th day samples

Figure: 30. Distribution of sugars in Jackfruit seeds during germination.

a) Axis tissue



b) Cotyledon tissue



But the sucrose was slightly reduced and maltose showed a slight increase than the control seeds. Raffinose was absent in the cotyledon on 5th day of germination. Cotyledon of this stage showed the presence of two unknown sugars (II and III).

On 10th day, the axis showed a marked increase in monosaccharides attaining the maximum amount of glucose and fructose which were about seven times than that of the control seeds (Table 15, Fig. 30a). Rhamnose was also increased. The sucrose and maltose were decreased than that of control seeds. Negligible quantity of raffinose was present in the axis. The total sugar content of the axis was very high about 49.7 percentage. This sample showed the maximum amount of monosaccharides, disaccharides, and total sugars compared to all other samples.

Glucose and fructose in the cotyledon of 10th day sample showed a slight decrease than the 5th day sample, but the rhamnose retained the same amount. Sucrose and maltose maintained almost the same quantities. Raffinose was present in negligible amount. Two unknown sugars were also present. The total sugar content was slightly reduced than the 5th day sample and was almost same as that of cotyledons of control seeds.

On 30th day of seedling growth, the cotyledon showed a more than four times increase in glucose and fructose contents than the 30th day sample (Table 15, Fig. 30b) reaching the maximum quantity in this sample but raffinose was same as that of 5th day sample. The sucrose and maltose showed a slight increase than the previous stage. The raffinose content was significantly increased. The total sugar content was increased to about 14.5%. Three unknown sugars were present in this sample.

In the cotyledon on 50th day of seedling growth, glucose and fructose were slightly reduced. Sucrose and maltose were almost same as that of the

previous stage and control seeds. The quantity of raffinose showed a significant increase which was maximum value in comparison with all other samples. Cotyledon showed the presence of two unknown sugars. The total sugar content was almost similar to that of the 30th day sample.

3.1.4.1. Unit Activity of Amylase

The cotyledon of control seeds showed 53.27 unit activity of β-amylase (pH-5.3) (Table 16, Fig. 31a). A 50% increase in activity was observed on 2nd day of germination. But on 5th day, 2 fold increase in activity was noticed compared to the control. The activity of β-amylase was reduced to half on 10th day. Thereafter the activity showed a gradual negligible increase throughout the germination period.

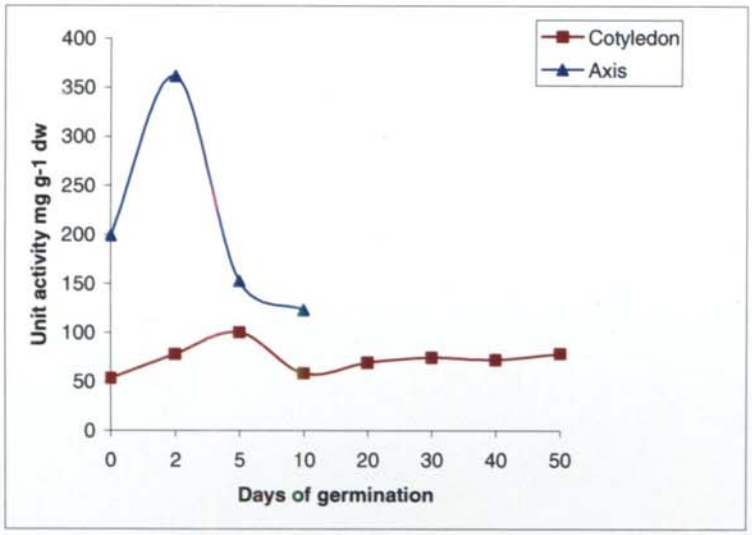
The β-amylase activity of control axis was about four times higher than that of cotyledon (Table 16, Fig. 31a). On 2nd day of germination, the activity was doubled compared to that of control axis. A sharp decline in the β-amylase activity was observed on the 5th day which was about less than half of the previous sample. But on 10th day the activity was slightly decreased.

The α-amylase (pH-8.0) activity of control cotyledon was slightly lower than that of β-amylase (pH-5.3) and the same activity was retained on the 2nd day also (Table 16, Fig. 31b). About 50% increase in activity was shown by cotyledon on 5th day and significant ($P<0.01$) reduction was observed on 10th day cotyledons. A slight significant increase ($P<0.01$) in activity was shown by cotyledon on 20th and 40th days, but a reduction was observed on 30th day.

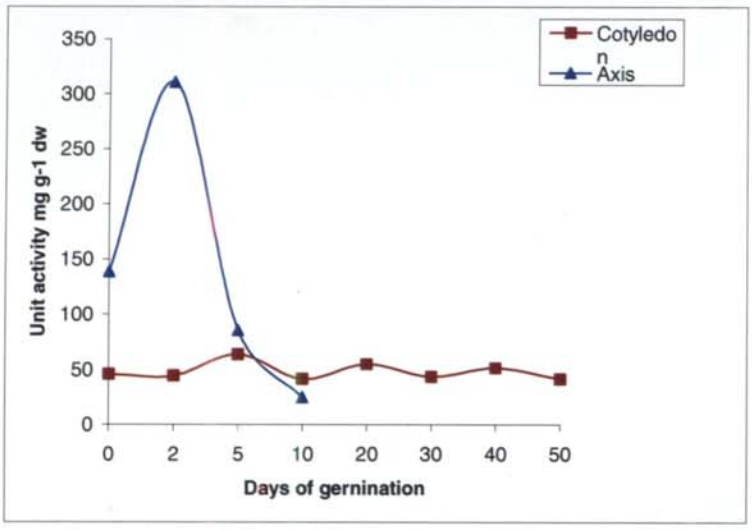
The unit activity of α-amylase (pH-8.0) in the control axis was lower than that of β-amylase (pH-5.3) (Table 16, Fig. 31b). On the second day of germination, the activity was sharply increased (more than double). A sharp

Figure: 31. Pattern of Amylase activity during germination of Jackfruit seeds.

a) At pH-5.3



b) At pH-8.0



decline in unit activity (one fourth) was observed in the axis on 5th day and it was significantly decreased on 10th day.

3.1.4.2. Specific Activity of Amylase

The specific activity of β-amylase in control cotyledon was 0.53. The activity was doubled on 2nd day (Table 16, Fig. 32a) and the same was maintained on 5th day also. The specific activity of this enzyme was decreased slightly ($P<0.01$) on 10th day and 20th day and increased significantly ($P< 0.01$) on 30th day. Thereafter it showed a decrease on 40th day but it was the highest on 50th day of seedling growth.

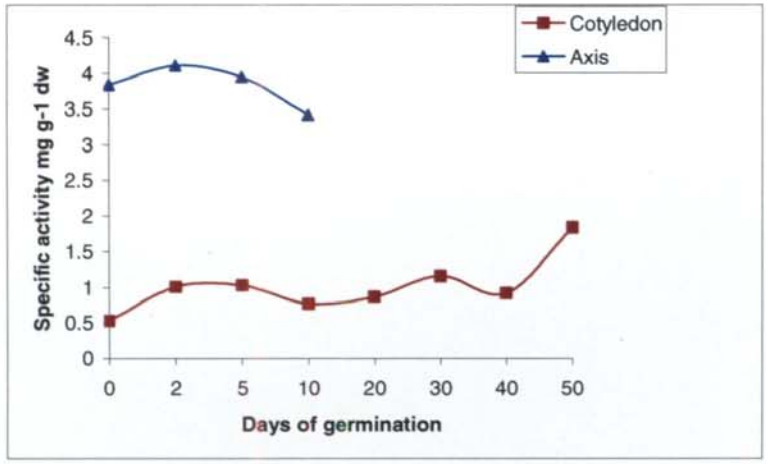
The control axis showed a very high specific activity of β-amylase than the cotyledon. On 2nd day, there was a slight increase in specific activity than control (Table 16, Fig. 32a). But on 5th day, the activity was almost same as that of the 2nd day sample. The activity was slightly decreased on 10th day.

The control cotyledon showed almost same α-amylase activity (pH-8) as that of β-amylase (pH-5.3). A slight insignificant increase in specific activity was observed on 2nd day (Table 16, Fig. 32b). On 5th day, the specific activity showed further insignificant increase. But on 10th day, the specific activity was reduced. Thereafter, it showed a gradual increase throughout the germination period. Maximum specific activity was shown by cotyledons on 50th day of seedling growth.

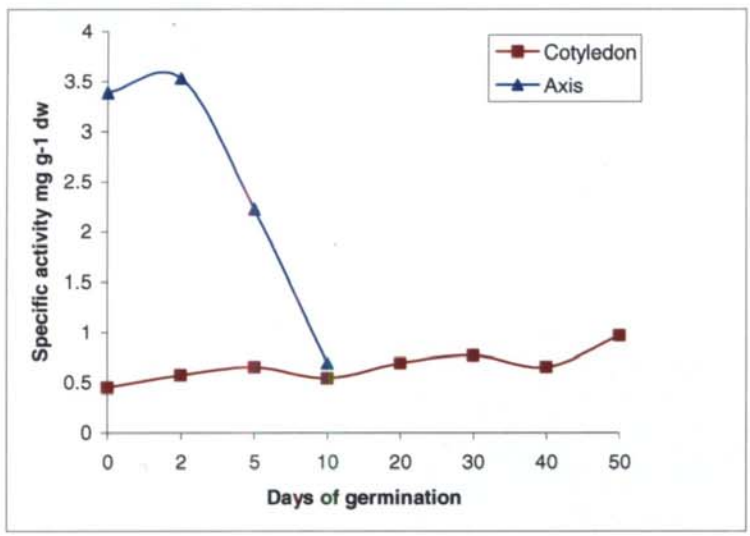
The specific activity of α-amylase in the control axis was almost same as that of β-amylase. The same specific activity was retained on 2nd day (Table 16, Fig. 32b). But on 5th day, it was insignificantly reduced than the 2nd day sample. A significant decrease in specific activity was observed in axis on 10th day.

Figure: 32. Pattern of Amylase activity during germination of Jackfruit seeds.

a) At pH-5.3



b) At pH-8.0



3.1.4. Total Protein

The control seeds showed only about 10% protein content in the cotyledons (Table 14, Fig. 33a). The total protein content was slightly decreased insignificantly on the 2nd day of germination. But on 5th day about 20% increase in total protein content was observed. The seeds on 10th day showed further reduction and after 10th day there was a sharp decline of total protein content in cotyledon and it continued upto 50 days.

The axis of control seeds showed about 18% of protein content (Table 14, Fig. 33a). Only a slight reduction occurred on 2nd and 5th days and it showed a gradual decline during the 10 days. On the 10th day of seedling growth, the total protein content was same in cotyledon and axis and both showed the same content as that of control cotyledon.

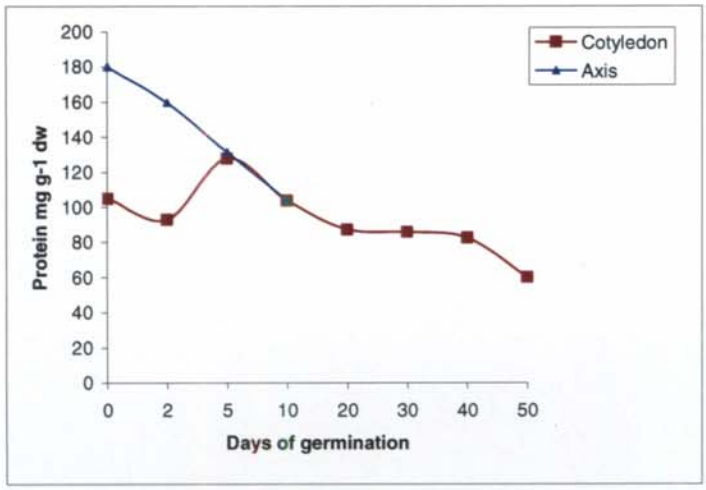
3.1.4.1. Soluble Protein

The soluble protein content in control seed cotyledon was same as that of total protein content (Table 14, Fig. 33b). The soluble protein content showed a gradual decrease throughout the germination period. The soluble protein content showed a significant ($P<0.01$) reduction on 2nd day of germination but it was increased by 5th day and again decreased on 10th day. Almost the same soluble protein content was present in the cotyledon upto 20 days, but again slightly increased on 40th day. On 50th day, only less than half of the protein content was retained when compared to the control. The soluble protein content was minimum on 50th day of seedling growth.

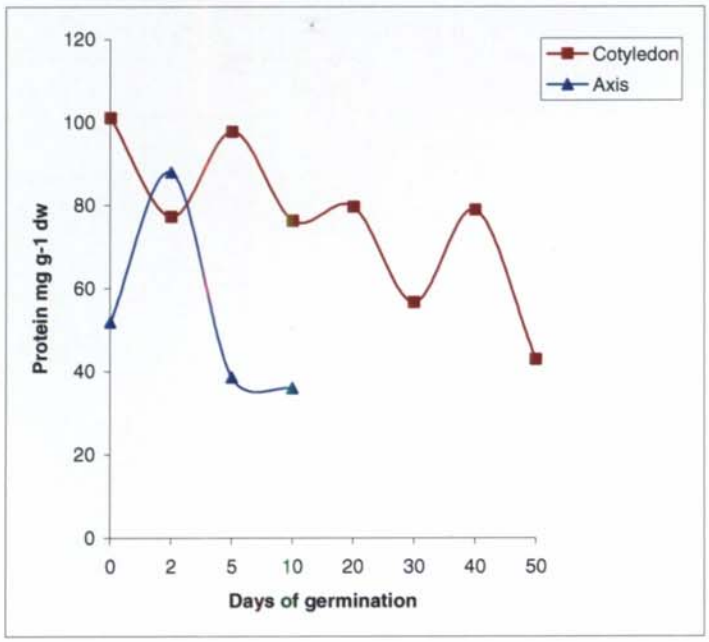
The axis showed about 5% of soluble protein which was only half the value as that of control cotyledons (Table 14, Fig. 33b). A significant increase was observed on the 2nd day of germination and was the maximum value of soluble protein in the axis. A sharp decline in soluble protein was observed on 5th day axis and the reduction continued

Figure: 33. Distribution of Protein content in Jackfruit seeds during germination.

a) Total Protein



b) Soluble protein



3.1.6. Lipid content

Lipid content of control seeds was very negligible (Table 14). There was a slight increase in lipid content during germination of seeds upto 10^{th} day. After that there was a slight decrease and maintained the same lipid content throughout the germination period.

