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Effect of Priming in Enhancing Viability of *Artocarpus heterophyllus* Seeds

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ABSTRACT: Recalcitrant seeds are the short lived ones, which do not survive drying and freezing during *ex-situ* conservation, thus their storage become problematic. Priming helps recalcitrant seeds of jackfruit (*Artocarpus heterophyllus*) to become more resistant to drought stress by enabling them to adapt with water deficiency during storage and thereby increase the viability. Two priming treatments - halopriming (using 75mM NaCl) and osmopriming (using 20% PEG) were imparted in jackfruit seeds, which counter the stress that develops in recalcitrant seed due to water loss. Moisture content and germination percentage of control and primed seeds decreased with increase in storage time. However, the decrease was much faster in control seeds under ambient conditions as compared to seeds stored after both the priming treatments. Priming in jackfruit seeds resulted in enhanced mitochondrial activity to overcome desiccation stress and also resulted in increased lipid peroxidation due to the effect of stress, imparted by the priming agents (NaCl and PEG). Concomitantly, primed seeds exhibited enhanced activity/content of enzymatic and non enzymatic antioxidants than in untreated seeds, indicating detoxify ROS operation by the well defined antioxidant machinery during desiccation or storage period. This resulted in the enhanced viability of primed seeds than control seeds as the desiccation stress imparted damages are repaired in the former. Because of the recalcitrant nature of jackfruit seeds, they are difficult to store and in this context, the extension of the viability period of jackfruit seed was an accomplishment, to achieve the storage of the same successfully.

Keywords: Jackfruit, *Artocarpus heterophyllus*, Recalcitrant seeds, Seed priming, Seed viability

Recalcitrant seeds are a category of seeds which cannot survive desiccation below a particular range (between 12 to 31%) of moisture content [1]. Recalcitrant (desiccation-sensitive) seeds are metabolically active when shed from the mother plant. Even under ambient temperature and low relative humidity, their post-harvest life is very short, either days or months, depending on the species [2]. Some common examples of plants that produce recalcitrant seeds include avocado, cocoa, coconut, jackfruit, leeches, mango, rubber, tea, *Avicennia* species, some horticultural trees, and several plants used in traditional medicine.

Recalcitrant seeds do not undergo dehydration during seed ripening; when shed from the mother plant, they have a high moisture content and active metabolism [2]. Such seeds quickly lose their germination capacity if they are dried below certain, relatively high moisture level, or if they are not stored in a hydrated state. So the seeds are difficult to be successfully stored and their *ex-situ* conservation is problematic. High moisture content nature encourages microbial contamination and results in more rapid seed deterioration [1]. Moreover, storage of recalcitrant seeds at freezing temperatures causes the formation of ice-crystals which disrupt cell membranes and causes freezing injury of seeds.

Rapid dehydration of excised embryonic axes from recalcitrant seeds to water contents approaching the level of non-freezable water permits retention of viability in short-term and this opens up the possibility of long-term conservation. For the long-term storage of recalcitrant seeds a general method has not yet been developed. In the past three decades, seed scientists have attempted several methods to store recalcitrant seeds, such as 'moist' storage, partially dry storage and cryostorage, but all these methods have their own limitations [2].

Priming treatment is known to activate signalling components or transcription factors required for stress perception and tolerance. It also results in activation of cellular defence responses. Priming allows the plants to activate the various defence responses more quickly and/ or effectively when exposed to biotic or abiotic stress [3]. Seed priming has emerged as a promising strategy in modern stress management due to its protection against various biotic and abiotic stresses without heavily affecting the fitness of the plants. Priming helps seeds to become more resistant to drought stress. It has been reported that seed priming prior to storage improved the longevity of seeds [4]. Hossain *et al.* [3] reported that priming before storage delayed the loss of seed viability. It enables seed to adapt with water deficiency during storage and thereby increase the viability period of seed.

The jackfruit (*Artocarpus heterophyllus* Lam.), is a recalcitrant species, belongs to the family Moraceae. It is a widely cultivated and popular food item throughout the tropical regions of the world. The jackfruit has played a significant role in Indian agriculture for centuries and also provides a potential solution to countries facing problems with food security. Because of the recalcitrant nature, the jackfruit seeds are difficult to store [5]. Water losses gradually during the storage period of jackfruit seeds and the seeds may die quickly if conditions are not suitable for germination. So in this context, the extension of the viability period of jackfruit seed is an accomplishment, to achieve the storage of the same successfully.

MATERIALS AND METHODS

Seed Collection and Processing

Mature jackfruits (*A. heterophyllus* Lam., local variety Pazhanchakka) belonging to the family Moraceae, were harvested and depulped without any injury to the seeds. Minimum 900 mature seeds were collected at a time. Of these, 300 seeds were kept in open trays at room temperature (~30° C) and designated as control. Of the remaining 600 seeds, two sets of 300 seeds were sorted for NaCl and PEG priming treatments, respectively. One set of seeds were immersed in 75mM NaCl and other in 20% PEG for 24 hours in open beaker. Then the seeds were washed twice and wiped, and kept in open trays at room temperature (~27±2° C).

