IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume **8** | Issue 2 | July, 2013 | 215-220

RESEARCH ARTICLE

Histochemical changes in the cotyledon and embryonic axis of jackfruit (*Artocarpus heterophyllus* Lam) seeds during desiccation

■ S. SHEELA AND NABEESA SALIM

SUMMARY

Jackfruit seeds are recalcitrant and hence desiccation sensitive. Fresh jackfruit seeds were subjected to desiccation by exposing to air drying at room temperature (30±2ºC). During desiccation the seeds retained viability only up to 12 days. Histological and histochemical changes during desiccation up to the loss of viability were studied at comparable intervals during a period of 16 days. Disappearance of starch grains was observed in the cotyledons and the tip of the embryonic axis during desiccation. The distribution of starch grains in the cotyledons and embryonic axis and the histological changes in the embryonic axes were found to be directly related to the loss of seed viability.

Key Words : Histology, Recalcitrant, Starch, Viability

How to cite this article : Sheela, S. and Salim, Nabeesa (2013). Histochemical changes in the cotyledon and embryonic axis ofjackfruit (*Artocarpus heterophyllus* Lam) seeds during desiccation. *Internat. J. Plant Sci.*, **8** (2) : 215-220.

Article chronicle : Received : 09.11.2012; **Revised :** 25.01.2013; **Accepted :** 20.02.2013

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). Several studies have inferred that recalcitrant seeds are sensitive to desiccation and lose viability within a short period of storage in many species including jackfruit seeds and longevity varies from plant to plant and conditions of storage. 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; 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) *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, 1995; Chien and Lin, 1997), *Garcinia* species (Malik *et al.*, 2005).

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
NABEESA SALIM, Department of Botany, Physiology and Biochemistry Division, University of Calicut, CALICUT (KERALA) INDIA
Email: nabeesasalim@gmail.com

Address of the Co-authors:
S. SHEELA, Department of Botany, Govt. Arts and Science College, KOZHIKODE (KERALA) INDIA

Even though desiccation sensitivity has been reported in a large number of recalcitrant seeds histochemical and anatomical changes during desiccation remain to be studied. So the present investigation is an attempt to analyze the histochemical changes of insoluble polysaccharides occurring during desiccation and the relationship between starch distribution and viability in jackfruit seeds which are starch rich. In order to demonstrate and elucidate the dependence or participation of seed viability on insoluble carbohydrates during desiccation the authors have tried to focus on the histochemical changes of insoluble carbohydrates particularly starch content of jackfruit seeds and the distribution of starch grains in the seed parts such as cotyledon and embryonic axis. Histological changes of the embryonic axis during desiccation stress also is an important objective of the present study.

MATERIAL AND METHODS

Jackfruits (*Artocarpus heterophyllus* Lam.) for the present study were collected from a specific (marked) tree growing at Chathannur Village in Kollam District, Kerala. The firm flesh variety (Anonymous, 2000) ofjackfruit was selected for the investigation. Fruits ripened on the mother plant were collected manually and brought to the laboratory, cut open and seeds were depulped, washed and wiped with clean towel. Surface sterilization was done by wiping with a clean towel wetted with 80 per cent ethyl alcohol and kept for desiccation in open trays at room temperature (30±2°C).

Seeds stored at open room condition were sampled for moisture content determination and viability studies at an interval of 8, 12, 14 and 16 days after storage. Fresh seeds immediately after collection served as the control (0 sample). Axes and cotyledons of control and desiccated seeds sampled at each interval 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 using safranin-iodine potassium iodide stain (Johansen, 1940).