3.2. Histochemical Studies

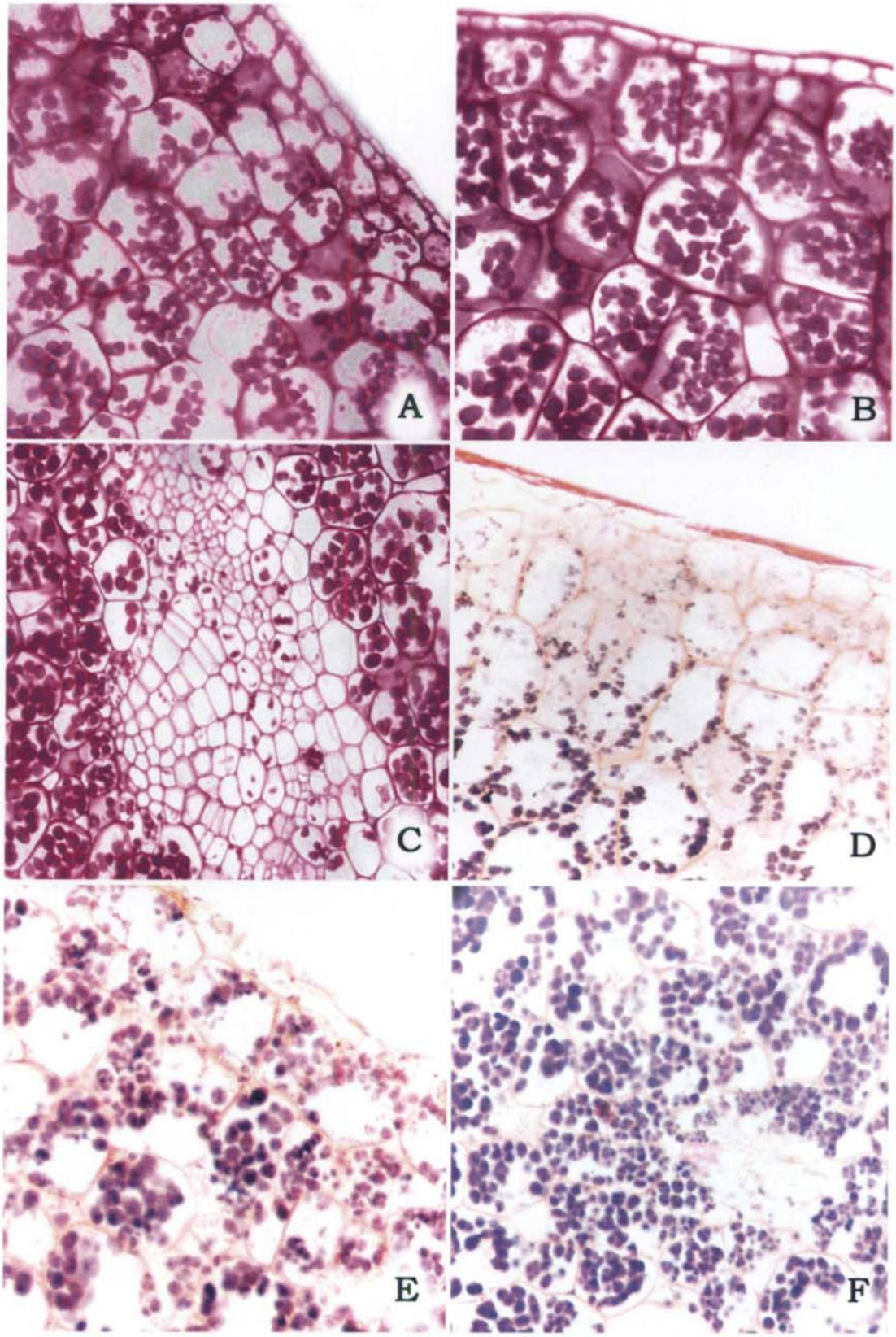
3.2.1. Localisation of Starch

Localisation of starch by PAS and safranin in combination with I_2 KI staining of cotyledon have shown that on 2^{nd} day of germination, the epidermal layer and the immediate layers on adaxial side were almost similar to that of cells in adaxial side of control cotyledon (Fig. 34, A, D). The cells were larger than the control cotyledon cells. A slight reduction in number of starch grains was observed.

The distribution of starch grains in the cells in abaxial side was similar to that of the adaxial side (Fig. 34, A, D). About 21 to 34 vascular bundles were present in the cross section of cotyledon on the 2^{nd} day of germination. The central vascular bundle was large and fan shaped. The starch grains in the parenchyma cells near vascular bundles were smaller in size. In the centre region of cotyledon, the cells were filled with starch grains (Fig. 34, C, F).

A rapid reduction of starch grains was observed in the cotyledon on 10^{th} day of germination. The epidermal cells on the adaxial side maintained the original size. The hypodermal cells and the parenchyma cells were about 3 to four times larger than the cotyledon cells of the control seeds (Fig. 35, A, D). The hypodermal cells were devoid of any starch grains. Starch grains of the inner layers were also declined.

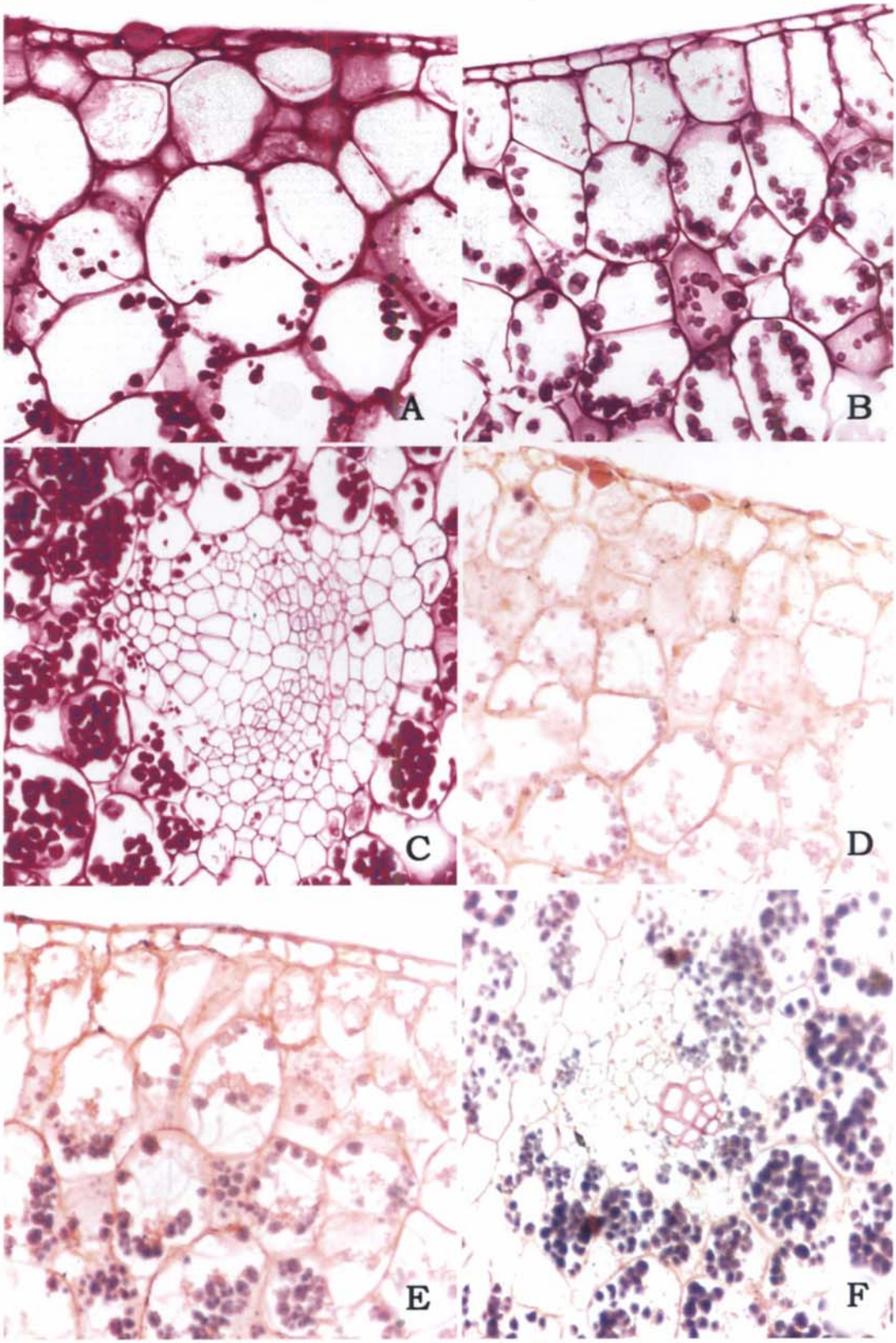
Figure: 34. Distribution of starch grains in the cotyledon of Jackfruit seeds on 2nd day of germination.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

Figure : 35. Distribution of starch grains in the cotyledon of Jackfruit seeds on 10thday of germination.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

In the abaxial side, the hypodermal and inner cells were enlarged. Starch grains of the hypodermal cells were disappeared but present in the cells inner to the hypodermis (Fig. 35, B, E).

Cross section of the cotyledon of 10^{th} day sample showed about 40 vascular bundles of different sizes. The central one was fan shaped. The parenchyma cells near the vascular bundles contained smaller starch grains (Fig. 35, C, F). The number and size of starch grains in the parenchyma cells in the centre region were reduced.

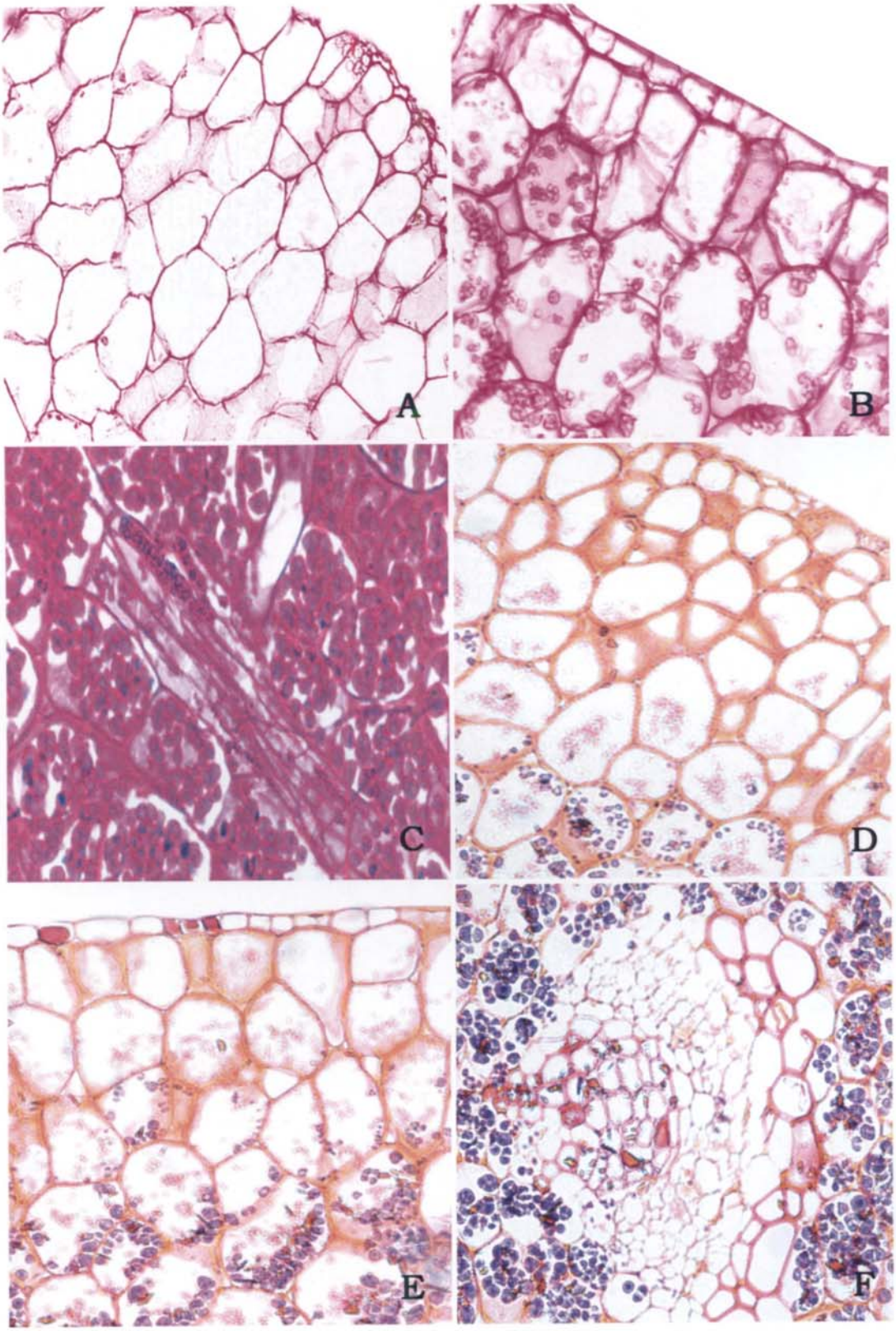
On 30^{th} day of seedling growth, the epidermal cells in the adaxial side were similar to that of the control seeds. Inner to epidermis, the cells in about 7-8 layers were devoid of any starch grains. Cells of central region contained few smaller starch grains (Fig. 36, A, D).

In the abaxial side, the cells of hypodermal and other layers were devoid of starch grains but present in cells of inner layers (Fig. 36, B, E). Cells in inner layers contained very few (3-4) small starch grains. The epidermal cells were enlarged.

Parenchyma cells in the centre region contained more number of starch grains which showed a slight reduction in number and size compared to the 10^{th} day sample. The starch grains in the parenchyma cells near vascular bundles were small (Fig. 36, C, F). The number of vascular bundles was almost similar to that of the 10^{th} day sample but the central fan shaped vascular bundle was much larger and cells were more differentiated.

On 50^{th} day of seedling growth, 10-12 layers of cells inner to the epidermis on adaxial side were devoid of any starch grains and other contents. These cells were almost empty (Fig. 37, A, D). The changes in abaxial side were similar to that in adaxial side. The epidermal cells of the abaxial side showed that some of them were enlarged to form epidermal hairs (Fig. 37, B, E). Starch grains were present only in cells near the vascular bundle. The

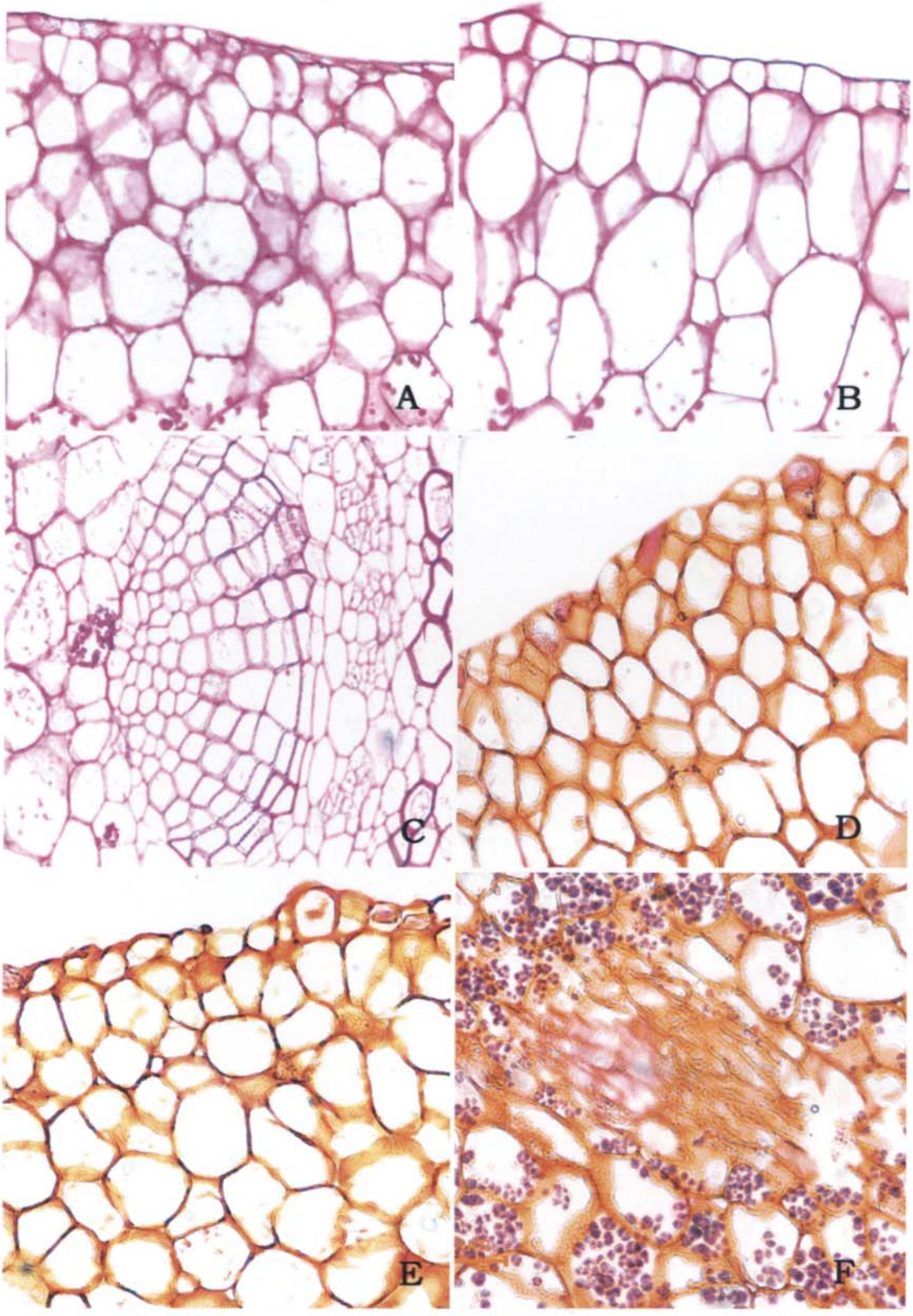
Figure: 36. Distribution of starch grains in the cotyledon of Jackfruit seeds on 30th day of seedling growth.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

Figure: 37. Distribution of starch grains in the cotyledon of Jackfruit seeds on 50th day of seedling growth.