Moisture Content Determination

Ten seeds of jackfruit each from control, NaCl and PEG primed seeds were taken at 1 week interval and after determining fresh weights, they were kept in hot air oven at 100°C for 1 hour. Later the temperature of oven was set at 60°C for the remaining period. Drying and weighing were repeated daily until constant values were obtained. Moisture content percentage was calculated as explained by International Seed Testing Association [6].

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Germination Studies

Germination percentage was studied as following procedure. Seeds of jackfruit each from control, NaCl and PEG primed seeds were sown for germination in polythene bags filled with soil on alternate days. Daily count of germinated seeds was taken and the percentage of germination was calculated as given below:

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

Physiological and Biochemical Studies

The different physiological and biochemical parameters of seeds (each from control, NaCl primed and PEG primed) were analysed from the first day of collection till the 28 d at an interval of seven days.

Mitochondrial isolation from seed tissue was carried out according to the protocol of Kolloffel [7]. Oxygen consumption by mitochondria was measured at 25°C using a Clark-type O_2 electrode as per the protocol of Schmitt and Dizengremel [8]. The mitochondrial activity was calculated in terms of µmol O_2 consumed min^{-1} mg^{-1} protein. Osmotic potential of seed cell sap was measured according to Hura *et al.* [9] using a vapour pressure osmometer (Wescor, 5520, USA).

Protein content of the seeds was estimated using Folin-Ciocalteu reagent according to the method of Lowry *et al.* [10]. Total sugar was determined according to the procedure of Montgomery [11]. Total free amino acids were determined according to the method of Moore and Stein [12]. Proline content was determined as described by Bates *et al.* [13]. Malondialdehyde content was estimated as described by Heath and Packer [14].

For the estimation of super oxide dismutase (SOD) activity the protocol of Giannopolitis and Ries [15] was adopted. Guaiacol peroxidase (GPX) activity was measured according to the protocol by Gaspar *et al.* [16].

The estimation of ascorbate and glutathione content was done according to the method of Chen and Wang [17]. Total phenolics were estimated according to the procedure described by Folin and Denis [18].

RESULTS AND DISCUSSION

Moisture content percentage decreased with increasing storage period in case of both control and primed seeds of jackfruit. During the initial days of storage (1- 7 d) moisture content in control and NaCl primed seeds was found to slightly increase. From 7 d onwards, the reduction in moisture content was gradual in both control and primed seeds. But the percentage reduction of moisture content was at a slower phase in both primed seeds with increase in drying period as compared to control (Figure 1A).

Germination percentage decreased with increasing storage period in case of both control and primed seeds of *A. heterophyllus*. Control jackfruit seeds showed >80% germination during the initial days of storage (1-7 d) and seeds remained viable only up to 21 d of storage. NaCl and PEG primed seeds showed 80% germination up to 11 d of storage and thereafter it declines to 20% germination on 28 d (Figure 1B). Though it was maintained up to 37 d and 39 d in NaCl primed and PEG primed seeds, respectively, but the germination fell close to zero so the data has not been given.

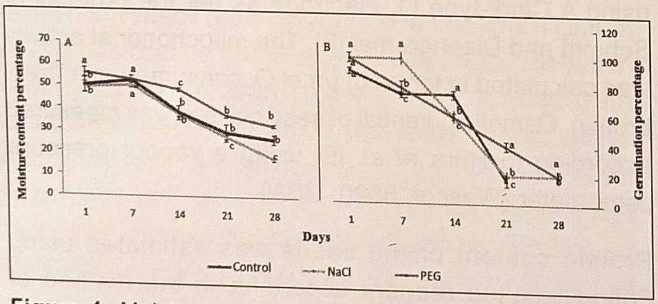

Figure 1. Moisture content percentage (A) and germination percentage (B) of *A. heterophyllus* seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Mitochondrial activity of the seeds of *A. heterophyllus* primed with NaCl and PEG showed an enhanced profile over the control seeds during storage. Control seeds showed maximum mitochondrial activity on 1 d and further it decreased. NaCl and PEG primed seeds showed increased rate of mitochondrial activity from 14-28 d of storage. As compared to the control, NaCl primed seeds showed maximum activity on the 21 d and PEG primed seeds showed maximum activity on the 14 d than control. PEG primed seeds recorded high mitochondrial activity than the control and NaCl primed seeds during the later stages of the storage period (14-28 d) (Figure 2).