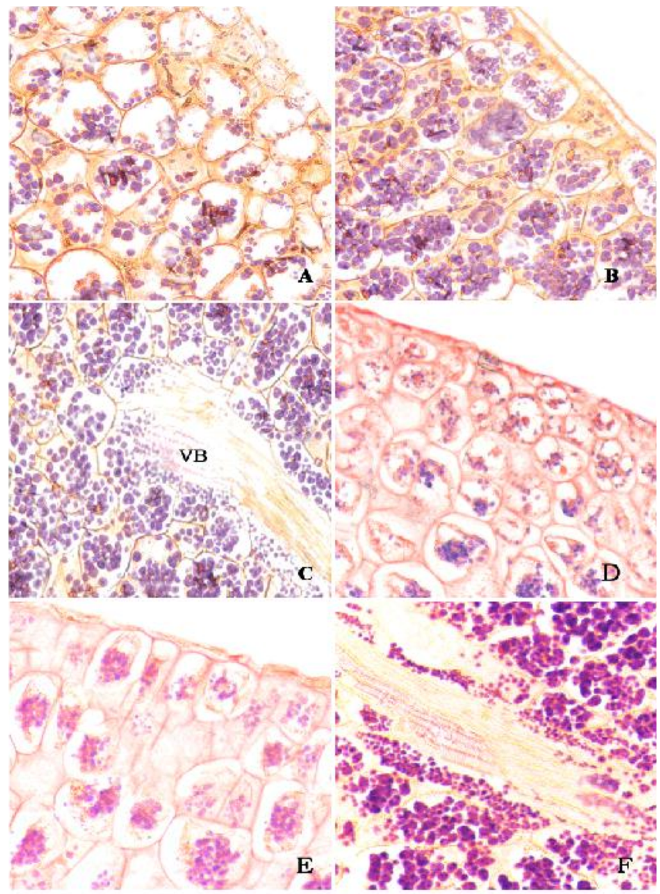
RESULTS AND DISCUSSION

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. The starch grains appear beautifully and even the hilum was visible in the centre of simple grains and individual grains of compound ones.

In cotyledon of control seeds, epidermal cells on the adaxial side were small, elongated and without any starch grains (Fig. 1 A). Epidermal and cortical cells were smaller and contained only 5-6 starch grains /cell. The starch grains showed a gradation of increase in size in the cells towards centre. Nucleus was present in all cells and 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. 1 B). Hypodermal cells contained about 10 to 14 starch grains with various degrees of aggregation. In the centre, cells were comparatively larger, compactly arranged and contained nucleus and about 25 to 40 starch grains per cell (Fig. 1 C). Simple and compound grains (aggregation of 2-10 grains) of different sizes and shapes were present in the central cells. Cross section of cotyledon showed 21 to 34 vascular bundles. Parenchyma cells near vascular bundles contained smaller grains which also showed various degrees of aggregation. Histochemical staining of embryonic axis of control seeds revealed that axis was well protected within the tegmen on one side and a portion of cotyledon on other side (Fig. 4, A, B). Starch grains were present at the extreme tip of radicle.

On the eighth day of desiccation, there were no significant changes in the starch grains compared to the control. 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. 1 D). Cells near the abaxial side contained slightly larger grains and they showed aggregation between all grains within the cells (Fig. 1 E).The cells in the centre of cotyledon were almost similar to that of the control seeds (Fig. 1 F). In the seeds desiccated for eight days, the structure of axis was same as that of the control seeds, but the number of starch grains in the apex showed a slight decrease (Fig. 4 C, D).

On the 12th day of desiccation, when the germination



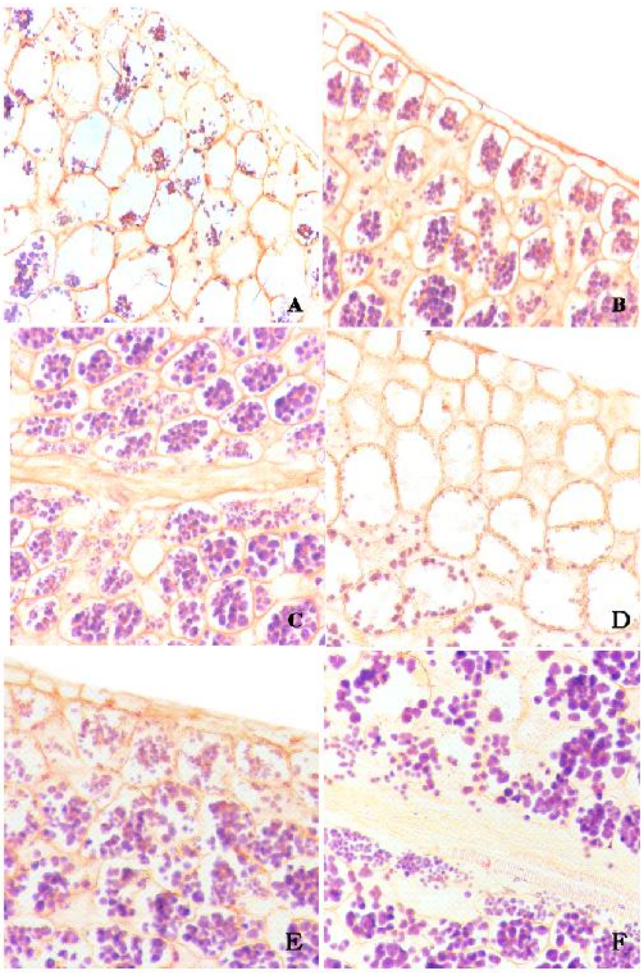
A. Adaxial side, B. Abaxial side, C. Centre } Control; D. Adaxial side, E. Abaxial side, F. Centre } 8 days