A. Adaxial side
B. Abaxial side
C. Centre
} PAS

D. Adaxial side
E. Abaxial side
F. Centre
} Safranin I_2KI

number and size of the starch grains were very much reduced containing only 10 to 15 very small grains. In this stage the cotyledon contained starch grains only in the parenchyma cells nearer to the vascular bundles (Fig. 37, C, F).

3.2.2. Localisation of protein

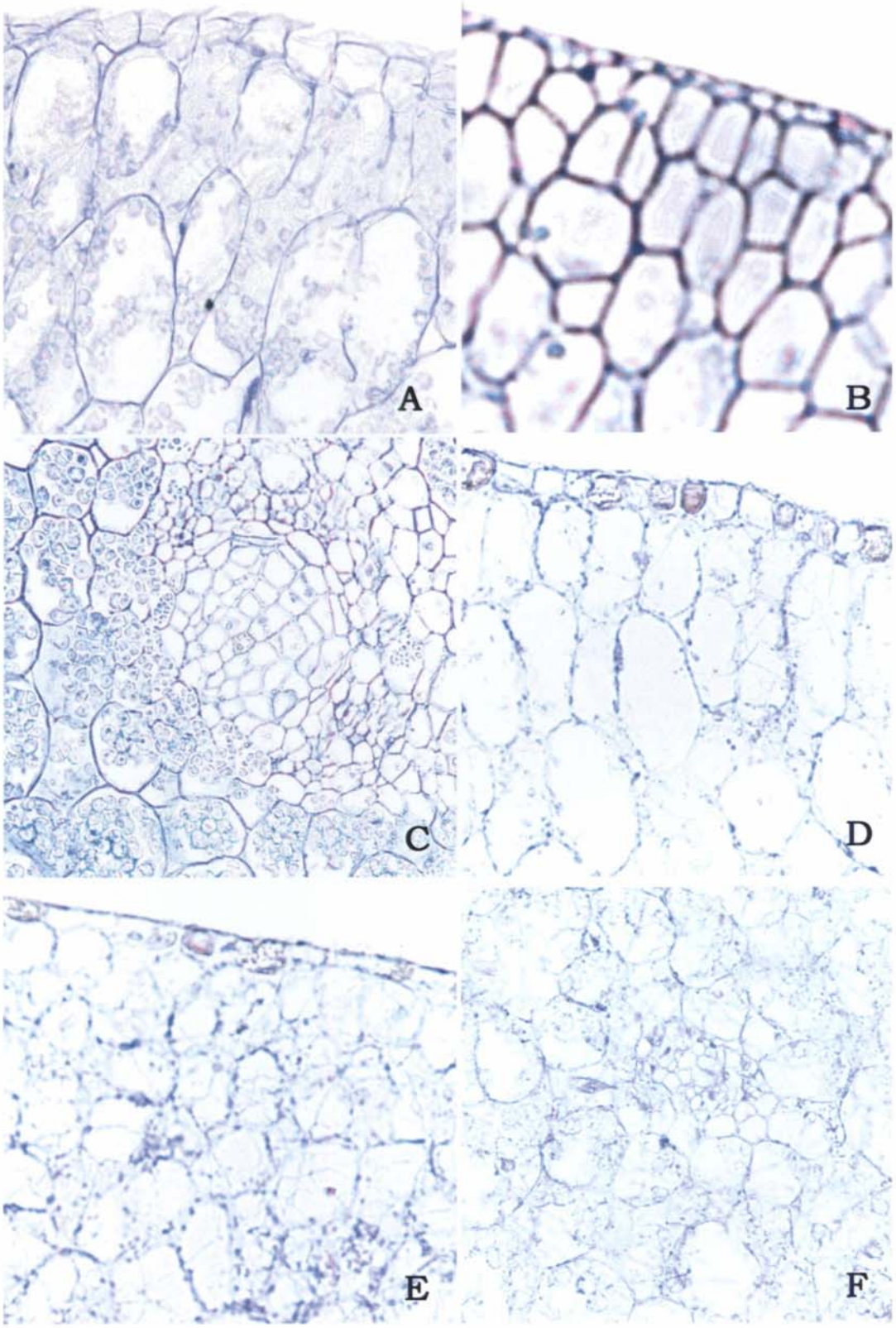
Sections of cotyledon of control and germinated seeds were stained with mercuric bromophenol blue. Protein appeared as blue mass and starch grains remained unstained and appeared as hyaline bodies

The protein in cotyledon of control seeds appeared as blue scattered mass in the cells. The starch grains were hyaline and the grains near the vascular bundles showed a reddish spot in the centre. The nucleus in the cells appeared red in colour. There was no much difference in the protein content in the adaxial and abaxial side of the cotyledon. On 2^{nd} day of germination, cells on the adaxial side showed the beginning of the receding of stained mass (protoplast) from the cell wall along with the contents and found localized to about half of the cell lumen (Fig. 38, A). But in the cells of abaxial side, the cells were filled with stained protoplast consisting of protein and hyaline starch grains. (Fig. 38, B). The cells in the centre region of the cotyledon were filled with the starch grains and protein mass. Fan shaped vascular bundle was visible in the centre (Fig. 38, C) and the cells surrounding it contained more protein content and nucleus was also visible in these cells.

Cotyledon of the 10^{th} day sample showed much change in protein content than the earlier sample. The cells on the adaxial side (Fig. 38, D) were with less protein content than the earlier sample. The decrease in protein content was also shown by the cells in the abaxial side (Fig. 38, E). The cells in the centre region (Fig. 38, F) also showed signs of disappearance of its contents and so were not completely filled with the contents.

In the cotyledon of the 30^{th} day sample, 4-6 layers of cells in the adaxial side were without the protein contents (Fig. 39, A). But traces of

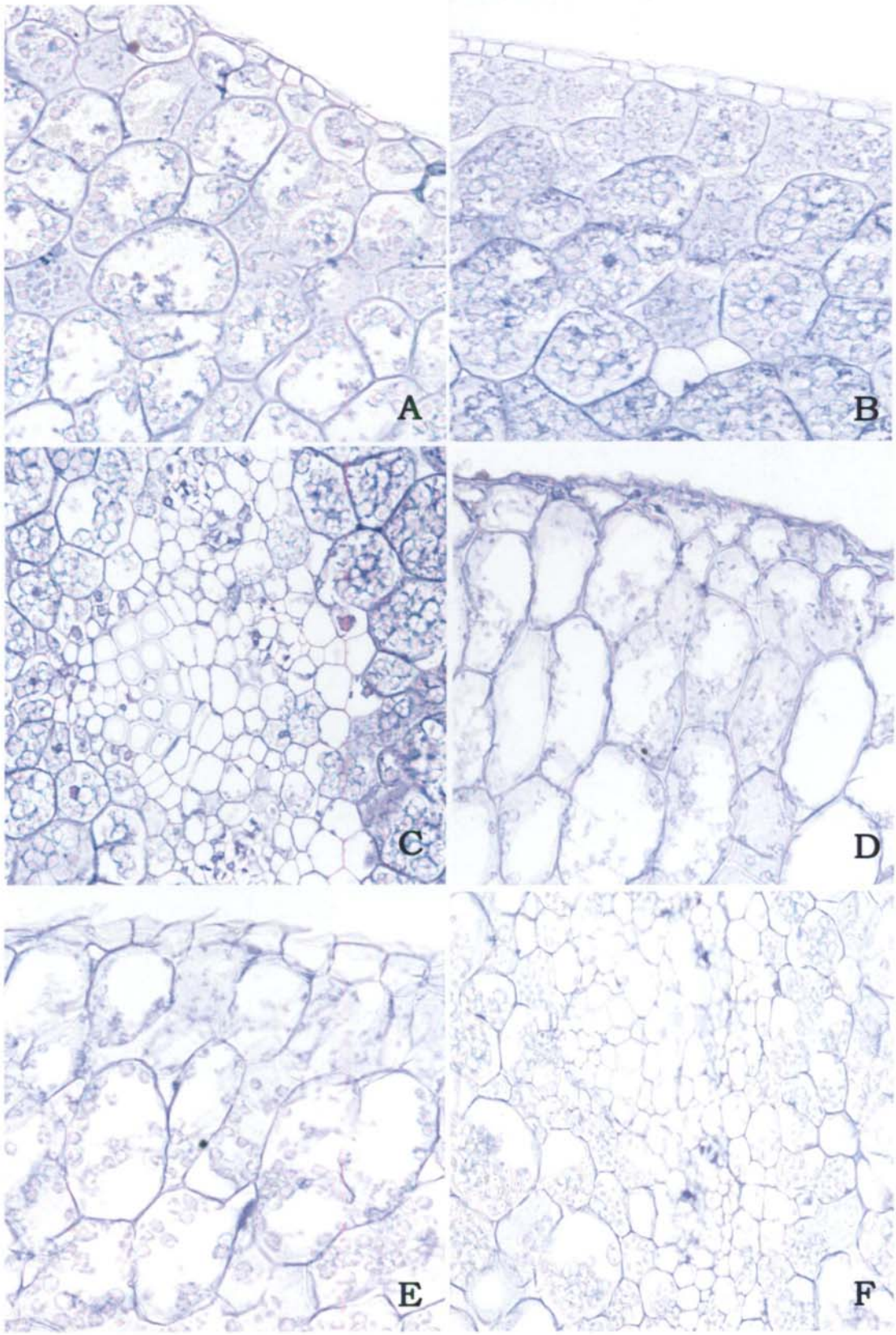
Figure: 39. Distribution of protein in the cotyledon of Jackfruit seeds on 30th and 50th day of seedling growth.



A. Adaxial side
B. Abaxial side
C. Centre
} 30th day

D. Adaxial side
E. Abaxial side
F. Centre
} 50th day

Figure: 38. Distribution of protein in the cotyledon of Jackfruit seeds on 2nd and 10th day of germination



A. Adaxial side
B. Abaxial side
C. Centre
} 2nd day

D. Adaxial side
E. Abaxial side
F. Centre
} 10th day

protein contents were present in the cells of abaxial side (Fig. 39, B). The cells in the centre region (Fig. 39, C) were almost similar to that in the 10^{th} day sample.

The protein content was almost declined in the cotyledon of seeds on 50^{th} day of seedling growth. The cells near epidermis of the adaxial side showed disappearance of protein mass along with the starch grains (Fig. 39, D). Three to four layers of cells inner to epidermis were almost empty. The inner cells showed reduced protein content. In the abaxial side, 5 to 6 layers of cells inner to epidermis were without protein contents (Fig. 39, E). In the centre region, almost all cells possessed protein contents (Fig. 39, F). Nucleus was also visible in these cells. A slight increase in protein content was visible in the cells near vascular bundles.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON JACKFRUIT SEEDS (*Artocarpus heterophyllus Lam.*) DURING STORAGE AND GERMINATION

Thesis
submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT

2007

Discussion

High moisture content maintenance at ambient temperature is necessary for retention of viability in recalcitrant seeds. As recalcitrant seeds are shed from parent plant at high moisture content, they are highly sensitive to desiccation (Chin *et al.*, 1981, 1984; Chin, 1988; Pammenter *et al.*, 1994; Farrant *et al.*, 1993; Chaitanya *et al.*, 2000 a, b; Greggains *et al.*, 2001). Hence life span of recalcitrant seeds is very short which varies from a few days to few months. Almost all studies on recalcitrant seeds invariably revealed that distribution of moisture content and life span (storability) of recalcitrant seeds are directly correlated.

Fresh seeds of ripened Jackfruit (*Artocarpus heterophyllus*) contain 50% moisture content. The viability percentage is getting reduced to less than 100 percentage during a period of 12 days open-air storage at room temperature (30±3°C) and the corresponding moisture content is in the range of 33 to 41% (Table 1, Fig. 1). These observations are suggestive of the highly recalcitrant nature of Jackfruit seeds whereas Chin *et al.*, (1984) reported that *Artocarpus heterophyllus* seeds lose viability when their moisture content decreases from 53% to 43%. Chandel *et al.*, (1995) suggested that embryonic axis of partially mature and mature Jackfruit seeds

retains viability when desiccated to moisture levels of 14% and 7% respectively. Smith *et al.*, (2001) reported that rapid drying of embryonic axes of *Artocarpus heterophyllus* seeds to water content 0.4g g^{-1} dry weight can retain cent percent viability and this view is in accordance with the findings of the present investigation.

Critical moisture content is the moisture content at which the seed viability is lost which varies from species to species (Corbineau and Come 1988; Farrant *et al.*, 1993; Pritchard *et al.*, 1995; Cunha *et al.*, 1995; Bonner, 1996 b; Connor and Bonner, 1998; Danthu *et al.*, 2000; Malik *et al.*, 2005). A linear correlation between moisture content and viability up to the attainment of critical moisture content is shown by Jackfruit seeds (Fig. 1), afterwards, there is a sharp fall in the viability but the concomitant loss of moisture content is insignificant. Desiccation sensitive seeds are characterized by a linear relationship between viability percentage and moisture content as reported in many recalcitrant seeds such as *Aesculus hippocastanum* (Tompsett and Pritchard, 1993; Pritchard *et al.*, 1996), *Inga* (Pritchard *et al.*, 1995; Faria *et al.*, 2004), *Machilus thunbergii* (Lin and Chen, 1995), *Quercus nigra* (Bonner, 1996 b), *Guarea guidonia* (Connor and Bonner, 1998) *Boscia senegalensis, Butyrospermum parkii, Cordyla pinnata* and *Saba senegalensis* (Danthu *et al.*, 2000), *Shorea robusta* (Chaitanya *et al* ., 2000 a, b), *Syzygium aromaticum* (Anilkumar *et al.*, 2000), *Avicennia marina* (Greggains *et al.*, 2001) *Avicennia alba* (Le Tam *et al.*, 2004) three species of *Garcinia* (Malik *et al.*, 2005).

Eventhough Jackfruit seeds come under highly recalcitrant category due to their high moisture content and short life span (Table 1), about 10 percent reduction in moisture content is not imposing any adverse effect on viability since 100% germination is retained upto 10 days when the moisture content is reduced to 41%. But further 5% reduction of moisture content

within 2 days after 10^{th} day resulted in significant reduction of viability. Studies on critical moisture content of recalcitrant seeds have shown that highly recalcitrant seeds which contain above 45-50% moisture content lose their viability when the moisture content reduces to 20-30% (Tompsett and Pritchard, 1993; Lin and Chen, 1995; Connor and Bonner, 1998; Danthu *et al.*, 2000; Chaitanya *et al.*, 2000 a, b; Anilkumar, *et al.*, 1998, 2000; Le Tam *et al.*, 2004 and Malik *et al.*, 2005). Critical moisture content of Jackfruit seeds comes under this range due to their highly recalcitrant behaviour. Greggains *et al.*, (2001) found an exceptional case, that *Avicennea marina* seeds containing 65% moisture content lost viability when the moisture content was reduced to below 60% and at 47% moisture content, all seeds became nonviable. It has been well established that recalcitrant seeds are metabolically active as they are shed with high moisture content (Chin *et* al., 1981, 1984; Pammenter *et al.*, 1994) and desiccation causes damage due to aberrant metabolic processes as these seeds are in highly hydrated conditions (Pammenter *et al.*, 1994, Pammenter and Berjak, 1999).