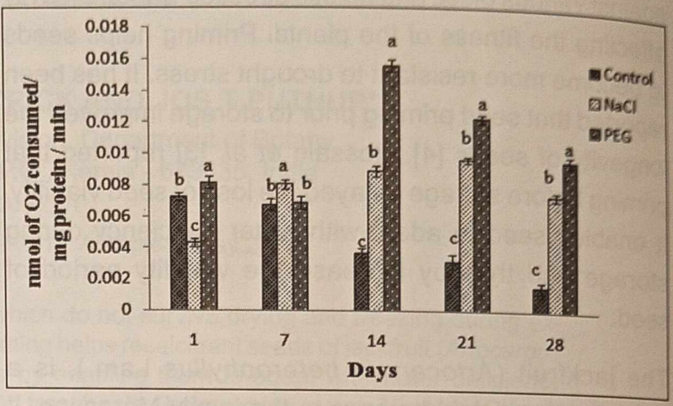

Figure 2. Mitochondrial activity of *A. heterophyllus* seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Osmotic potential (ø) of control seeds decreased with increase in storage period of seeds and this trend remained same in both NaCl and PEG primed seeds. The highest decrease in ø was observed on 28 d and it was 95, 86 and 102% in control, NaCl primed and PEG primed seeds, respectively, as compared to the seeds of 1 d (Figure 3).

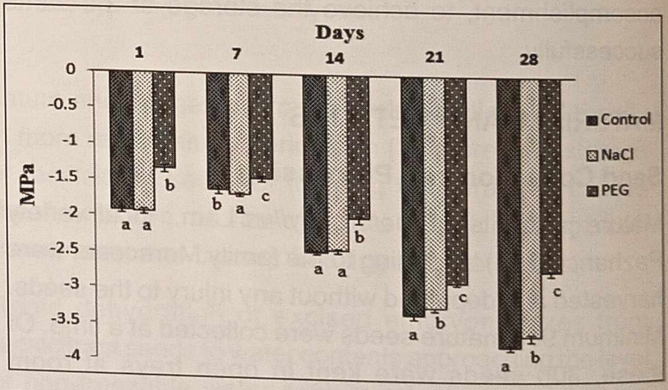

Figure 3. Osmotic potential of *A. heterophyllus* seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Total protein content of control and primed seeds decreased during storage period. Maximum protein content was showed on the 1 d, in which NaCl primed seeds showed 50% increase and PEG primed seeds showed 40% increase as compared to the control seeds. Further, on 7 d there was a drastic reduction of protein in seeds of all treatments and protein content of primed seeds was less than that of control and from 14 d onwards the protein content in primed seeds enhanced above than that of the control. An enhancement in sugar content was recorded in both control and primed seeds with increase in the storage period. Primed seeds exhibited higher accumulation of sugar than control seeds during initial days of storage period (up to 14 d). Further, from 21 d onwards, reduction in sugar content accumulation was recorded in primed seeds as compared to control seeds. NaCl and PEG priming induced a significant increase in the total free amino acid (FAA) content in the seeds of *A. heterophyllus* during storage. Control seeds showed an initial increase of FAA on 7 d and then a gradual reduction occurred. As compared with the control seeds, FAA content in NaCl primed seeds was less whereas in PEG primed seeds, it was higher than control and NaCl primed seeds, throughout the storage period. Proline content increased significantly during 7 and 14 d of the storage period in control and primed seeds. Thereafter, the proline content decreased (Table 1).

The MDA content in seeds of *A. heterophyllus* increased during storage. Although, initially a decrease in MDA content was recorded in control as well as primed seeds, a gradual progression of MDA content was recorded up to 28 d. From 7 d onwards the seeds subjected to priming showed higher MDA content than that of control seeds (Figure 4).

The activity of SOD was almost equal in both control and primed seeds and further exhibits a slight increase during storage period. The activity of SOD enhanced in seeds of *A. heterophyllus* when exposed to PEG priming treatment on 21 d and the increase was to the extent of 28% (Figure 5A). Guaiacol peroxidise activity of control and primed seeds increased during storage period. During the initial days of storage, activity of GPX was very less and from 14 d onwards it showed a sudden increase (Figure 5B).