Fig. 1: Distribution of starch grains in cotyledon of controljackfruit seeds and desiccated for 8 days

percentage was below 100 per cent desiccation stress was expressed (Table 1). The cells in adaxial side showed smaller grains. Starch grains in the cells inner to the epidermis, showed aggregation into a single mass around the nucleus (Fig. 2 A). The starch grains were closely aggregated around the nucleus (Fig. 2 B). The radicle of seeds stored for 12 days showed the beginning of desiccation induced effects. The cells of radicle tip were slightly distorted (Figs. 4 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 at this interval showed 78 per cent viability (Table 1).

Table 1 : Relationship between moisture content and viability of Jackfruit seeds during desiccation (room-open storage)

Days of desiccation	MC %	Germination %
0	50.19±1.59	100
4	47.99±1.84	100
8	43.15±1.46	100
12	33.58±2.10	78.00±3.46
14	30.21±0.89	41.60±3.24
16	25.70±1.15	8.00±1.80

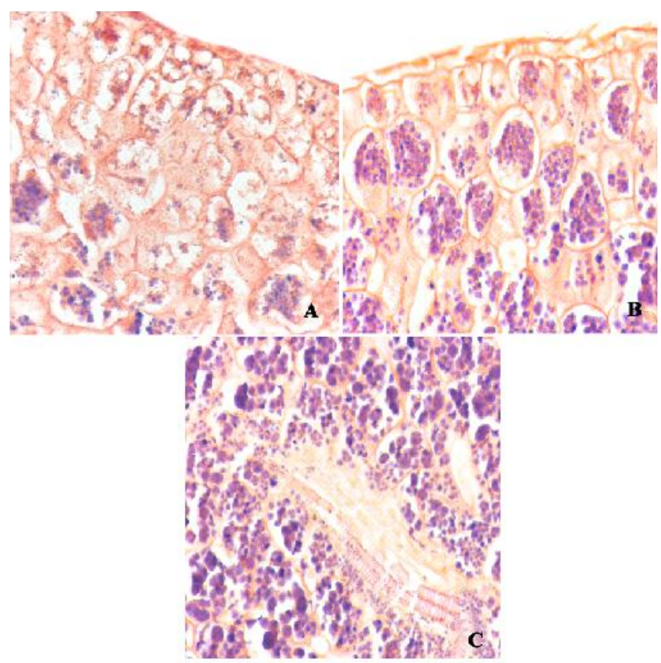


A. Adaxial side, B. Abaxial side, C. Centre } 12 days
D. Adaxial side, E. Abaxial side, F. Centre } 14 days

Fig. 2: Distribution of starch grains in cotyledon of jackfruit seeds and desiccated for 12 days and 14 days

The cotyledon of seeds desiccated for 14 days showed the disappearance of almost all starch grains and nuclei in the cells near the adaxial side (Fig. 2 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 compared to the previous sample (Fig. 2 E). In the centre region, there was a slight beginning of coalescence of grains but they maintained their identity (Fig. 2 F). There was a reduction in number of grains per cell. Here the grains in cells adjacent to the vascular bundles were smaller. In the seeds desiccated for 14 days, radicle showed a clear desiccation induced morphological changes. At the extreme tip, beginning of invagination was observed (Figs. 5 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 per cent viability.