Many studies on recalcitrant seeds have shown that positive correlation do exist between moisture content and viability but the range of moisture content varies from plant to plant (Chin *et al.*,1981; Hor *et al.*,1984; Nautiyal and Purohit, 1985 a). According to Nautiyal and Purohit (1985 a) loss of viability in Sal (*Shorea robusta*) seeds during desiccation is due to loss of membrane integrity and it is reported that loss of moisture content causes increased permeability of cell membranes which ultimately allows cellular constituents to leach out during rehydration process of germination.

The response of recalcitrant seeds to desiccation depends not only on inherent characteristics of the species but also in the developmental status of the seed and condition under which the seeds are dried. Freshly harvested Jackfruit seeds with 50% moisture content show sensitivity towards

desiccation only when the moisture content is reduced to 33% during a period of 12 days. So the desiccation sensitivity is based on the water potential equivalents to 33% moisture content i.e. the seeds become intolerant to desiccation at this level. According to Vertucci and Leopold (1986), Vertucci *et al.,* (1994) desiccation sensitivity of recalcitrant seeds varies from species to species and the principles are firmly based on the properties of water in the seeds at the various hydration levels corresponding to specific water potential ranges and on the physiological status of the seeds.

The mode of rehydration phase is very important in assessing the response of recalcitrant seeds to desiccation (Leprince *et al.*, 1998). Those authors showed that coalescence of oil bodies in the cotyledons of recalcitrant Cocoa seeds occurred during reimbibition on damp filter paper, rather than drying. It is suggested that the effect of rehydration rate has a similar basis to the effect of drying rate and during a low rehydration the tissue spends extended time periods, at intermediate water contents, permitting further damage to occur, whereas rapid rehydration by plunging into liquid water prevents or slows the accumulation of damage (Peran *et al.,* 2004). In Jackfruit seeds, desiccation under room open condition (not under controlled relative humidity) shows short storability probably due to rapid dehydration and slow rehydration as the seeds are placed in between moist filter paper for germination. This behavior of Jackfruit seeds is in conformity with the view of Peran *et al.*, (2004).

Eventhough desiccation sensitivity is the most studied aspect of recalcitrant seeds, metabolic aspects such as distribution of metabolites and enzyme activity during desiccation are not much known.

Amylase activity of Jackfruit seeds showed two peaks at pH-5.3 and 8 indicating the occurrence of β- and α-amylases respectively in control, stored and desiccated seeds. More activity is at pH-5.3 compared to pH-8.0 (Table 6,

Fig. 6). Enhanced activity of β-amylase compared to α-form in recalcitrant Jackfruit seeds during desiccation seems to be unique because enzymic changes of carbohydrate metabolism of recalcitrant seeds have not yet been investigated. According to Bewley and Black (1994), β-amylase is present in starch rich seeds and become active during germination, but can act only on the products of α-amylase which is synthesized during germination. β-amylase cannot hydrolyse native starch grains, but it cleaves successive maltose molecules from the non reducing ends of large oligomers released by prior α-amylase attack. In seeds of Jackfruit, α-amylase activity in the control sample indicates the degradation of starch by α-amylase which is found to be constitutive since it is active in fresh seeds resulting in the formation of large oligomers on which the β-amylase is acting during germination-associated metabolisms in the seeds since the metabolism of seeds when shed from the mother plant is in continuum with germination in recalcitrant seeds. During the short life span of Jackfruit seeds under open air condition, gradual increase of both α- and β-amylases having maximum activity on 12^{th} day of desiccation (Table 6, Fig. 6) resulting in a concomitant reduction of starch content was observed. All these changes are coincided with the initiation of viability loss i.e. the seeds become intolerant to desiccation as the viability is reduced from cent percent. Nevertheless, considerable activity of amylase at both pHs is observed in seeds showing enhanced rate of viability loss and so also starch depletion (Table 4, 6; Fig. 4a, 6). Similarly sugars also exhibit significant qualitative and quantitative variations in their distribution during desiccation. During viable period i.e. upto 12^{th} day, gradual increase of sugars occurred and glucose and fructose are comparatively high (Table 5; Fig. 4b). Afterwards abundant occurrence of rhamnose, glucose, fructose, sucrose and maltose indicates impaired utilization due to reduced respiration in desiccated seeds (Nautiyal and Purohit, 1985; Vertucci, 1989; Leprince *et al.*, 1999). Notwithstanding, the significant accumulation of maltose on 12^{th} day can be

correlated to the protective mechanism rendered by this disaccharide against desiccation induced membrane damage. According to Kaplan and Guy (2004) amylase induction and the resultant maltose accumulation may function as a compatible-solute stabilizing factor in the chloroplast starch of (*Arabidopsis* variety *Columbia*) in response to acute temperature stress and maltose has the ability to protect protein, membranes, and phototsynthetic electron transport chain at physiologically relevant concentrations.

Distribution of protein also varied and correlated to desiccation induced viability loss in Jackfruit seeds. Significant depletion of total and soluble protein coincides with desiccation induced viability loss of seeds after 12 days of desiccation (Table 1, 4; Fig. 1, 7). Peak values of both α- and β-amylases activity and maximum protein content shown by Jackfruit seeds on 12^{th} day indicate synthesis or activation of more enzyme in viable seeds whereas in desiccated non viable seeds, despite the depleted protein content, amylase is very active (Table 6; Fig. 6).

When desiccation induced viability loss was initiated on 12^{th} day (desiccated-viable) of desiccation, both α- and β-amylases showed maximum activity. But in desiccated (non-viable) seeds also, these enzymes are more active than the control seeds. This observation is presumably due to the stability of enzyme protein already synthesized during viable period because impaired protein synthesis has been reported in desiccating seeds due to dehydration induced decline in RNAse activity and transcription and translation processes of nucleic acids (Chaitanya *et al.*, 2001).

Interaction of raffinose family of oligosaccharides in the manifestation of desiccation tolerance has been put forwarded and accepted by many investigators (Koster and Leopold, 1988; Bernal-Lugo and Leopold, 1992, 1995; Blackman *et al.*, 1992; Steadman *et al.*, 1996). Sucrose is getting reduced though not gradually during the entire period of desiccation and

culminates in the highest amount when seed viability is lost (Table 1, 5; Fig. 1, 4b). Similarly monosaccharides are increased proportional to the viability period (Fig. 4b). The presence rather involvement of sucrose as a factor contributing to desiccation tolerance in seeds has been reported (Koster and Leopold, 1988; Black *et al.*, 1996, 1999; Buitink *et al.*, 2000). Similarly role of sucrose in inducing desiccation tolerance has been established by culturing isolated protoplasts of embryonic tissue of pea seeds in medium supplemented with sucrose / raffinose to enhance desiccation tolerance (Halperin and Koster, 2006). Distribution of monosaccharides in desiccating seeds of Jackfruit is comparable with the behavior of germinating pea, soybean and maize embryos having tolerance towards desiccation and increased concentration of monosaccharides (Koster and Leopold, 1988).

The role of raffinose, maltose and one unidentified oligosaccharide (I) in the induction of slight desiccation tolerance after 8 days (Fig. 4 b) cannot be ruled out in Jackfruit seeds because considerable increase of these sugars occurs during this period compared to the control. Given the enhanced activity of amylases after 8 days of desiccation, resultant maltose accumulation is not evident. However more raffinose compared to the control may be due to the utilization of maltose for sucrose synthesis and the sucrose is acted upon by the sucrose synthetase (Keller and Pharr, 1996) resulting in the formation of galactosyl sucrose (raffinose) which is the important carbohydrate playing a role in the desiccation tolerance. In desiccated (non-viable) seeds, these sugars except maltose are significantly reduced. In desiccated (non-viable) seeds, maltose is significantly accumulated compared to viable seeds due to the stable activity of amylases and lack of hydrolysis to form monosaccharides or any other catabolic reactions. The maltose accumulation is only due to enhanced α- and β-amylases activity as already discussed earlier but no protective role can be attributed to this maltose accumulation since the seeds are non-viable.

In orthodox seeds, accumulation of non-reducing sugars is shown to be associated with acquisition of desiccation tolerance (Leprince *et al.*, 1999; Blackman *et al.*, 1992; Black *et al.*, 1999; Corbineau *et al.*, 2000). Oligosaccharides facilitate the formation of aqueous glasses (Koster and Leopold, 1988) or substitute for water, there by preventing phase transition in the lipid bilayer (Crowe *et al.*, 1992). In Pea seeds, acquisition of desiccation tolerance was coincident with an accumulation of raffinose and stachyose and desiccation tolerant seeds are characterised by a high amount of sucrose and a low content of monosaccharides (Bailly *et al.*, 2001).

Accumulation of sugar that stabilize membranes and other cellular structures have been proposed to play an important role in conferring desiccation tolerance and storability in seeds. Desiccation tolerance mechanisms in maturing orthodox seeds such as *Zea mays* (Bernal-Lugo and Leopold, 1995; Brenac *et al.*, 1997), *Triticum aestivum* (Black *et al.*, 1999), *Phaseolus vulgaris* (Bailly *et al.*, 2001) and in germinating orthodox seeds like *Glycine max*, *Zea mays* and *Pisum sativum* (Koster and Leopold, 1988) 19 species of various families (Horbowicz and Obendorf, 1994), *Brassica oleracea* (Hoekstra *et al.*, 1994) 18 orthodox species (Steadman *et al*, 1996) *Impatiens walleriana* and *Capsicum annum* (Buitink *et al.*, 2000) has been explained and many of the views are contradictory. Nevertheless, role of sucrose and raffinose family of oligosaccharides in various combination rather ratios, put forth by many investigators include ratio of sucrose: raffinose showing progressive decline with increased storage (Bernal-Lugo and Leopold, 1995) oligosaccharide: sucrose ratio, varies between orthodox, intermediate and recalcitrant seeds (Steadman *et al.,* 1996), oligosaccharide: total sugar coming between 0-0.7 related to longevity of seeds (Buitink *et al.*, 2000) values of sucrose : raffinose ratio less than 20 : 1 in desiccation tolerant seeds (Brenac *et al.*, 1997). However, the ratio proposed by Koster and Leopold (1988) is comparable and agreeable to the distribution pattern of

soluble sugars in Jackfruit seeds, when the seeds show desiccation sensitivity. Oligosaccharides are comparatively very low in control and seeds desiccated upto 8th day with an enhancement during 12th day when the seeds are on the verge of viability loss. On 14th day, sucrose and comparatively more raffinose help to confer desiccation tolerance to Jackfruit seeds and afterwards, the raffinose is reduced significantly. According to Koster and Leopold (1988), loss of desiccation tolerance is coincident with loss of oligosaccharides: sucrose, is less in desiccation sensitive seeds. On 12th day the ratio is 0.4: 1 in Jackfruit seeds and in these seeds, desiccation tolerance is only to a very limited extent and the ratio is decreased further on 14th day and is due to the desiccation sensitivity of Jackfruit seeds. Association between desiccation tolerance and higher raffinose family of oligosaccharide content has been described in *Vicia faba* (Lahuta *et al.*, 2000) *Acer* species (Pukacka and Wojkiewicz, 2002) and Lupin seeds (Pinheiro *et al.*, 2005).

Desiccation of Jackfruit seeds under room-open condition can be equated with maturation drying of orthodox seeds on the mother plant. According to Corbineau *et al.*, (2000) the oligosaccharide biosynthesis is regulated by the rate of water loss. The slower the dehydration, higher the oligosaccharide content in pea seeds. Sucrose and oligosaccharides (raffinose family) are involved in the stabilization of membranes during dehydration or impairing glass formation (Vertucci and Farrant, 1995).

Histochemical localization of starch in the small cotyledon of Jackfruit seeds (control) showed abundant occurrence of starch grains around the nucleus of the cells indicating metabolically active state of seed despite their storage function (Fig. 9, A-F). Eventhough cent percent viability is shown by seeds on 8th day and slightly lowered on 12th day, distribution of starch grains exhibited slight reduction on 12th day (Fig. 10, A-F). An important cellular character associated with desiccation tolerance in plant tissue is conversion of

soluble metabolites to insoluble in order to maintain osmoticum and the insoluble metabolites are stored in vacuoles (Oliver and Bewely, 1997). Contradictory to the view of Oliver and Bewely (1997) in seeds of Jackfruit starch grains (insoluble) are depleted as the seeds became desiccation intolerant. Starch grain disappearance from the cotyledon during desiccation can also be correlated to germination-associated metabolic changes (Pammenter *et al.*, 1994) occurring in Jackfruit seeds until they become nonviable. Slight shrinkage of cells was observed in the cotyledon of non-viable seeds. Starch grains in the cells of adaxial side of cotyledon of these samples were almost disappeared. These observations clearly indicate that desiccation effect of open air drying to which external surface of cotyledon (adaxial side) is directly exposed. Reduction and / or disappearance of starch grains can be correlated to mobilisation during the early period of desiccation which is almost similar to the germination-associated changes whereas shrinkage of cells and losing their integrity is related to the desiccation induced damages of cells in which the cell inclusions along with nuclei are disappeared (Fig-13). Desiccation associated changes at ultrastructural levels have been described in *Hevea braziliensis* (Chin *et al.*, 1981). Cells of *Hevea* seeds during desiccation, showed absence of distinctive nucleus and damaged cell membranes in the axis due to the detrimental effect of drying.

An interesting observation of histochemical localisation of insoluble polysaccharides along with the disappearance of starch from the adaxial side of cotyledonary cells is disintegration of cell wall in the sample of 16^{th} day of desiccation (non-viable), when the viability was only 8% whereas on 14^{th} day, eventhough starch grains are almost disappeared, cell wall appears intact. As a result of cell wall disintegration, cotyledonary cells stained with PAS and safranin-I_2 KI reagent appear as distorted (Fig. 12, 13).

Ruhl (1996) found that in Cocoa seeds, short period of drying induces the damages to the radicle. The ultrastructural study revealed that the double membranes enveloping the amyloplasts break up exactly in the critical moisture range and this phenomenon was preceded by the reduction of the ribosomes in the endoplasmic reticulum enveloping amyloplasts. The breakage of amyloplast membrane at critical moisture content may result in the abundant availability of starch for the enhanced amylolytic activity during 12^{th} day of desiccation in Jackfruit seeds.

Starch depletion during desiccation, coincident with loss of viability in Jackfruit seeds can be correlated with enhanced amylase activity particularly at pH-5.3 (Fig. 6). Distribution of sugars shows a hike in rhamnose on 14^{th} and 16^{th} day of desiccation and cell wall disintegration of cotyledonary cells (Fig. 12, 13) also is superimposed on this condition of desiccating seeds. Accumulation of rhamnose is presumably due to cell wall disintegration by the hydrolysis of pectins which are the most soluble of the cell wall polysaccharides and can be extracted with hot water (Taiz and Zeiger, 2002). So during desiccation, pectins are solubilised resulting in the formation of rhamnose which is the deoxy sugar molecule constituting pectins of cell wall.