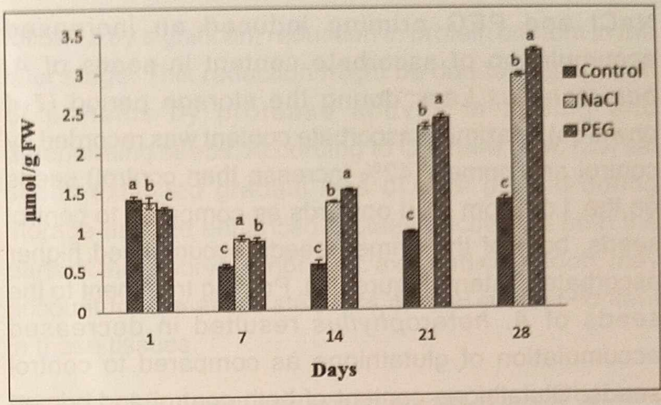

Figure 4. MDA content of *A. heterophyllus* Lam. seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

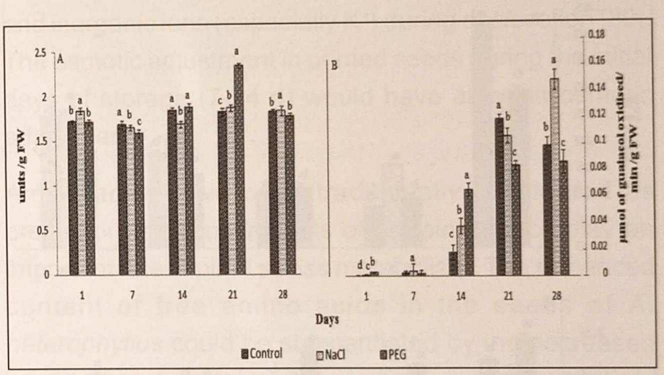

Figure 5. Super oxide dismutase (A) and guaiacol peroxidise activity (B) of *A. heterophyllus* Lam. seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Table 1. Total protein, total sugar, total free amino acid and proline content of *A. heterophyllus* Lam. seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Parameters	Treatment/Days	1	7	14	21	28
Total protein	Control	1481.25±28.3^{c}	718.74±13.2^{a}	650.76±12.2^{b}	663.82±12.2^{c}	688.27±12.5^{c}
	NaCl	2217.18±42.3^{a}	637.5±10.9^{b}	609.37±10.7^{c}	684.52±12.6^{b}	714.84±13.2^{b}
	PEG	2072.77±39.4^{b}	589.05±10.1^{c}	693.75±12.8^{a}	728.25±13.3^{a}	743.73±13.5^{a}
Sugar	Control	0.46±0.01^{b}	19.04±0.04^{c}	20.75±0.02^{b}	48.72±0.04^{a}	84.71±0.03^{a}
	NaCl	0.42±0.04^{c}	45.09±0.02^{a}	21.44±0.03^{b}	32.43±0.01^{c}	64.61±0.04^{b}
	PEG	0.961±0.01^{a}	26.05±0.03^{b}	32.3±0.04^{a}	33.96±0.01^{b}	38.65±0.03^{c}
Amino acid	Control	128.33±3.2^{a}	391.65±6.4^{a}	357.42±5.8^{b}	350.78±5.4^{b}	327.77±5.6^{b}
	NaCl	118.61±3^{b}	262.22±5.7^{c}	195±3.8^{c}	218.45±3.9^{c}	253.88±5.4^{c}
	PEG	75.83±2.7^{c}	373.61±6^{b}	519.99±8.4^{a}	523.83±8.2^{a}	525.83±8.3^{a}
Proline	Control	0.55±0.03^{c}	2.35±0.04^{c}	4.404±0.02^{b}	3.76±0.02^{b}	2.97±0.07^{b}
	NaCl	1.71±0.02^{b}	3.61±0.07^{b}	4.77±0.04^{a}	3.99±0.04^{a}	2.86±0.05^{c}
	PEG	2.68±0.05^{a}	5.58±0.03^{a}	3.69±0.03^{c}	3.61±0.05^{c}	3.25±0.05^{a}

NaCl and PEG priming induced an increased accumulation of ascorbate content in seeds of *A. heterophyllus* Lam. during the storage period (7 d onwards). Maximum ascorbate content was recorded by control and primed (42% increase than control) seeds on the 1 d. From 21 d onwards as compared to control seeds, both of the primed seeds accumulated higher ascorbate content (Figure 6A). Priming treatment to the seeds of *A. heterophyllus* resulted in decreased accumulation of glutathione as compared to control seeds. Glutathione content of both control and primed seeds decreased gradually during storage period (Figure 6B). Significant decrease in the accumulation of phenolic content was recorded in *A. heterophyllus* seeds subjected to NaCl and PEG priming over the control seeds during initial days of storage (0-7 d). Further, 14 d onwards there was an enhancement of phenolic content recorded in primed seeds than control seeds (Figure 6C).