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. 3 A). 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 14th day sample and the nucleus and the grains showed a sort of aggregation (Fig. 3

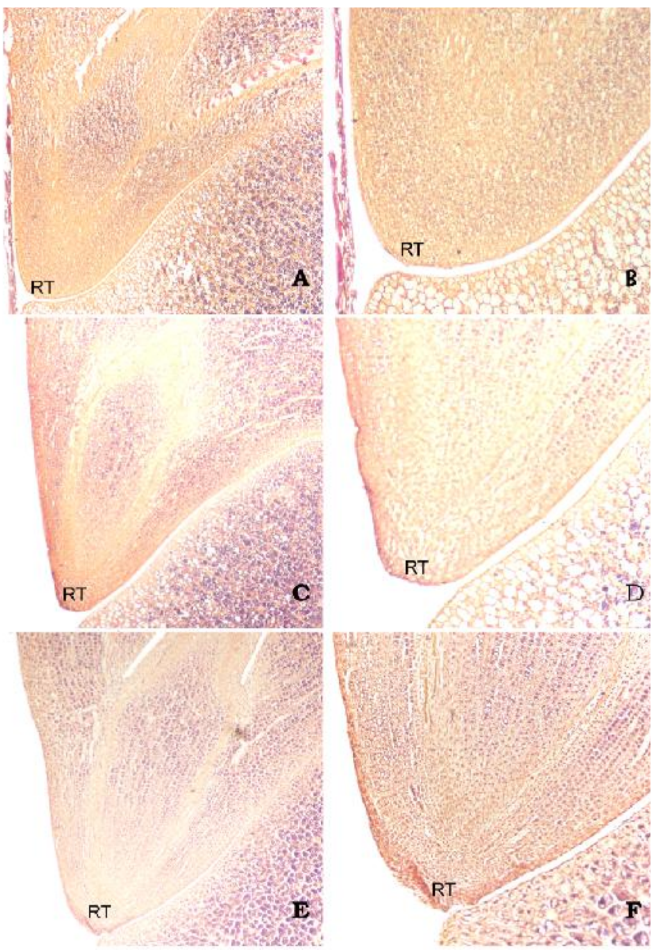


A. Adaxial side
B. Abaxial side
C. Centre

Fig. 3: Distribution of starch grains in cotyledon of jackfruit seeds desiccated for 16 days

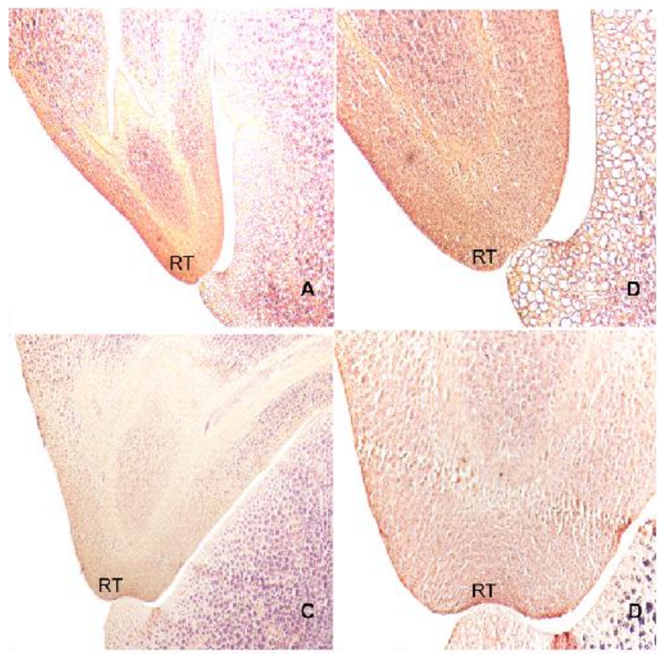
B). The cells in the central region were damaged resultantly changes in shape and loss of integrity of cell wall were occurred. Starch grains in the central cells showed sign of disintegration (Fig. 3 C). The seeds desiccated for 16 days showed more prominent desiccation induced histochemical characters. 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 (Figs. 5 C, D). The cells of apical region were distorted and showed complete disappearance of starch grains. Seeds desiccated for 16 days were non-viable and the viability was only eight percentage.