Histochemical study on the embryonic axis of Jackfruit seeds subjected to desiccation showed that when desiccation induced viability loss is occurred, radicle tip shows slight shrinkage and apical cells are distorted since the cells lose the ability to withstand the mechanical stress associated with the volume / size reduction during desiccation. A similar observation was reported in *Avicennia marina* in which the disintegration of the hypocotyls where the meristematic root primordia are situated, suffer lethal damages at water content below 33% wet mass basis (Farrant *et al.*, 1997). In desiccation induced non-viable Jackfruit seeds, shape of the radicle tip was concave and apical dome shape was lost (Fig-18). Disappearance of starch grains and

nucleus from the radicle tip is very evident in desiccated seeds. This observation confirms the reduction of starch content consequent to viability loss as shown by starch content depletion and increased amylase activity as described earlier. In the radicle tip of non-viable seeds, the bilobed appearance due to the closely appressed cotyledonary portion seems to be related to the overall shrinkage of seeds during desiccation as the moisture content is reduced to only one half (Table 1) in cotyledon of Jackfruit seeds. Desiccation resulted in the disintegration of apical cells, disappearance of nucleus and breakage of cell walls (Fig. 18). According to Ruhl (1996), the primary site of desiccation damage in Cocoa seeds is the cotyledon since a short period of drying enabled the cotyledon to induce secondary changes in the axis. In Jackfruit seeds, both cotyledons and axis are equally vulnerable to desiccation because desiccation-associated cellular / histochemical changes are evident in both tissues.

Histochemical localization of protein in the cotyledon of Jackfruit seeds, showed shrinkage of cells which are more prominent in the cells of adaxial side compared to the abaxial and centre region of cotyledon when desiccation induced viability loss is initiated (Fig. 15, A, B, C). Staining intensity which is proportional to the protein content does not change much and hence mobilization of protein is not presumed to occur during desiccation probably due to comparatively lower quantity of protein and starch rich nature of Jackfruit seeds.

During desiccation of Jackfruit seeds, SDS-PAGE studies revealed the disappearance of few high molecular weight protein bands (more than 66 kDa) at the initiation of desiccation damage, more bands are found to disappear with the advancement of desiccation. It appears that high molecular weight protein bands are involved in the maintenance of desiccation tolerance and thereby viability. More or less similar observations were made in *Shorea*

robusta (Nautiyal *et al.*, 1985). However, those authors correlated the disappearance of low molecular weight proteins with desiccation intolerance in *Shorea robusta*. On the contrary in Jackfruit seeds, the disappearance of high molecular weight proteins is correlated with desiccation intolerance or loss of viability.

In recalcitrant seeds, the effect of drying rate on desiccation tolerance is associated not only with the regulation of metabolism (physico-chemical aspects) but also with the physical processes of dehydration (mechanical aspects). In Cocoa embryonic axis, rapid drying at low relative humidity was less harmful compared to slow drying at high relative humidity (Liang and Sun, 2000). Several authors suggested that under slow drying condition, the seed tissue has to stay longer time at intermediate water content at which aqueous-based deleterious processes are due to coordinated regulation of metabolism (Pammenter *et al.*, 1991; Pritchard and Manger, 1998; Pammenter and Berjak, 1999). In some recalcitrant seeds, slow drying may permit the inhibition of germination so that seeds become increasingly sensitive to desiccation (Farrant *et al.*, 1985). Jackfruit seeds under room-open condition, undergo a slow drying at comparatively low relative humidity (not under controlled relative humidity) and the desiccation effect is protracted resulting in the damage of tissues as shown by histochemical studies such as cell distortion and dislocation of starch grains occur in the cotyledonary tissue (Fig. 9, 12, 13).

Desiccation sensitivity of recalcitrant seeds has been interpreted as a direct result of dehydration through lipid phase transition (Leopold and Vertucci, 1986). In the present study, desiccation induced changes can only be correlated with changes centred on carbohydrate metabolism and desiccation induced mechanical / cellular changes in embryonic axis and cotyledon. However, molecular aspect of desiccation of recalcitrant seeds has already

been interpreted in terms of lipid phase change (Leopold and Vertucci, 1986) and accumulation of free radicals by peroxidation of lipid and protein components are considered as the probable immediate causes of membrane damage (Hendry *et al.*, 1992, Leprince *et al.*, 1993). Nevertheless, in the present study this aspect is not undertaken.

Because of their high moisture content and desiccation sensitivity, moist or wet storage have been practiced in recalcitrant seeds (Chin *et al.*, 1981; Bewley and Black, 1994; Copeland and McDonald, 1995; Baskin and Baskin, 2001). Moist storage of recalcitrant seeds at low temperature also (10-20°C) has been suggested by many investigators (Chin *et al.*, 1984; Anilkumar, 1998; Anilkumar *et al.*, 1996, 2002; Danthu *et al.*, 2000; Decruse and Seeni, 2003). Storage of Jackfruit seeds (room-polythene), employed in the present study can be considered as a sort of moist storage and found to be an effective method of storage because viability is retained upto about four months (Table 7, Fig. 21). During storage under this condition, only negligible fluctuations in moisture content is observed maintaining more or less same moisture content throughout storage period. In the storage studies on *Boscia senegalensis, Butyrospermum parkii, Cordyla pinnata* and *Saba senegalensis,* Danthu *et al.*, (2000) showed that storage in wet air tight containers resulted in maintenance of viability upto a few months retaining their moisture content above the critical level. More or less similar observations are obtained in Jackfruit seeds under room-polythene storage.

Generally, recalcitrant seeds are vulnerable to microbial infection during storage at ambient temperature due to high moisture content and high sugar contents (Bewley and Black, 1994, Baskin and Baskin, 2001). But in seeds of Jackfruit, during prolonged storage in room-polythene condition, microbial infection is very rare. This can be attributed to the comparatively thick waxy glazing testa. Chin *et al.*, (1984) stated that *Artocarpus*

heterophyllus seeds are having thin and membraneous, impermeable coats and seeds are difficult to dry. Contrary to the above statement, the present author observed that even though the seeds possess glazing testa, they lose viability within 13 days of desiccation. Control as well as slightly desiccated seeds germinate within one to two days when moistened due to the permeability of testa. So the impermeable nature of testa that are harder to dry in *Artocarpus heterophyllus* seeds suggested by Chin *et al.*, (1984) is not acceptable.

Under room-polythene storage condition that prevents water loss, viability is lost only after 110 days. A current view on hydrated storage of recalcitrant seeds (Pammenter *et al.*, 1994) is that recalcitrant seeds are metabolically active at the time of shedding and hence undergo germination-associated changes in storage. So it is suggested that under storage, recalcitrant seeds are exposed to an initially mild but increasingly severe water stress which ultimately lead to death of seeds (Farrant *et al.*, 1986, 1988; Pammenter *et al.*, 1994).

Jackfruit seeds stored in room-polythene condition exhibited longevity upto 110 days without any significant reduction of moisture content. But unlike many other recalcitrant seeds which germinate when stored in air tight containers at ambient or low temperatures (Farrant *et al.,* 1988, 1995; Finch-Savage *et al.*, 1992; Berjak *et al.*, 1993) Jackfruit seeds never show visible sign of germination under room-polythene storage or at low temperature storage. Notwithstanding, these seeds are metabolically active showing turgid cells with conspicuous nucleus, moderate amylase activiy and the seeds germinate within 1-2 days without any triphasic imbibition that occur in orthodox seeds (Bewely and Black, 1994).

Pammenter *et al.*, (1994) excellently reviewed the causes of recalcitrant behaviour of seeds and suggested that hydrated storage show degeneration process during long term storage but during short term storage,

only non-degenerative changes occur since the seeds are metabolically active and show germination-associated changes. Jackfruit seeds stored in room-polythene and refrigerator can be considered as storage under "hydrated" condition because moisture content registered only negligible reduction *vis-a vis* no sign of germination even after 100-110 days (Table 7, Fig. 20, 21) and evidently no desiccation or chilling stress as the case may be is imposed. Hence, eventhough visible symptoms of germination are not seen, the most probable reason for seed death can be attributed to germination-associated changes initiated during earlier days of storage and later the metabolism associated to the germination get impaired due to lack of oxygen, sufficient water etc. in the closed polythene bags.

Distribution pattern of moisture content of Jackfruit seeds during storage in room-polythene and refrigerator is almost similar and their values show gradual insignificant reduction upto 110 and 100 days in room-polythene and refrigerator respectively (Table 7, Fig. 20, 21) and at these stages, loss of viability is initiated and some sort of water stress is seemed to be imposed on the seeds under storage. According to Pammenter *et al.*, (1994) there are two components to the water stress brought about by germination-associated metabolism during hydrated storage of recalcitrant seeds i.e. the level of stress and the duration of stress. In this context, a better rather suitable comparison can be made between the water stress suffered by stored seeds and the same effect suffered by seeds during dehydration i.e. desiccation under open air conditions. In this comparison, the period of stress is an important criterion because seeds undergo drastic dehydration (desiccation) and lose viability within 13 days in room-open while the seeds under 'hydrated storage' (both room-polythene and refrigerator) remain viable upto 100-110 days (Table 1, 7, Fig. 1, 21). Pammenter *et al.*, (1994) opined that while the intensity of water stress suffered by stored seeds is milder than that imposed in dehydration studies and the duration of the stress is

considerably extended. Storage behaviour of Jackfruit seeds in both conditions, compared to the desiccation behaviour is inconsistent with the view of Pammenter *et al.*, (1994) because only mild desiccation stress is shown during storage.

In Jackfruit seeds, comparison of metabolic changes during storage and desiccation can only be made upto 12 days because seeds become intolerant to desiccation under open air resulting in the loss of viability beyond that period. Notwithstanding, it is to be emphasized that seeds under storage retain their viability upto 100-110 days. So the duration of stress is considerably extended. According to Pammenter *et al.*, (1994) in many desiccation studies of recalcitrant seeds, the rate or level of imposition of the stress is considered only in the context of whether a tolerance mechanism is induced or not. As tolerance mechanism is not induced or existing in recalcitrant seeds, the duration of the stress should not be overlooked. This view is in conformity with the findings of Jackfruit seed behaviour under storage because desiccation tolerance is not induced under storage as well as open air desiccation. But only after a prolonged period of storage (100-110 days), the seeds lose viability presumably due to the accumulation of damages during the prolonged duration of water stress compared to the enhanced level of stress (short term severe stress) suffered by the seeds under desiccation during a short period of 12 days (Table 1, 7, Fig. 1, 20, 21).

Events occurring in seeds stored in room-polythene condition can more or less be compared to that occurring during germination as per the concept put forth by Pammenter *et al.*, (1994). So present author proposes a comparison of metabolic changes between seeds stored in room-polythene and fresh (control) seeds germinated under laboratory conditions eventhough the sampling is not done at comparable intervals since storage period is 120

days and germination period is only 50 days (due to growth retardation under laboratory conditions after 50 days).

Distribution of moisture content in these two seed samples showed that (Table 1, 7) it remained unchanged in the seeds of room-polythene storage whereas gradual but significant increase was observed in control seeds during germination. Metabolites like starch in the seeds of room-polythene storage, (Table 8, Fig. 22) showed only negligible decrease upto 50 days. On the contrary germinating seeds showed reduction in starch content to about half as that of control seeds, on 50^{th} day (Table 13). Sugar content in room-polythene seeds remained unchanged during 40 days of storage. But during 50 days of germination, three fold increase in total sugar content in the cotyledon is observed. A negligible fluctuation in the distribution of protein is observed in room-polythene seeds whereas gradual reduction in protein content was observed during 50 days of germination. The changes in carbohydrates in germinating seeds are evidently due to the degradation / utilization of starch during seedling growth and reserve mobilization upto 50 days of seedling growth whereas stored seeds lack seedling development as well as mobilization of reserves.

Both α- and β-amylases activity (unit and specific) also showed more or less similar trend with negligible fluctuations in the seeds stored in room-polythene. However, during germination, activity of β-amylases exhibited slight increase, but α-amylases activity remained unchanged, comparatively enhanced maltose in the germinating seeds reveals the increased activity of β-amylases because this enzyme cleaves maltose from the oligomers formed earlier due to α-amylases activity. (Bewely and Black, 1994)

Eventhough the germination-associated metabolism is believed to occur in Jackfruit seeds during storage under room-polythene, compared to

the germinating seeds, significant variations are observed. Starch depletion was confined only to germination owing to the mobilization to the developing seedlings, whereas in stored seeds only slight changes occurred (Table 8, 14; Fig. 22, 28). Amylase activity in general and specific activity in particular is enhanced in germinating seeds enabling hydrolysis of starch and resultant depletion of the same due to mobilization. Similarly enhanced metabolism of carbohydrates is evident in germinating seeds by transient accumulation of soluble sugars (Table 9, 15, Fig. 24, 30). Seeds under room-polythene storage also showed metabolic changes but the depletion was insignificant owing to the lack of mobilization as the seedling development is inhibited despite germination-associated changes do occur.

Metabolism induced damage associated with dehydration of recalcitrant seeds has been well interpreted by Berjak *et al.*, (1993) and Come and Corbineau (1996) according to whom free radical generation as a consequence of uncoordinated metabolism may be an injurious factor during desiccation. Eventhough antioxidant protective mechanism has been suggested against free radical damage (Hendry *et al.*, 1992; Finch-Savage *et al.*, 1993, 1994), this antioxidant system become ineffective to protect tissue from uncoordinated metabolism during dehydration.

Jackfruit seeds contain only very low protein (10%) and significant changes are not occurring in seeds during storage as well as germination (Table 12, 14). In general, metabolic changes during storage and germination are more or less alike except enhanced amylase activity and starch depletion leading to the mobilization of metabolites to the developing seedling in germinating seeds. The metabolic changes eventhough very mild, occurring for prolonged periods (110 days under room-polythene) of storage are directly related to accumulation of gradual detrimental changes resulting in loss of viability. However, a dominant role of water stress due to the reduced

moisture content over the metabolite induced stresses cannot be ruled out in seeds of Jackfruit during storage. These characteristics of Jackfruit seeds are in consistent with the view of Finch-Savage (1992) according to whom desiccation of storage tissues may lead to metabolic disturbances and may have an indirect effect on seed longevity.

Seeds of Jackfruit under room-polythene storage suffer desiccation stress, as moisture content is reduced slightly on 100^{th} day onwards, whereas seeds stored in refrigerator show reduction of moisture content on 90^{th} day onwards. At this critical levels of moisture content, sucrose and oligosaccharides, raffinose in particular are getting increased presumably to confer desiccation tolerance. There is some discrepancy in the values of raffinose content of the seeds stored in refrigerator due to the lack of sugar analysis data on 90^{th} day which coincides with initiation of viability loss (Table 9, Fig. 24). However, seed samples of 80^{th} day show very high raffinose content as a pre-requisite for desiccation tolerance and on 100^{th} day, the seeds show less than cent percent viability and very low raffinose content since the seeds are intolerant to desiccation and hence show no storability. The behavior of Jackfruit seeds under storage is directly related to desiccation stress imposed by reduction of moisture content and at this juncture desiccation tolerance is imposed which is associated with co-operative accumulation of raffinose *vis a vis* moderate sucrose content. Koster and Leopold (1988) suggested that sucrose without high oligosaccharide do not confer desiccation tolerance in imbibed orthodox seeds of soybean, maize and pea. Presence of sucrose is considered as an important factor contributing to desiccation tolerance in seeds (Black *et al.*, 1996, 1999; Buitink *et al.*, 2000; Halperin and Koster, 2006).

The role of sucrose and raffinose in enhancing desiccation tolerance by stabilizing membrane structure during dehydration and rehydration has been

reported by Crowe *et al.*, (1992), Koster *et al.*, (2000). According to Bryant *et al.*, (2001) sucrose plays an important role in membrane protection at low hydration and loss of membrane permeability during desiccation is thought to be resulted from the damaging effect of hydration forces that become large when hydrophilic surfaces of membranes are brought into close apposition. The presence of sugars between membranes helps to limit the close approach of their surface during hydration and helps to stabilize the membranes during desiccation (Koster *et al.*, 2000). Desiccation studies of protoplast isolated from germinating Pea (*Pisum sativum*) revealed that desiccation tolerance is enhanced by isolation and drying in hypertonic sucrose / raffinose solution. Protection of protoplast membranes may result from sucrose uptake into the protoplast and elevated level of sucrose can hinder the close approach of membranes and other cellular structures during dehydration (Halperin and Koster, 2006).