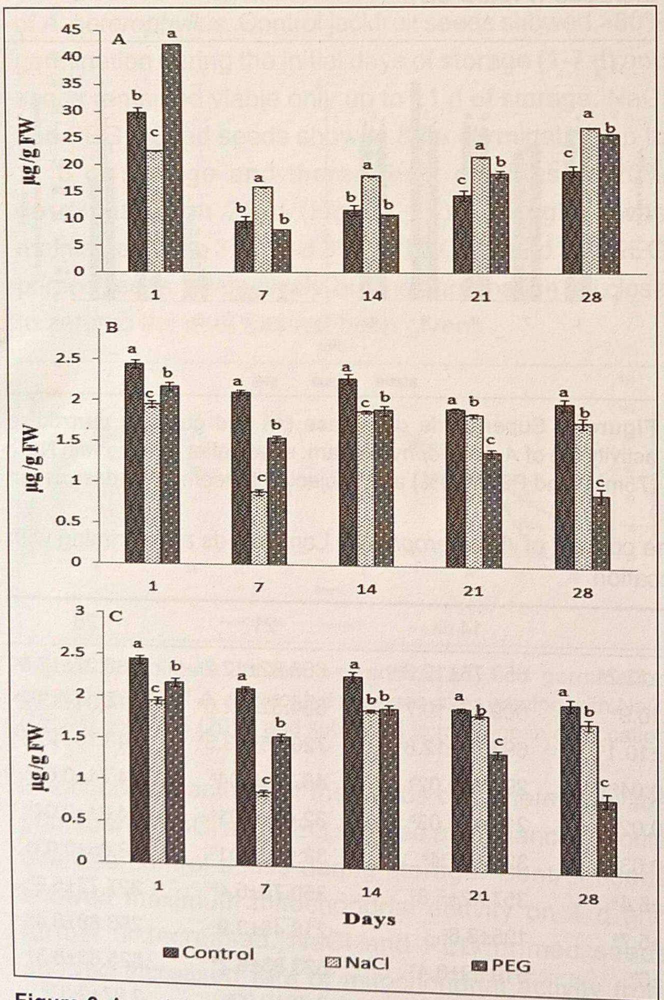

Figure 6. Ascorbate content (A), glutathione content (B) and total phenolics content (C) of *A. heterophyllus* Lam. seeds after priming with NaCl (75mM) and PEG (20%) and subjected to room-open desiccation

Recalcitrant seeds are rich in water content since they are shed from the mother plant without maturation drying. When fresh recalcitrant seeds begin to dry, viability is reduced as its moisture content is lost [19]. The fresh seeds of *A. heterophyllus* Lam. contain ~50% moisture content and its viability is lost when desiccated below 25% moisture content [20]. In our study, moisture content in control seeds reaches nearly 25% by 21 d, below which no germination occurs indicating the total loss of viability. Primed seeds show more than 25% moisture content even on 28 d. Thus it is clear that both priming equip the seeds with the capacity for upholding moisture content for a longer period and thereby improving the viability, which extended up to 28 d.

Generally, recalcitrant seed bearing plant species face the problem of early loss of seed viability due to the intolerance towards desiccation and *A. heterophyllus* Lam. belongs to this category. According to Pammenter and Berjak [2], the rapid loss of viability of recalcitrant seeds is due to loss of water from embryonic axes with increasing desiccation period. As water is lost, ROS are generated due to unbalance of aqueous based metabolism [21]. Jackfruit seeds exhibit typical recalcitrant behaviour during storage because these seeds are shed at high moisture content, highly sensitive to desiccation and viability declines with water loss [5].

Singh *et al.* [22] reported that seed priming is an effective and easy technique for increasing the capacity of seeds for tolerating stress. It also has advantages like reduction in the leakage of metabolites from seeds, repairs the deteriorated seed parts etc. Specific stress-responsive system may also be enhanced in primed seeds, for example, osmopriming induced accumulation of stress proteins, such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSP) in tomato plants [23]. Studies conducted by Jose *et al.* [24] in seeds of *Eucalyptus* sp. found that priming of the seeds enhance stress tolerance of the plantlets emerging from it.

Mitochondrial activity in seeds provides energy and carbon skeletons for other cellular processes. Mitochondrial activity in desiccating control seeds was found to decrease during storage. Pammenter and Berjak [2] reported that increased mitochondrial organisation in the seeds of recalcitrant species indicate increased respiratory activity. But seed deterioration can affect the oxidative properties of mitochondria which will result in impaired ATP production. Cytochrome C oxidase activity of *Antiaris toxicaria* axis mitochondria decreased with dehydration, it was assumed that the mitochondria membranes gradually deteriorated with dehydration [25]. *A. heterophyllus* seeds primed with NaCl and PEG exhibited an enhanced mitochondrial activity over the control seeds from 14 d onwards. Outer and inner membrane integrity of the mitochondria was identified as the primary target for desiccation stress and priming can improve the integrity of the outer membrane of mitochondria and also increase the number of mitochondria in plant tissues [4] and hence the mitochondrial activity was higher in primed seeds than control.