Histochemical staining using Safranin-Iodine potassium iodide stain of the cotyledons of fresh jackfruit seeds (control) showed abundant starch grains around the nucleus of the cells indicating metabolically active state of seed despite the storage function of starch (Fig. 1 A-C). Even though cent per cent 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. 2 A-C). 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 which are very important characteristic feature of recalcitrant seeds (Pammenter *et al*., 1994) occurring in jackfruit seeds until they become non-viable. Starch grains in the cells of adaxial side of cotyledon of these samples were almost disappeared. These observations clearly indicate that external surface of cotyledon (adaxial side) is directly exposed to the open air drying and hence desiccation stress is more in these cells compared to inner (abaxial) side of the cotyledon. Reduction and / or disappearance of starch grains can be correlated to mobilization 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 found to disappear (Fig.3). Desiccation-associated changes at ultrastructural levels have been described in *Hevea braziliensis* seeds during desiccation and the results showed absence of distinctive nucleus and damaged cell membranes



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

Fig. 4: Changes in embryonic axis of jackfruit seeds during desiccation



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

Fig. 5: Changes in embryonic axis of jackfruit seeds during desiccation for 14 and 16 days

in the embryonic axis due to the detrimental effect of drying (Chin *et al*.,1981).

An interesting observation of histochemical localization of insoluble polysaccharides along with the disappearance of starch from the adaxial side of cotyledonary cells is disintegration of cell wall in the non-viable seeds of 16^{th} day of desiccation, whereas in the samples of 14^{th} day, cell wall appears intact even though starch grains are almost disappeared. As a result of cell wall disintegration, cotyledonary cells stained with safranin-I_2 KI reagent appear as distorted (Fig.3). Ruhl (1996) found that in cocoa seeds, short period of drying induced damages to the radicle and 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 in desiccating seeds may result in an abundant availability of starch for the enhanced amylolytic activity (unpublished data) during 12^{th} day of desiccation in jackfruit seeds (Sheela, 2007).

Histochemical study on the embryonic axis ofjackfruit 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 open air storage. A more or less similar observation was reported in *Avicennia marina* in which the disintegration of the hypocotyls where the meristematic root primordia are developed, suffer lethal damages at water content below 33 per cent wet mass basis (Farrant *et al*., 1997). In desiccation induced non-viable jackfruit seeds, shape of the radicle tip become concave and apical dome shape is lost (Fig. 5). Disappearance of starch grains and nucleus from the cells of radicle tip is very evident in desiccated seeds. This observation confirms the reduction of starch content consequent to loss of viability of desiccated jackfruit seeds which showed starch content depletion and increased amylase activity (Sheela, 2007). 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 one half compared to the initial MC in cotyledon of jackfruit seeds (Sheela, 2007). The overall adverse effect of desiccation is found to be the disintegration of apical cells, and concomitant disappearance of nucleus and breakage of cell walls (Fig. 5). 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 which are detrimental to the seed viability. But in jackfruit seeds, both cotyledons and axis are equally vulnerable to desiccation because desiccation-associated cellular / histochemical changes do occur in both organs / tissues simultaneously.

Acknowledgement :

The first author is grateful to the Head of the Dept. of Botany, University of Calicut for providing necessary facilities and the UGC for granting financial assistance for doing Ph.D. research under Faculty Improvement Programme.

REFERENCES

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

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

Chandel, K.P.S., Choudhary, R., Radhamani, J. 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., 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.

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

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.

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. (1992). Seed development in the recalcitrant species *Quercus robur* L.: germinability and desiccation tolerance. *Seed Sci. Res.,* **2**:17-22.

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.

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.

Johansen, D.A. (1940). *Plant microtechnique*. Mc. Graw Hill, NEW YORK. 523pp.

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.

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.

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.

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

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

Pammenter, N.W., 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.

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.

Sheela, S. (2007). Physiological and biochemical studies onjackfruit *(Artocarpus heterophyllus* Lam.) seeds during storage and germination**.** Ph.D.Thesis, University of Calicut, Calicut, KERALA (INDIA).

Smith, J.W., Pammenter, N.W., Berjak, P. 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.

■ Webliography

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.

8th Year
★★★★★ of Excellence ★★★★★