Seed vigour of Jackfruit seeds, showed variations in seeds under desiccation and different types of storage. Eventhough cent percent viability was exhibited by seeds desiccated upto 12 days under open air condition, seed vigour was significantly reduced (Table 2, Fig. 2). During desiccation, viability was reduced geometrically while seed vigour index was reduced logarithmically. Seed vigour index does not change upto 50 days o f storage, afterwards gradual reduction was observed whereas viability loss was initiated when seed vigour index showed significant reduction. Refrigerator stored seeds also showed almost similar values of seed vigour as that of room-polythene (Table 2, Fig. 2). But in general, seeds stored in refrigerator showed lower seed vigour values. Reduced seed vigour has been reported in *Hevea braziliensis* (Chin *et al.*, 1984) during desiccation. This observation is in agreement with the results obtained by Xia *et al.*, (1992) according to whom the seed vigour index of just harvested Lychee (*Litchi chinensis*) and longan (*Euphoria longan*) seeds could be increased only by slight desiccation.

Conversely, Chaitanya *et al.*, (2000 a) 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. In Jackfruit seeds, however, the seed vigour as well as the protein content decreased during the entire period of desiccation.

The processes of water relations occurring in seeds under hydrated storage can be viewed in terms of model of Vertucci (1990, 1993) describing the thermodynamic states of water in seed tissues. Because the seeds are able to initiate germination when shed i.e. seeds probably possess 'type 4 and 5' water, which are the water with properties of dilute solutions. Jackfruit seeds under room-polythene storage, show no change in moisture content, although metabolic changes in general and starch depletion and concomitant sugar accumulation in particular upto 110 days and consequent viability loss occur. It can be presumed that the properties of 'type 4 and 5' water with the properties of dilute solutions get changed due to further dissolution of metabolites, particularly sugars resulting in the depletion of water potential. However viability loss is initiated when the moisture content exhibit only slight decline but significant reduction of water potential cannot be ruled out owing to the concentration of sugars during this stage of storage (Table 7, 9, Fig. 20, 24a).

Even though, recalcitrant seeds are chilling sensitive, (Hor *et al.*, 1984; Corbineau and Come., 1988; Chin *et al.*, 1984; Kovach and Bradford, 1992; Cunha *et al.*, 1995; Danthu *et al.*, 2000; Le Tam *et al.*, 2004; Varghese *et al.*, 2004; Malik *et al.*, 2005) low temperature storage has been practiced (Danthu *et al.*, 2000; Anilkumar, 2000; Connor and Sowa, 2002; Le Tam *et al.*, 2004; Faria *et al.*, 2004). Seeds of Jackfruit stored in refrigerator retained viability upto 100 days which is more or less similar to that of moist storage at room temperature (room-polythene). In both storage conditions

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moisture content was retained in similar pattern (Table 7, Fig. 20). An interesting observation of room-polythene and refrigerator storage is that the 100% viability was shown by seeds having moisture content almost same as that of the control seeds. This behaviour of Jackfruit seeds shows the crucial role of moisture content in the maintenance of viability irrespective of the mode of storage / damage.

Distribution of individual sugars of Jackfruit seeds during storage in room-polythene show a significant increase in monosaccharides and reduction in sucrose during 80 days when the seeds are having cent percent viability (Table 7, 9). However, as storage proceeds to 100-110 days, the monosaccharides are reduced and sucrose content showed an increase. Similarly accumulation of raffinose and maltose also is observed during this period. All these characters are positively correlated one way or the other to desiccation stress under storage of Jackfruit seeds. Notwithstanding, distribution of moisture content (Table 7) shows no significant reduction during storage. The overall carbohydrate metabolism in terms of starch depletion, sugar contents and amylase activity of seeds is similar or comparable to the germination-associated changes which are considered as a character of recalcitrant seeds (Farrant *et al.*, 1992; Lin and Chen, 1995). However, during 100-110 days, as the viability loss is initiated, the distribution of sugars in general and raffinose family of oligosaccharides in particular can also be correlated to desiccation stress (Table 7, 9). The desiccation stress is not observed during storage since moisture content remains unchanged. However, it is concernable that eventhough moisture content is not changed, the soluble sugars contribute towards the reduction of water potential and thereby inducing desiccation stress. But the major sugar content is maltose and sucrose which are known to be protective against desiccation induced damages. Hence the role of sugar metabolism as described earlier can be considered as a protection against desiccation as the

seeds show cent percent viability. Desiccation sensitivity of recalcitrant seeds in storage may also result from the initiation of germination-associated events and may therefore be analogous to the desiccation sensitivity of germinating seeds of orthodox species (Berjak *et al.*, 1984; Bernal-Lugo and Leopold, 1992, 1995; Steadman *et al.*, 1996).

As reported earlier, Jackfruit seeds are starch rich and both embryonic axis and cotyledon contain starch, more than 70% and 49% respectively. Quantitative estimation and histochemical localization of starch in desiccating seeds show that until viability loss, only gradual reduction of starch occur and when viability percentage is less than 50% (14^{th} day onwards) starch depletion (10%) was highly significant (Table 4, Fig. 4). Similarly during storage also, starch depletion is directly correlated to loss of viability. In seeds stored in room-polythene, when viability declined to less than cent percent, starch content showed 11% reduction on 110^{th} day of storage. In seeds stored in refrigerator also, more or less similar relationship between viability and starch is observed (Table 7, 8). In the case of refrigerated storage, viability is reduced to 80% on 100^{th} day and the starch content reduction is 11%. These observations reveal that irrespective of the mode of desiccation / storage, 10-11% starch depletion in the seeds desiccated under open air, stored in room-polythene and in refrigerator result in loss of viability after 12, 110 and 100 days respectively and the respective moisture content of seeds are 33, 48 and 42%. As described earlier, the starch depletion and enhanced amylolytic activity resulting in the formation of sugars cause lowering of the magnitude of water potential which is detrimental to seeds. So it is conceivable that a metabolic scenario of specific range of water potential controls the seed viability rather than the moisture content, mode of desiccation / storage, and / or duration of treatments. In this context, it is recalled that the desiccation sensitivity of recalcitrant seeds is firmly based on 1) the properties of water at various hydration levels corresponding to specific water potential ranges, 2)

on the physiological status and metabolic processes occurring in seeds at various water potential (Vertucci and Leopold, 1986; Vertucci, 1993; Vertucci and Farrant, 1995). Similarly in their excellent review Pammenter and Berjak (1999) stated that it is more meaningful to consider the water status of seed in terms of water potential rather than water content though those two measures are loosely but not linearly related.

Storage under refrigerator condition resulted in seed viability loss earlier than that of room-polythene condition. During 40-80 days of refrigerated storage, monosaccharides, (glucose and fructose) exhibited a significant increase compared to control as well as that of room-polythene, presumably due to reduced rate of respiratory metabolism under low temperature (Table 9, Fig. 30b). An important observation of sugar distribution in the seeds under refrigerator storage is an enhanced accumulation of disaccharides (sucrose and maltose) compared to the previous stage as well as in comparison with that of seeds under room-polythene storage and concomitantly monosaccharides are reduced during this period. Sucrose synthesis by utilizing the monosaccharides and maltose formation due to amylolytic activity in accordance with the view of Kaplan and Guy (2004) who reported high β-amylase activity resulted in maltose accumulation in *Arabidopsis* (variety *Columbia*) cultivated under cold condition.

Unlike the seeds stored in room-polythene, starch depletion and accumulation of sugars in the seeds stored in refrigerator are related each other, but without any significant increase in amylase activity. A plausible role of starch phosphorylase can be attributed to the hydrolysis of starch and resultant accumulation of sugars. But this enzyme cannot attack starch grains which must be partly degraded first by other enzymes (Ashford and Gubler, 1984; Bewley and Black, 1994). However, the seeds stored in refrigerator

during 80-90 days show considerable amylase activity as a pre-requisite for phosphorylase activity. Phosphorylase releases glucose-1-phosphate from amylose / amylopectin by incorporating a phosphate moiety and the resultant glucose-1-phosphate can be directly used as substrate for sucrose synthesis by the enzyme sucrose synthetase. However, synthesis of glucose-1-phosphate requires consumption of energy which may be generated by the enhanced respiration by utilizing monosaccharides evidenced by the marked reduction of monosaccharides at this stage (Table 9). The accumulation of sucrose in the seeds stored in refrigerator during 90-110 days (Table 9) seems to be the ultimate product of starch phosphorylase and sucrose synthetase and the role of this disaccharide in imposing desiccation tolerance is well established as described earlier.

In addition to sucrose, maltose was accumulated in seeds stored in refrigerator during 90-100 days. Activity of ß-amylase (pH-5.3) was comparatively higher in seeds stored in refrigerator during this interval, maltose is the immediate product of ß-amylase activity and this enzyme is found to be more active in refrigerator stored seeds playing a protective role against temperature intolerance. A close association between amylase activity and maltose accumulation and temperature stress has been established in plants. Nielsen *et al.*, (1997) reported that ß-amylase in potato tubers and pea is induced by storage at low temperatures with an increase in maltose. Two specific ß-amylases are induced by cold shock in *Arabidopsis* (variety *Columbia*) and maltose accumulation was also occurred (Kaplan and Guy, 2004). According to those authors, the maltose accumulation may be an important factor helping plants to cope up with temperature stress. Because maltose production is a single step reaction, plants (tissues) with adequate starch levels could produce significant quantities in very short time and this maltose accumulation could act as a compatible solute to protect proteins of membranes (compatible protectant / osmoprotectant / osmolyte).

Occurrence of raffinose during 100-120 days of storage in both room-polythene and refrigerator can be correlated to the desiccation (dehydration)-induced stress since moisture content is significantly reduced in these samples during this period. Role of raffinose associated with desiccation tolerance is well established in recalcitrant seeds (Koster and Leopold, 1988; Bernal-Lugo and Leopold, 1992; Hoekstra, 1994; Horbowicz and Obendorf, 1994).

A significant reduction of moisture content during 90-100 days, coinciding with initiation of viability loss is an important observation of seed behavior under refrigerator storage. So the manifestation of desiccation is due to the superimposed effect of reduced moisture content and low water potential contributed by abundance of total sugars during this period. Nevertheless, metabolic processes such as accumulation of raffinose family of oligosaccharides and low ratio of sucrose: raffinose which is involved in the induction of desiccation intolerance in recalcitrant seeds as suggested by many authors (Horbowicz and Obendorf, 1994; Bernal-Lugo and Leopold, 1995) are not shown by Jack seeds under cold storage and seeds do not show much tolerance to chilling despite germinability is exhibited. Eventhough seeds are characterized by radicle protrusion, the potential for seedling establishment is lost due to cold storage and seed decay is frequently observed. Seed vigour also is very low in the seeds stored in refrigerator as a result of chilling injury affected to the seeds, cotyledons in particular as suggested by Chin *et al.*, (1981).

Irrespective of the conditions of desiccation / storage, more or less a common pattern of carbohydrate metabolism is exhibited by Jackfruit seeds to cope with desiccation stress when seeds stored at open-air conditions (room temperature), in closed polythene bags (room temperature) and in refrigerator. Depletion of starch, enhanced amylolytic activity (both α- and β-amylase) and

resultant maltose accumulation occur when the seeds become sensitive to desiccation on 12-14 days, 100-110 days and 90-100 days during desiccation / storage under room-open, room-polythene and refrigerator respectively. Since Jackfruit seeds are starch rich, constitutive α- and β-amylase are getting induced under low and ambient temperature, and maltose and / or sucrose could play a compatible role to protect membrane proteins against the desiccation stress. Nevertheless, this tolerance mechanism is not prevailed in Jackfruit seeds for prolonged time presumably due to high recalcitrant nature. However, this proposed pattern of carbohydrate metabolism poses an interesting question concerning the role of starch, amylase and maltose on imposing desiccation tolerance in recalcitrant seeds.

Germination is frequently considered as the index or marker of almost all investigations on recalcitrant seeds, to describe and interpret salient features such as desiccation intolerance, chilling injury and short life span of recalcitrant seeds. According to Farrant *et al*, (1986, 1988) in recalcitrant seeds, germination is considered as a continuum of development. However, metabolic changes during germination are not yet been well interpreted in recalcitrant seeds. Jackfruit seeds are included under recalcitrant category of seeds (Chin *et al.*, 1984; Chandel *et al.*, 1995; Smith *et al.*, 2001; Peran *et al.*, 2004) and starch rich (Anonymous, 1994, 2000, 2003).

The starch rich cotyledonary cells of Jackfruit seeds show wide variation in the shape and structure of starch grains. Comparatively smaller grains and compound grains containing 2-11 individual grains (Fig. 19F) are localised in the cells of peripheral region of cotyledons and these compound grains are getting degraded during reserve mobilization after germination. This heterogeneity of starch grain may be an inherent character unique to Jackfruit seeds.

Fresh Jackfruit seeds contain 73% and 49% starch in cotyledon and axis respectively on dry weight basis. The metabolic changes during germination of Jackfruit seeds particularly amylase activity (β- and α-amylases) at pH-5.3 and 8 is increased to more than double resulting in significant depletion of starch (less than half) in the axis tissue (Table 16, Fig. 31, 32) can be compared to the typical characteristics of germination-related reserve mobilization in orthodox seeds reported in many seeds (Khan, 1977; Bewley and Black, 1982, 1994; Mayer and Poljakoff-Mayber, 1989; Baskin and Baskin, 2001). But in orthodox seeds, such an elaborated starch hydrolysis occurs only after a few days of germination and the duration varies from species to species (Mayer and Poljakoff-Mayber, 1989). In Jackfruit seeds, these changes are occurring on 2nd day onwards showing immediate germination-associated metabolism and reserve mobilization as a continuum of development. Significant activity of α- and β-amylases and resultant starch reduction occur in the cotyledon despite the depletion of starch in the axis tissue (Fig. 27, 31) which show drastic reduction (50%) on 2nd day and only about 10% is retained on 10th day. This behaviour of Jackfruit seed embryonic axis is similar to the metabolism reported in embryonic axes of orthodox seeds (Murray, 1984). On 10th day, a marked reduction of starch content in the axis tissue was observed due to the activity of β-amylase (Fig. 27, 31) indicating the specific involvement of β-amylase in the hydrolysis of starch due to availability of oligomers formed as a result of α-amylase activity and no other tissue showed such a peak value at pH- 5.3.

Unlike the orthodox seeds in which hydrolytic enzyme such as β-amylase, protease, phosphatase etc. are sequestered in organelles (lytic bodies) or vacuoles and are released at appropriate time after germination (Bewley and Black, 1994), amylolytic activity is very high in fresh Jackfruit seeds (control) which are starch rich. Significant increase of amylolytic activity is observed both in the axis and cotyledon and a concomitant

depletion of starch on 2nd day, as germination proceeded, starch content of the axis is depleted rather exhausted, but that of cotyledon remains unchanged upto 30 days of seedling growth (Fig. 27, 31). Starch content is reduced to one half on 40th and 50th days without significant increase in β-amylase activity (pH-5.3). Starch phosphorylase is another enzyme involved in starch catabolism in seeds during germination (Bewely and Black, 1994) and in the significant reduction of starch in Jackfruit seeds during 30-50 days of seedling growth, phosphorylase activity cannot be ruled out. On the contrary histochemical localization of starch revealed that during seedling growth, starch grains of the cotyledonary cells of both adaxial and abaxial sides (Fig. 37, A-F) are completely disappeared and only smaller grains are present in vicinity of vasculature. This disparity is probably due to several reasons: 1) Quantitative estimation of starch values show very high standard deviation (even after repetition of experiments more than ten times) (Fig. 27) indicating the heterogeneity of samples because, for biochemical estimation samples were taken from pooled tissue of a minimum four seeds each after chopping into small pieces to which big cotyledon contribute more tissue due to the very small size of the smaller cotyledon whereas for histochemical studies only smaller cotyledon was selected for convenience of tissue preparation for microscopic observations. 2) Starch depletion may occur preferentially from the smaller cotyledon due to close proximity.