In primed seeds of jackfruit, the reduction of osmotic potential (OP) as compared to control seeds was recorded on 21 d of storage (desiccation/water stress). There was no reduction of OP in NaCl and PEG primed seeds than control (but it was higher especially in PEG primed seeds) at early days of storage and it could be because of early adjustment of osmoticum as a preparative step towards countering water stress, in response to tissue dehydration. Sevik and Cetin [26] studied the effects of osmopriming on *Phaseolus vulgaris* seeds and observed that the seeds presented a progressive decrease of osmotic potentials, which became more negative, indicating an effective osmotic adjustment.

The increase of total proteins during early days of desiccation in seeds of jackfruit may be due to the expression of new stress related proteins. Control and primed seeds showed maximum protein content on first day of storage, in which NaCl primed seeds recorded 50% increase and PEG primed seeds recorded 40% increase as compared to that of control. This increase in protein content of primed seeds could be due to production of dehydrin proteins which are responsible for desiccation tolerance. A specific class of dehydration proteins that become prevalent in desiccation tolerant seeds are Em-proteins, LEA proteins and ABA-responsive (Rab) proteins [27]. Moreover, lectins such as jacalin and artocarpin contents also would be high in the initial stages, which have a significant role in maintaining seed quality. Jagtap and Bapat [28] suggested that jacalin and artocarpin has a wide range of properties, which increase the seed quality in jackfruit seeds. Priming induced protein synthesis of *A. heterophyllus* seeds in the initial days of treatment was followed by significant reduction in protein content in the later stage. This reduction might be due to degradation of proteins by protease activity in ageing and deteriorating seeds. According to Chandra *et al.* [29] sal seeds exhibited gradual loss of total protein during storage and the enhanced protease activity in both the parts of the embryo, embryonic axis and cotyledon, may account for the associated loss of total protein content in these tissues.

Primed jackfruit seeds showed increase in sugar content than control seeds during initial days of storage. Increased accumulation of sugar content recorded in the primed (NaCl and PEG) seeds of *A. heterophyllus* on 7-14 d of storage would have aided in maintaining the membrane integrity under stressed conditions. Most of the osmotic adjustment of cell was accounted by increases in concentration of a variety of common solutes, including sugars, organic acids, amino acids, and inorganic ions (especially K^+) during desiccation [30]. The osmotic adjustment in primed seeds during the initial days of storage (7-14 d) would have aided in osmotic adjustment.

Amino acids have been traditionally considered as precursors and constituents of proteins, which play an important role in plant stress metabolism. The enhanced content of free amino acids in the seeds of *A. heterophyllus* could be substantiated by the decreased production of proteins upon exposure to desiccation. Amino acid content increased during the storage period in the case of NaCl and PEG primed seeds while, in control no significant increase was recorded. This reveals the direct role of amino acid in the metabolic process related to tolerance of desiccation stress in seeds especially those subjected to priming.

The increased rate of proline accumulation in the seeds of *A. heterophyllus* under NaCl and PEG priming treatments indicates that it may play a significant role in stress tolerance. In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses. Proline has the ability to mediate osmotic adjustment, stabilize sub cellular structures and scavenge free radicals. The elevated level of proline content observed in *Vigna radiata* seeds subjected to halopriming indicates its prominent role in osmotic stress tolerance [4].

Generation of ROS is a common process in plant metabolism under normal physiological condition. Its production in various sites of plant cell (viz. chloroplast, mitochondria, plasma membrane, peroxisomes, apoplast, ER and cell wall) is increased to harmful levels during dehydration [3]. Malondialdehyde is the final product of lipid peroxidation which is described to cause oxidative damage and thus measurement of MDA levels is routinely used as a sensitive index of oxidative stress. The increased MDA production in recalcitrant seeds indicates the induction of secondary oxidative damage in the tissue. Priming results in increase of lipid peroxidation, which is due to the effect of stress, imparted by the priming agents NaCl and PEG.

Enhanced activities of antioxidant enzymes result in quick scavenging of ROS and thus help in avoiding the oxidative stress. SOD and GPX are two of the major enzymes involved in maintaining seed longevity during storage. SOD functions to catalyse the dismutation of superoxide anion radical ($O_2^{\cdot-}$) with great efficiency, resulting in the production of H_2O_2 and O_2 [31]. The primed jackfruit seeds maintained SOD level throughout the storage period at a level which was recorded on the initial day and which was almost equal to the level of SOD activity generally shown by plants under stress. Therefore, the SOD activity recorded right from the beginning of desiccation in jackfruit seeds would be sufficient to take care of the super oxides synthesised during desiccation stress and hence, no significant increase of SOD activity was recorded during further storage period, neither in the control nor the primed seeds.

Activity of SOD produces hydrogen peroxide (H_2O_2) that is converted into water either by catalase or ascorbate/guaiacol peroxidase. There was also a reduction in GPX activity during the early stages of storage (until 7 d). Jisha *et al.* [4] suggested that the down regulation of protective programs during priming could be restored by slow-drying of primed seeds, which is consistent with our results. Moore *et al.* [32] stated that the increasing protection afforded by seeds of *Quercus robur* by the enzymatic defences in the cotyledons may have had a significant role in minimizing lipid peroxidation in these tissues.

Antioxidants like ascorbate, glutathione and phenolics are the most important non-enzymatic ROS scavenging compounds which performs very critical role in the defence system and prevents oxidation of vital components. Ascorbate is a ubiquitous soluble antioxidant in plant cells which can directly scavenge ROS and act as a reducing substrate for APX and GPXs to detoxify H_2O_2 [33]. The increased ascorbate content recorded in *A. heterophyllus* under NaCl and PEG priming might be playing a major role in counteracting/neutralizing the harmful effects of ROS produced in the cells.

The data suggests that antioxidant activities in seeds were tightly associated with the physiological status. For example, the overall antioxidant activities, especially that of AsA-GSH cycle, decreased with a lesser germination percentage in both control and primed seeds. Thus, it is possible that a renewal of antioxidant system may be initiated with the repair of cell membranes and organelle development, which are required for complete germination and growth demands of the seedlings. Like ascorbate, glutathione which is termed as ubiquitous or universal redox buffer, plays an important role in several physiological processes including regulation of sulphate transport, signal transduction, several growth and development related events and is also a potential scavenger of O_2 and H_2O_2 [34]. Glutathione have participation in decomposition of H_2O_2 generated by SOD in different cellular organelles. Thus, the reduction of glutathione content in jackfruit seeds, especially after priming may be due to the enhanced activity of GPX and supplementation of glutathione (GSH) towards GPX for maintaining the enhanced activity.

Phenolics are heterogeneous group of secondary metabolites which are originated in the endoplasmic reticulum and located in cell vacuoles and also located in cell walls. In general, the metabolism of phenolics in recalcitrant seeds is very important because these compounds are involved in the activity of cell especially during desiccation. Primed seeds have lower phenol content than control because in primed seeds desiccation stress is not immediately visible because of its increased desiccation tolerance.

Enhanced viability was shown by primed seeds than control because the damages imparted by desiccation stress get repaired in the former. The increased accumulation of protein in primed seeds confirms that they exhibit tolerance towards desiccation stress by the increased production of various stress related proteins. Priming results in increase of lipid peroxidation, which is due to the effect of the stress imparted by the priming agents (NaCl and PEG). Concomitantly, primed seeds exhibited enhanced activity/content of enzymatic and non enzymatic antioxidants than control seeds, indicating that well defined antioxidant machinery is operating in order to detoxify ROS generated during desiccation or storage period. The increased levels of antioxidants mainly GPX, ascorbate and phenolics could prevent oxidative damage and enhance the tolerance of *A. heterophyllus* seeds towards desiccation stress ultimately aiding in enhancing the viability of seeds.