HPLC studies of sugars in Jackfruit seeds during germination and seedling growth were done only at certain intervals due to unavailability of facilities, so continuous changes of sugars during seedling growth cannot be drawn. However, distribution of metabolisable sugars in the axis show that monosaccharides - glucose and fructose are present in considerable quantities and are increased due to high metabolism related to growth and differentiation. A simultaneous reduction of sucrose in the cotyledon may be due to the translocation to the metabolically active growing axis in which

sucrose is comparatively low in germinating phases. Absence of raffinose and other oligosaccharides in the embryonic axis tissue is found to be a characteristic feature of recalcitrant Jackfruit seeds because in orthodox seeds, raffinose family of oligosaccharides are the first carbohydrates to be utilized by hydrolytic cleavage of α-galactosidic bond to yield sucrose and galactose (Bewley and Black, 1994). Similarly the absence of galactose in the axis and cotyledon also may be a characteristic of recalcitrant seeds during germination. Raffinose and other oligosaccharides are absent in the embryonic axis of Jackfruit seeds and this observation can be correlated with the absence of maturation drying in recalcitrant seeds. But in orthodox seeds occurrence of raffinose and its metabolic role as an important reserve readily available on germination have already been reported (Keller and Pharr, 1996: Peterbauer and Richter, 2001).

As a result of very high activity of both α- and β-amylases during early days of germination and maximum activity of β-amylases on 2nd day (Fig. 30), maltose is the most abundant sugar in the embryonic axis of control seeds and it is reduced gradually during germination where as in the cotyledon, maltose is very low due to comparatively feeble amylolytic activity (Table 16, Fig. 30). The utilization / hydrolysis of maltose by the enzyme α-glucosidase (maltase) which is present in the seeds and getting increased during germination (Bewley and Black, 1994) induces the amylase activity by avoiding the end product inhibition of the enzyme.

Raffinose and three unidentified sugars present in the cotyledon of fresh Jackfruit seeds disappeared during germination and reappeared at certain interval of seedling growth. The occurrence of raffinose family of oligosaccharides in the fresh seeds can be considered as a pre-requisite for imposing desiccation tolerance during post-harvest period since the role of raffinose family of oligosaccharides in the induction of desiccation tolerance

in recalcitrant as well as orthodox seeds is well established (Koster and Leopold, 1988, Bernal-Lugo and Leopold, 1992, 1995; Horbowicz and Obendorf 1994; Bailly *et al.*, 2001). Total sugar content in the cotyledonary tissue which is increasing as growth advanced to 50 days, indicate retarded mobilization to the growing axis because the germination studies are conducted in the dark and during this prolonged period seedlings are not well developed or established rather seedlings are etiolated with impaired metabolism.

Histochemical staining of cotyledonary tissue of 30^{th} and 50^{th} day samples showed intact and comparatively thick cell walls in spite of the disappearance of almost all starch grains emptying the lumen of the cells (Fig. 36, 37). On the contrary, orthodox seeds in general and cereals in particular, accompanying mobilization of starch, carbohydrates of cell wall also get hydrolysed which promotes easier access by starch degrading enzymes (Bewley and Black, 1994). In Jackfruit seeds, all cells of cotyledon contain conspicuous nucleus and other cell inclusions and appear turgid and even after 50 days of seedling growth, the cells are intact and the cotyledons appear fresh with compact structural / textural morphology. This character can be attributed to the recalcitrant nature of the seeds i.e. high moisture content, turgidity of cells and high metabolic activity.

Jackfruit seeds contain only 10% protein (total) in the cotyledons and 18% in the axis whereas soluble protein is only less than one third of total protein. Only negligible changes occurred in the total and soluble protein upto 10 days with a significant increase of total protein on 5^{th} day of germination (Table 14, Fig. 33). This hike is coincident with high metabolic activity and related synthesis of metabolites in the cotyledons during germination (Table 16, Fig. 31). Similar observations have been made in Pea seedling by Davis (1979) who suggested that α-amylase activity during germination is

proportional to enzyme protein synthesis. After 10 days, total and soluble protein did not change much with a reduction on 50^{th} day.

During germination of Jackfruit seeds, ß-amylase in the axis tissue of 2^{nd} day sample, was very active, (Fig. 31a, 32a) compared to α-amylase activity. But the specific activity of the enzyme α-amylase was declined sharply with a slight increase on 2^{nd} day and very feeble activity on 10^{th} day (Fig. 32b). The difference in the specific activity of axis tissue can be correlated to the hike in soluble protein content of the axis tissue. Comparatively very high specific activity of ß-amylase can be correlated to the soluble protein hike.

An interesting observation of protein metabolism in the germinating seeds of Jackfruit is the relationship between the soluble protein and corresponding amylase activity in the cotyledon and that of the axis. Axis tissue of fresh (control) seeds is characterised by very high activity of α- and ß-amylases, but soluble protein is comparatively less than the cotyledons. Similarly sugars particularly maltose are also more in the axis. In addition to α- and ß-amylases, other hydrolyzing enzymes such as α-glucosidase (maltase) hydrolysing maltose to glucose, debranching (R) enzyme, starch phosphorylase etc. are synthesized for complete hydrolysis of starch during germination (Ashford and Gubler, 1984; Bewley and Black, 1994). All these metabolic changes are related to the metabolically active embryonic axis of fresh (control) Jackfruit seeds which is starch rich and during germination, enhanced synthesis of amylase enzyme protein as reported in legumes (Davis, 1979) is presumed to occur because of very high activities of α- and ß-amylases. However, maltose content is not accumulated during germination but abundant quantity of glucose is accumulated as one of the product of α-amylase activity along with maltose formation, as well as degradation products of newly formed maltose due to maltase activity. Bewley and Black

(1994) suggested that the product of starch hydrolysis mostly glucose and fructose which are converted into sucrose for translocation or used as energy source during respiration.

The reserve mobilization pattern of Jackfruit seeds during seedling growth observed in the present investigation is not comparable with reserve mobilization pattern expected in orthodox seeds because Jackfruit seeds are recalcitrant and germination is a continuum of development. Another disparity of comparison is the germination experiment conducted in the dark at room temperature and so seedling growth cannot be considered as normal.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON JACKFRUIT SEEDS (*Artocarpus heterophyllus Lam.*) DURING STORAGE AND GERMINATION

Thesis
submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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2007

Conclusions

1. **R**ecalcitrant behaviour of *Artocarpus heterophyllus* is established due to the following characters.

   a) Seeds contain high moisture content (50%) and when it is reduced to 33% (critical moisture content) during desiccation, viability is lost within 13 days so the seeds are desiccation intolerant.

   b) Short life span (longevity) of seeds under storage in polythene bags (without loosing moisture content) at room-temperature for 110 days and in refrigerator for 100 days only.

   c) Fresh seeds begin to germinate within 24 hours since germination is in continuum with development.

2 The embryonic axes and cotyledons are starch rich. Starch depletion during desiccation coincident with loss of viability is correlated with enhanced amylolytic activity especially β-amylases.

3. Histochemical study reveals that desiccation injury in embryonic axes is exhibited by the invagination of radicle tip due to desiccation which coincides with viability loss. Cotyledons show desiccation intolerance by

disappearance of starch grains from adaxial side. Embryonic axis and cotyledon are equally vulnerable to desiccation.

4. The histochemical and SEM studies show the presence of simple and compound starch grains of different sizes, shapes and number of individual grains and this heterogeneity of starch grains is unique to Jack fruit seeds.

5. A close relationship is evident between moisture content, depletion of starch, maximum activity of α- and β-amylases, increased sugar content and viability loss of seeds during desiccation and storage.

6. Raffinose family of oligosaccharides, maltose and sucrose are more in seeds when the initiation of desiccation sensitivity was shown and these sugars function as a protective measure against desiccation induced membrane damage leading to loss of viability. But due to high recalcitrant nature of Jack seeds, desiccation tolerance is not shown after 12 days of open-air desiccation.

7. Significant depletion of total and soluble proteins coincides with desiccation induced viability loss after 12 days of desiccation. Peak values of amylase activity and maximum protein content on 12^{th} day indicate enhanced synthesis of enzyme (amylase) protein.

8. Protein profile shows disappearance of high molecular weight protein bands followed by low molecular weight protein bands during desiccation showing their vital role as a requisite for viability.

9. Seeds stored in room-polythene retained viability upto 110 days and that of refrigerator upto 100 days only. Moisture content remains unchanged in room-polythene and refrigerator stored seeds until viability loss which is coincident with the depletion of starch content, accumulation of maltose,

sucrose and raffinose family of oligosaccharides which are involved in imposing desiccation tolerance at these intervals.

10. As viability declines from cent percent, starch depletion is 10% and 11% in desiccated (room open) and stored (room-polythene and refrigerator) seeds respectively.

11. Maltose and sucrose accumulation occurs in seeds stored in room-polythene and refrigerator due to the activities of amylase and phosphorylase respectively and these disaccharides are involved in the compatible protection of membrane and protein damage during storage.

12. Hike in amylase activity in axis tissue on 2^{nd} day of germination with concomitant depletion of starch content and moderate activity of both α- and β-amylases are indicative of the concept that germination is continuous with development in recalcitrant seeds.

13. Histochemical localization of starch during germination shows the pattern of starch grain hydrolysis and their disappearance which starts from adaxial and abaxial sides of cotyledon.

14. During desiccation / storage stress, the constitutive amylases (both α- and β-amylases) are getting induced and their enhanced activity resulting in the formation of maltose and sucrose along with existing raffinose family of oligosaccharides are found to be a requisite for imposing desiccation tolerance at some critical periods such as 12^{th} day of open air desiccation, 100-110 days of room-polythene storage and 90-100 days of refrigerator storage at least for a transient period.

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References

Anilkumar, C. 1998. ***Physiological and Biochemical Aspects of Seed Storage and Viability in Syzygium species.*** Ph.D. Thesis, University of Calicut.

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

Anilkumar, C., Krishnan, P.N. and Nabeesa Salim 2000. Seed viability of *Syzygium aromaticum* (L) Merril and Perry during storage. ***Centennial Conference on Spices and Aromatic Plants*** 20-23.

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

Anonymous.1994. ***The Useful Plants of India***, Publication and information directorate, Vol. **I.** pp 57 CSIR, New Delhi.

Anonymous 2000. ***The Wealth of India*-First supplement series** (Raw materials) Vol. **I.** 97-98. CSIR, New Delhi.

Anonymous 2003. ***The Wealth of India.*** Raw materials Vol. **I** pp 447-453 CSIR, New Delhi.

Ashford, A.E. and Gubler, F. 1984. Mobilization of polysaccharide reserves from endosperm. In: Murray, D.R. (Ed.) ***Seed Physiology*** **Vol. 2.** ***Germination and Reserve Mobilization.*** Academic press, Tokto. 117-162.

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 related to acquisition of drying tolerance and seed quality. ***J. Expt. Bot.*** **52:** 701-708.

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

Berjak, P. and Dumet, D. 1996. Cryopreservation of seeds and isolated embryonic axes of neem (*Azadirachta indica*). ***Cryo-Letters*** **17:** 99-104.

Berjak, P., Dini.M. and Pammenter, N.W. 1984. Possible mechanism underlying the differing dehydration responses in recalcitrant and orthodox seeds; desiccation-associated subcellular changes in the propagules of *Avicennia marina.* ***Seed Sci. Technol.*** **12**: 365-384.

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

Berjak, P., Farrant, J.M. and Pammenter, N.W. 1990. Recalcitrant (homoiohydrous) seeds; the enigma of their desiccation sensitivity. ***Seed Sci. Technol.*** **18**: 297-310.

Berjak, P., Vertucci, C.W. and Pammenter, N.W. 1993. Effects of developmental 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. ***Botanical Microtechnique and Cytochemistry***. The lowa State University Press. Ames, Iowa. pp.326.

Bernal-Lugo, I. and Leopold, A.C. 1992. Changes in soluble carbohydrates during seed storage. ***Plant Physiol.*** **98:** 1207-1210.

Bernal-Lugo, I. and Leopold, A.C. 1995. Seed stability during storage: raffinose content and seed glassy state. ***Seed Sci. Res.*** **5**: 75-80.

Bernfeld, P. 1955. Amylase, K and 2. In : S.P. Colowich and N.O.Kaplan (Ed.) ***Methods in Enzymology*. Vol. 1.** Academic Press. Inc., New York. 149-158.

Bewley, J.D. and Black, M. 1982. ***Physiology and Biochemistry of Seeds in Relation to Germination*, Vol. 2. Viability, *Dormancy and Environmental Control.*** Springer- Verlag, New York. pp. 306.

Bewley, J.D. and Black, M. 1994. ***Seeds: Physiology of Development and Germination***. (2^{nd} edn.). Plenum Press, New York. pp. 445.

Black, M., Corbineau, F., Grzesik, M., Guy, P. and Come, D. 1996. Carbohydrate metabolism in the developing and maturing wheat embryo in relation to its desiccation toleance. ***J. Expt. Bot.* 47**: 161-169.

Black, M., Corbineau, F., Gee, H. and Come, D. 1999. Water content, raffinose, and dehydrins in the induction of desiccation tolerance in immature wheat embryos. ***Plant Physiol.* 120:** 463-472.

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

Bonner, F.T. 1996a. Commercial seed supply of recalcitrant and intermediate seed: present solutions to the storage problem. Papers presented. www. ipgri.c giar. org / publications / HTML Publications 67/ch 02. htm. 513K.

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

Brenac, P., Horbowicz, M., Downer, S.M., Dickerman, A.M., Smith, M.E. and Obendorf, R.L. 1997. Raffinose accumulation related to desiccation tolerance during maize (*Zea mays* L.) seed development and maturation. ***J. Plant Physiol.* 150:** 481- 488.

Bryant, G., Koster, K.L. and Wolfe, J. 2001 Membrane behaviour in seeds and other systems at low water contents: the various effects of solute. ***Seed Sci. Res.*** **11**: 17-29.

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.

Chaitanya, K.S.K. and Naithani, R. 1994. Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seed of *Shorea robusta* Gaertn. ***New Phytol.*** **126**: 623-627.

Chaitanya, K.S.K, Keshavkanth, S. and Naithani,S.C. 2000a. Changes in total protein and protease activity in dehydrating recalcitrant Sal (*Shorea robusta*) seeds. ***Silva Fennica*** **34 (1):** 71 -77.

Chaitanya, K.S. K., Naithani, R and Naithani, S.C. 2000b. Ascorbic acid metabolism in ageing recalcitrant Sal. (*Shorea robusta* Gaertn. f.) seeds. ***Indian J. Expt. Biol.*** **38:** 1031-1035.

Chaitanya, K.S. K., Varghese, B. and Naithani, S.C. 2001. Changes in nucleic acid content and RNAse activity in recalcitrant Sal seeds. ***Indian J. Plant Physiol.*** **6**: 335-341

Chandel, K.P.S., Choudhary, R., Radhamani and Malik, S.K. 1995. Desiccation and freezing sensitivity in recalcitrant seeds of Tea, Cocoa and Jackfruit. ***Ann. Bot.*** **76:** 443-450.

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. pp28.

Chin, H.F and Roberts, E.H. 1980. ***Recalcitrant Crop Seeds.*** Kuala Lumpur, Malaysia, Tropical Press, pp. 152.

Chin, H. F., Aziz, M., Ang, B. B. and Hamzah, S. 1981. The effect of moisture and temperature on ultrastructure and viability of seeds of *Hevea braziliensis*. ***Seed Sci. Technol.*** **9**: 411-423.

Chin, H.F., Hor, Y.L. and Mohd Lassim, M.B. 1984. Identification of recalcitrant seeds. ***Seed Sci. Technol.*** **12:** 429-436.