REFERENCES

1. ROBERTS EH (1973). Predicting the storage life of seeds. *Seed Science and Technology,* **1**: 499-514.
2. PAMMENTER NW AND P BERJAK (2013). Development of the understanding of seed recalcitrant and implications for *ex situ* conservation. *Biotecnología Vegetal,* **13**(3): 131-144.
3. HOSSAIN MA, S BHATTACHARJEE, SM ARMIN, P QIAN, W XIN, HY LI AND LSP TRAN (2015). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Frontiers in Plant Science,* **6**:1-19. doi: 10.3389/fpls.2015.00420.
4. JISHA KC, K VIJAYAKUMARI AND JT PUTHUR (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum,* **35**(5): 1381-1396.
5. SHEELA S AND N SALIM (2013). Dynamics of water relations and recalcitrant behaviour of jackfruit (*Artocarpus heterophyllus*) seeds. *International Journal of Plant Science,* **8**(2): 288-293.
6. ISTA (1985). International rules for seed testing. *Seed Science and Technology,* **13**: 338-341.
7. KOLLOFFEL C (1967). Respiration rate and mitochondrial activity in the cotyledons of *Pisum sativum* during germination. *Plant Biology*, **16**: 111–122.
8. SCHMITT N AND P DIZENGREMEL (1989). Effect of osmotic stress on mitochondria isolated from etiolated mung bean and sorghum seedlings. *Plant Physiology and Biochemistry,* **27**: 17-26.
9. HURA T, K HURA, A OSTROWSKA, M GRZESIAK AND K DZIURKA (2013). The cell wall-bound phenolics as a biochemical indicator of soil drought resistance in winter triticale. *Plant Soil Environment*, **59**: 189–195.
10. LOWRY OH, NJ ROSEBROUGH, AL FARR AND RJ RANDALL (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry,* **1**: 265-75.
11. MONTGOMERY R (1957). Determination of glycogen. *Archives of Biochemistry and Biophysics*, **67**: 373–386.
12. MOORE S AND WH STEIN (1948). Photometric ninhydrin method for use in chromatography of amino acids. *Journal of Biological Chemistry,* **176**: 367-388.
13. BATES LS, RP WALDREN AND IK TEARE (1973). Rapid determination of free proline for water studies. *Plant Soil*, **39**: 205.
14. HEATH RL AND L PACKER (1968). Phytoperoxidation in isolated chloroplasts I- kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics,* **125**: 189-198.
15. GIANNOPOLITIS CN AND SK RIES (1977). Superoxide dismutases: I. occurrence in higher plants. *Plant Physiology,* **59**: 309-314.
16. GASPAR T, C PENEL AND H GREPPIN (1975). Peroxidase and isoperoxidase in relation to root and flower formation. *Plant Biochemistry Journal*, **2**: 33-47.
17. CHEN JX AND XF WANG (2002). Guide to plant physiological experiments, Guangzhou: South China University, Technology Press. pp: 123–127.
18. FOLIN O AND W DENIS (1915). A colorimetric method for the determination of phenols (and phenol derivatives) in urine. *Journal of Biological Chemistry*, **22**: 305-308.
19. ASOMANING JM, NS OLYMPIO AND M SACANDE (2011). Desiccation sensitivity and germination of recalcitrant *Garcinia kola* Heckel seeds. *Research Journal of Seed Science,* **4**(1): 15-27.
20. MALIK SK, R CHAUDHURY AND Z ABRAHAM (2005). Desiccation- freezing sensitivity and longevity in seeds of Garcinia *indica, G. cambogia* and *G. xanthochymus. Seed Science and Technology,* **33**(3): 723-732).
21. BERJAK P, B VARGHESE AND NW PAMMENTER (2011). Cathodic amelioration of the adverse effects of oxidative stress accompanying procedures necessary for cryopreservation of embryonic axes of recalcitrant seeded species. *Seed Science Research,* **21**(3): 187-203.
22. SINGH BK, Y RAMAKRISHNA AND VK VERMA (2015). Chow-Chow (*Sechium edule*): Best alternative to shifting cultivation in Mizoram. *Indian Journal of Hill Farming,* **28**(2): 158-161.
23. WANG W, B VINOCUR AND A ALTMAN (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* **218**(1): 1-14.
24. JOSE AC, NCN SILVA, JMR FARIA AND WVS PEREIRA (2016). Influence of priming on *Eucalyptus* sp. seeds tolerance to salt stress. *Journal of Seed Science,* **38**(4): 329-334.
25. CORTES-ROJO C, M ESTRADA-VILLAGOMEZ, E CALDERÓN-CORTES, M CLEMENTEGUERRERO, R MEJÍA-ZEPEDA, I BOLDOGH AND A SAAVEDRA-MOLINA (2011). Electron transport chain dysfunction by H_2O_2 is linked to increased reactive oxygen species production and iron mobilization by lipoperoxidation: studies using *Saccharomyces cerevisiae* mitochondria. *Journal of Bioenergetics and Biomembranes,* **43**(2): 135-147.
26. SEVIK H AND M CETIN (2015). Effects of water stress on seed germination for select landscape plants. *Polish Journal of Environmental Studies,* **24**(2): 689-693.
27. BATTAGLIA M AND AA COVARRUBIAS (2013). Late embryogenesis abundant (LEA) proteins in legumes. *Frontiers of Plant Science,* **4**: doi: 10.3389/fpls.2013.00190.
28. JAGTAP UB AND VA BAPAT (2010). *Artocarpus:* a review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethanopharmacology,* **129**: 142-146.
29. CHANDRA J, M TANDON AND S KESHAVKANT (2015). Increased rate of drying reduces metabolic inequity and critical

water content in radicles of *Cicer arietinum* L. *Physiology and Molecular Biology of Plants,* **21**(2): 215-223.

30. VERSLUES PE AND EA BRAY (2005). Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential induced ABA and proline accumulation. *Journal of Experimental Botany,* **57**(1): 201-212.

31. HAYYAN M, MA HASHIM AND IM ALNASHEF (2016). Superoxide ion: generation and chemical implications. *Chemical Reviews,* **116**(5): 3029-3085.

32. MOORE JP, NT LE, WF BRANDT, A DRIOUICH AND JM FARRANT (2009). Towards a systems-based understanding of plant desiccation tolerance. *Trends in Plant Science,* **14**(2): 110-117.

33. RACCHI ML (2013). Antioxidant defences in plants with attention to *Prunus* and *Citrus* spp. *Antioxidants,* **2**(4): 340-369.

34. OZGUR R, B UZILDAY, AH SEKMEN AND I TURKAN (2013). Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology,* **40**(9): 832-847.