Clatterbuck, 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.Quedraogo, K.Poulsen and F. Stubsgard (eds.) ***Intermediate / Recalcitrant Tropical Forest Tree Seeds***. International Plant Genetic Resources Institute. 83-97.

Connor, K.F. and Bonner, F.T. 1998. Physiology and Biochemistry of recalcitrant *Guarea guidonia* (L.) Sleumer seeds. ***Seed Technol.*** **20**: 32-42.

Connor, K. F. and Sowa, S. 2002. Recalcitrant behavior of temperate forest tree seeds: Storage, biochemistry and physiology. Proceedings of the eleventh biennial southern silvicultural research conference, Gen. Tech. Rep.SRS-48, Asheville, NC, US. 47-50.

Copeland, L.D. and McDonald, M.B. 1995. ***Seed Science and Technology*** (3rd edn.) Chapman and Hall, New York: pp. 409.

Corbineau, F. and Come, D. 1988. Storage of recalcitrant seeds of four tropical species. ***Seed Sci. Technol.*** **16**: 97-101.

Corbineau, F., Pichard, M.A., Fougereux, J.A., Ladonne, I. and Come, D. 2000. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. ***Seed Sci. Res.*** **10**: 329-339.

Crowe, J.H., Hoekstra, F.A. and Crowe, L.M. 1992. Anhydrobiosis. ***Ann. Rev. Physiol.*** **54**: 579-599.

Cunha, R. Eira, M.T.S. and Rita, I. 1995. Germination and desiccation studies on wild nutmeg seed (*Virola surinamensis*). ***Seed Sci. Technol.*** **23:** 43-49.

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

Davis, B. D. 1979. Influence of cotyledons upon α-amylase activity in Pea embryonic axes. ***Plant Physiol.*** **63**: 1010-1015.

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

Duarte, O., Suchini.G and Huete, M. 2001. Seed storage time increase of Lychee (*Litchi chinensis*). Proceedings of Interamerican society for Tropical Horticulture. **44**: 92-94.

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

Farnsworth 2000. The ecology and physiology of viviparous and recalcitrant seeds. ***Ann. Rev. ecol. sys.*** **31**: 107-138.

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

Farrant, J. M., Berjak, P. and Pammenter, N.W. 1992. Proteins in development and germination of desiccation sensitive (recalcitrant) seed species. ***Plant growth regul.*** **11**: 257-265.

Farrant, J. M., Berjak, P. and Pammenter, N.W. 1995. Studies on development of desiccation sensitive (recalcitrant) seeds of *Avicennia*

marina (Forsk) Vierh. The acquisition of germinability and response to storage and dehydration. ***Ann. Bot.*** **71:** 405-410.

Farrant, J. M., Pammenter, N.W. and Berjak, P. 1986. The increasing desiccation sensitivity of recalcitrant *Avicennia marina* seeds with storage time. ***Physiol. Plant.*** **67:**291-298.

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

Farrant, J. M., Pammenter, N.W. and Berjak, P. 1993. Seed development in relation to desiccation tolerance. A comparison between desiccation sensitive (recalcitrant) seeds of *Avicennia marina* and desiccation tolerant types. ***Seed Sci. Res.*** **3**: 1-13.

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

Finch-Savage, W.E. 1991. Embryo water status and survival in the recalcitrant species *Quercus robur* L.: Evidence for a critical moisture content. ***J. Expt. Bot.*** **43:** 663-669.

Finch-Savage, W.E. 1992. Seed development in the recalcitrant species *Quercus robur* L.: 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. Papers presented. www. ipgri.c giar. org / publications / HTML Publications 67/ch 02.htm.513K.

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 Work Shop on Seeds: Basic and Applied Aspects of Seed Biology.*** ASFIS, Paris, 723-730.

Finch-Savage, W.E., Pramanik, S.K. and Bewley, J.D. 1994. The expression of dehydrin proteins in desiccation sensitive (recalcitrant) seeds of temperate trees. ***Planta*** **193**: 1994.

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

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.

Groot, S.P.C., van pijlen, J.G., Bergervoet, J.H.W., Kraak H.L. and Bino R.J. 1996. Seed storage behavior in relation to cell cycle progression. Papers presented. www. ipgri.c giar. org / publications / HTML Publications 67/ch 02.htm.513K.

Halperin, S.J. and Koster, K.L. 2006. Sugar effects of membrane damage during desiccation of pea embryo protoplasts. ***J. Expt. Bot.*** **57:** 2303-2311.

Hanson, J. 1984. The storage of seeds of tropical tree fruits. In: J.H.W. Holden and J.T. Williams, (eds.) ***Crop Genetic Resources: Conservation and Evaluation.*** George Allen and Unwin. London. pp 53-62.

Hay, F.R., Probert, R.J. and Coomber, S.A. 1997. Development of desiccation tolerance and longevity in seeds from detached capsules of foxglove (*Digitalis purpurea* L). ***Ann. Bot.*** **79**: 639-647.

Hendry, G.A.F., Finch-Savage, W.E., 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.

Hoekstra, F.A., Haigh, A.M., Tetteroo, F.A.A. and van Roekel. 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. 1996. A protocol to determine seed storage behaviour. IPGRI Technical bulletin No.1.

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

Horbowicz, M. and Obendorf, R.L. 1994 Seed desiccation tolerance and storability: dependence on flatulence producing oligosaccharides and cyclitols- review and surgery. ***Seed Sci. Res.*** **4**: 385-405.

ISTA (1985). International Rules For Seed Testing. ***Seed Sci. Technol.*** **13**: 338-341.

Jisha Mathew. 2006. ***Metabolic Changes of Mango (Mangifera indica Linn.) Seeds During Desiccation and Germination.*** Ph.D. Thesis, University of Calicut.

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

Kabir, S. and Daar, A.S. 1995. Maturation of immunoglobulin- A binding proteins in the seeds of jackfruit. ***Biochem. Mol. Biol. Int.*** **35(5)**: 1049-1057.

Kaplan, F. and Guy, C.L. 2004. *ß*-amylase induction and the protective role of maltose during temperature shock. ***Plant Physiol.*** **135:** 1674-1684.

Keller, F., and Pharr, D.M. 1996. Metabolism of carbohydrates in sinks and sucrose galactosyl-sucrose oligosaccharides. In: Zamski E, Schaffer AA, eds. ***Photoassimilate Distribution in Plants and Crops*** NewYork, NY: Marcel Dekker, 157-183.

Khan, A.A. 1977. (Ed.) The ***Physiology and Biochemistry of Seed Dormancy and Germination,*** North- Holland, Amsterdam. (multiauthor contributions).

King, M.W. and Roberts, E.H. 1979. ***The Storage of Recalcitrant Seeds – Achievements and Possible Approaches.*** IBPGR, Rome.

Kneen, E., Sandsledt, R.M. and Hollenback, C.M. 1943. The differential stability of malt amylases, separation of α- and β- components. ***Cereal Chem.*** **20:** 399-425.

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

Koster, K.L., Lei, Y.P., Anderson, M., Martin, S., and Bryant, G. 2000. Effects of vitrified and non-vitrified sugars on phosphatidylcholine fluid-to-gel phase transitions. ***Biophys. J.*** **78**: 1932-1946.

Kovach, D.A. and Bradford, K.J. 1992. Imbibitional damage and desiccation tolerance of wild rice (*Zizania palustris*) seeds. ***J. Expt. Bot.*** **43**: (747-757).

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**: 231-246.

Krishnapillay, D.L. and Englemann, F. 1996. Alternative methods for the storage of recalcitrant and intermediate seeds: slow growth and cryopreservation. Papers presented. www. ipgri.c giar. org / publications / HTML Publications 67/ch 02.htm.513K.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T_4. ***Nature.*** **227:** 680-685.

Lahuta, L.B., Login, A, Rejowski, A., Socha, A. and Zalewski, K. 2000. Influence of water deficit on the accumulation of sugars in developing

field bean (Vicia faba var. minor) seeds. ***Seed Sci. Technol.* 28**: 93-100.

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.

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., van Aelst, A.C., Pritchard, H.W. and Murphy, D.J. 1998. Oleosins prevent oil-body coalescence during seed imbibition as suggested by a low temperature scanning electron microscope study of desiccation-tolerant and sensitive oilseeds. ***Planta* 204:** 109-119.

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.

Le Tam, V.T., Hong, T.D., Ellis, R.H. and Ngoc-Tam, B.T. 2004. Seed storage of *Avicennia alba* Bl. ***Seed Sci. Technol.* 32:** 531-536.

Li, C. and Sun, W.Q. (1999). Desiccation sensitivity and activities of free radical scavenging enzymes in recalcitrant *Theobroma cacao* seeds. . ***Seed Sci. Res.*** 9: 209-217.

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**: 1911-1922.

Lin. T.P. and Chen, M.H. 1993. Desiccation intolerance in seeds of *Machilus kusanoi* Hay. Taiwan ***For. Res. Ins.* 8**: 143-147.

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

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

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

Magill, W., Deighton, N., Pritchard, H.W., Benson, E.E. and Goodman, B.A. 1994. Physiological and biochemical studies of seed storage parameters in *Carica papaya.* Proceedings of the Royal Society of Edinburgh, **102B:** 439-442.

Malik, S.K., Choudhary, R. and Abraham, Z. 2005. Desiccation-freezing sensitivity and longevity in seeds of *Garcinia indica, G. cambogia* and *G. xanthochymus.* ***Seed Sci. Technol.*** **33:** 723-732.

Mayer, A.M. and Poljakoff-Mayber, A. 1989. ***The Germination of Seeds***, 4th Edn. Pergamon Press, Oxford. pp. 270.

Mazia, D., Brewer, P.A. and Alfert, M. 1953. The cytochemical staining and measurement of protein with mercuric bromopheenol. ***Biol. Bull.*** **104:** 57-67.

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

Murray, D.R. 1984. Axis-cotyledon relationships during reserve mobilization. In: Murray, D.R. (Ed.). ***Seed Physiology*** **vol. 2.** ***Germination and Reserve Mobilization.*** Academic press, Tokto. 247-280.

Muthulakshmi,P. 2003. ***Genetic Diversity and Canopy Management in Jackfruit (Artocarpus heterophyllus Lam).*** Ph.D. thesis, Kerala Agricultural University.

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

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

Nautiyal, A.R., Thapliyal, A.P. and Purohit, A.N. 1985. Seed viability in Sal.1V. Protein changes accompanying loss of viability in *Shorea robusta*. ***Seed Sci. Technol.*** **13:** 83-86.

Nielsen, T.M., Deiting, U. and Stitt, M. 1997. A beta-amylase in potato tubers is induced by storage at low temperature. ***Plant Physiol.*** **113:** 503-510.

Oliveira, L.M.Q. and Valio, I.F.M. 1992. Effects of moisture content on germination of seeds of *Hancornia speciosa* Gom. (Apocyanaceae). ***Ann. Bot.*** **69**: 1-5.

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

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., Vertucci, C.W. and Berjak, P. 1991. Homeohydrous (recalcitrant) seeds: dehydration: the state of water and viability characteristics in *Landolphia kirkii*. ***Plant Physiol.*** **96:** 1093-1098.

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

Peran, R., Pammenter, N.W., 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.

Peterbauer, T. and Richter, A. 2001. Biochemistry and physiology of raffinose family of oligosaccharides and galactosyl cyclitols in seeds. ***Seed Sci. Res.*** **11**: 185-197

Pinheiro, C., Rodrigues, A.P., de Carvelho, I.S., Chaves, M.M. and Ricardo, C.P. 2005. Sugar metabolism in developing lupin seeds is affected by a short-term water deficit. . ***J. Exp. Bot.*** **56**: 2705-2712.

Pritchard, H.W. (1995). Cryopreservation of seeds. In: J.G. Day and M.R. McLellan. ***Methods in Molecular Biology***. Vol. **38.** Humana Press Inc. Totowa. 133 - 143.

Pritchard, H.W. 1996. Biochemical, biophysical and physiological methods for the assessment of recalcitrant and intermediate seed quality. *In:* A.S.Quedraogo, K.Poulsen and F. Stubsgard (eds.) ***Intermediate / Recalcitrant Tropical Forest Tree Seeds***. International Plant genetic resources Institute. 40 - 43.

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-letters*** **19** (1):23-30.

Pritchard, H.W., Haye, A.J., Wright, W.J. and Steadman, K J. 1995. A comparative study of seed viability in *Inga* species desiccation tolerance in relation to physical characteristics and chemical composition of the embryo. ***Seed Sci. Technol.*** **23:** 85-100.

Pritchard, H.W., Tompsett, P.B. and Manger, K.R. 1996. Development of a thermal time model for quantification of dormancy loss in *Aesculus hippocastanum* seeds. ***Seed Sci. Res.*** **6**: 127-135.

Pritchard, H.W., Steadman, K J., Nash, J.V. and Jones, C. 1999. Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds. ***J. Expt. Bot.*** **50:** 1507-1514.

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

Pukacka, S. and Wojkiewicz, E. 2002. Carbohydrate metabolism in Norway maple and sycamore seeds in relation to desiccation tolerance. ***J. Plant Physiol.*** **159**: 273-279.

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

Roberts, E.H, King, M.W., and Ellis, R.H. 1984. Recalcitrant seeds: their recognition and storage. In: ***Crop Genetic Resources: Conservation and Education.*** London: Allen and Unwin, 38-52.

Ruhl, G. 1996. Microscopic methods for subcellular examination of desiccation and chilling sensitivity. Papers presented. www. ipgri.c giar. org / publications / HTML Publications 67/ch 02.htm.513K.

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

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.

Smith, J.W., Pammenter, N.W. and Walters, C. 2001. The effects of two drying rates on the desiccation tolerance of embryonic axes of recalcitrant jack fruit (*Artocarpus heterophyllus* Lam.) seeds. ***Ann. Bot.* 88**: 653-664.

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- 674.

Still, D.W., Kovach D.A. and Bradford, K.J. 1994. Development of desiccation tolerance during embryogenesis in rice (*Oryza sativa*) and wild rice (*Zizania palustris*). ***Plant Physiol.* 104:** 431- 438.

Suda, C.N.K. and Giorgini, J.F. 2000. Seed reserve composition and mobilization during germination and initial seedling development of *Euphorbia heterophylla*.R. ***Bras Fisiol Veg*, 12(3)**: 226-245.

Taiz, L. and Zeiger, E. 2002. ***Plant Physiology*** 3rd Edn. Sinamer Associates, Inc. Publishers. Sunderland, Massachusetts. pp. 690.

Tommasi, F., Paciolla,C. and Arrigoni,O. 1999. The ascorbate system in recalcitrant and orthodox seeds. ***Physiol. Plant.* 105**: 193-198.

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.

Tompsett, P.B. and Pritchard, H.W. 1998. The effect of chilling and moisture status on the germination, desiccation tolerance and longevity of *Aesculus hippocastanum* L. seeds. ***Ann. Bot.* 82**: 249-261.

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

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

Vertucci, C.W. 1990. Calorimetric studies of the state of water in the seed tissues. ***Biophys. J.* 58** : 1463-1471.

Vertucci, C.W. 1993. Predicting optimum storage conditions for seeds using thermodynamic principles. ***J. Seed Res.* 17:** 41-53.

Vertucci, C.W. and Leopold, A.C. 1986. Physiological activities associated with hydration level in seeds. In: Leopold A.C. (Ed.) ***Membranes Metabolism and Dry Organisms***, Ithaca, London, Comstock. 35- 49.

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

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

Whelan, W.J. 1955. Starch, glycogen, fructosans and similar polysaccharides. *In*: M.N.V. Tracey (Ed.) ***Modern Methods of Plant Analysis*** Vol.**13**A, Springer-Verlag, Berlin. pp. 684-699.

Wood, C.B., Pritchard, H.W. and Amritphale, D. 2000. Desiccation-induced dormancy in papaya (*Carica papaya* L.) seeds is alleviated by heat shock. ***Seed Sci. Res.*** **10:** 135-145.

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