RESEARCH ARTICLE

Role of sucrose and raffinose in the desiccation sensitivity of *Theobroma cacao* seeds

■ ABIS V. CHERUSSERY, P.G. RADHA, NABEESA SALIM AND K.M. JAYARAM

SUMMARY

Freshly collected seeds of cocoa were desiccated at room temperature and germination rate moisture content, soluble carbohydrates etc were analysed in the samples collected at regular intervals. Cocoa seeds are highly recalcitrant and total lose of viability occurs within 6 days of storage at room temperature. There is progressive deterioration of integrity of the membrane component of the cellular system during desiccation. This is manifested as the enhanced leakage of electrolytes from the tissues. A significant increase in sucrose content was observed in the cocoa seed tissues during desiccation. Raffinose content in the seeds declined rapidly during desiccation. Sucrose is suggested to have a critical role in maintaining the integrity of the cell membrane during dehydration. HPLC analysis of sucrose and raffinose through successive stages of desiccation does not impart tolerance to dehydration. This is because of the crystallization of sucrose and the deficiency of raffinose to ameliorate it. The sucrose to raffinose ratio is critical to seed viability in cocoa than their absolute quantum. When the ratio goes beyond 8:1 the viability of the cocoa seeds commence to decline rapidly.

Key Words : Sucrose, Raffinose, Desiccation sensitivity, *Theobroma cacao* seeds

How to cite this article : Cherussery, Abis V., Radha, P.G., Salim, Nabeesa and Jayaram, K.M. (2015). Role of sucrose and raffinose in the desiccation sensitivity of *Theobroma cacao* seeds. *Internat. J. Plant Sci.*, **10** (1): 38-42.

Article chronicle : Received : 26.06.2014; **Revised :** 16.11.2014; **Accepted :** 30.11.2014

S eeds of a large number of tropical fruit trees are recalcitrant, being extremely vulnerable to dehydration in contrast to the desiccation tolerant orthodox seeds

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(Roberts, 1973). Upon desiccation, such seeds lose viability even at relatively high moisture content and hence, pose a problem for long term conservation. Cocoa (*Theobroma cacao* L.) is one such fruit tree whose seeds are classified as recalcitrant (King and Roberts, 1982).

The membrane system is the most vulnerable cellular component of the seed tissues to damages upon withdrawal of water during desiccation (Koster and Leopold, 1988) and irreversible changes occur in the structural and functional integrity of the cell membranes and proteins (Crowe *et al.,* 1998), eventually leading the seeds to the non- viable state. In orthodox desiccation tolerant seeds, certain sugars are found to replace the water around the polar residues in membrane phospholipids and proteins, thereby maintaining their integrity in the absence of water. Caffrey *et al.* (1988) suggested that sucrose forms a substitute for water at the membrane surface. However, sucrose in nearly dry conditions crystallizes, thereby limiting its availability. The

oligosaccharide, raffinose serves as an effective inhibitor of sucrose crystallization, thus, contributing to desiccation tolerance in orthodox seeds implying an interaction between membrane phospholipids and sugars with a direct relevance to the viability of dry seeds (Leprince *et al*., 1993; Buitink *et al*., 2000).

The sucrosyl oligosaccharides, raffinose and stachyose occur in relatively lower quantities in recalcitrant seeds than in orthodox seeds and in general orthodox and recalcitrant seeds have tissues with sucrosyl-oligosaccharide to sucrose mass ratio 1:7 and 1:12, respectively (Steadman *et al.,* 1996). The gradual acquisition of desiccation tolerance during maize seed development is found to occur only after an accumulation of raffinose and with the sucrose-to-raffinose mass ratio remaining less than 20:1 and full desiccation tolerance is associated with a sucrose-to-raffinose mass ratio of less than 10:1 (Brenac *et al.,* 1997).

Reports have shown that cocoa seeds are recalcitrant (King and Roberts,1982). Though a wide range of factors such as morphological, physiological and biochemical are attributed to desiccation sensitivity in recalcitrant seeds (Vertucci and Farrant, 1995; Berjak and Pammenter, 1997), the real causes remain imprecise. While the occurrence and distribution of sugars in seeds, irrespective of orthodox or recalcitrant behaviour, is no longer in doubt, their metabolic role in the control of desiccation sensitivity/tolerance still remains little understood. The objective of this study was to evaluate the qualitative and quantitative distribution and interaction of sugars in cocoa seeds during desiccation and the resultant loss of seed viability.

MATERIAL AND METHODS

Cocoa seeds were extracted from freshly collected pods and cleaned with saw dust. The seeds were allowed to desiccate at open air conditions at the laboratory temperature of $28 \pm 2^{\circ}C$. Samples were drawn at regular intervals of 12 hours. Seed moisture content and percentage of germination were measured. Seed moisture content was determined gravimetrically on seeds oven-dried for 16 hours at $102 \pm 2^{\circ}C$. Germination tests were carried out by keeping the seeds between moist paper towels. The emergence of the radicle to a length of 5.0 mm was taken as a criterion for germination.

The electrolyte leakage from the seeds at different stages of storage was measured as an indicator of membrane damage. The electric conductivity of leachate was estimated using the method of Mullet and Wilkinson (1979). Five seeds each were drawn in six replicates at 12 hour intervals, weighed and soaked in 25 ml distilled water. After 24 hours, the leachate was collected and conductivity measurements were made.

Samples for HPLC analysis of sugars were prepared according to the modified protocol of Wilson *et al.* (1981). One gram of seed tissue was homogenised in 80 per cent ethanol, refluxed for 2 hours and then centrifuged at 16000×g.

The supernatant was collected and the pellet was re-extracted in 80 per cent (v/v) alcohol, centrifuged and the supernatant was collected again. The combined supernatant was evaporated to dryness and the residue was dissolved in 2.0 ml of distilled water and passed through a column of Dowex 50 W- X 8 cation exchanger. The sugar fraction was eluted with 10 ml of distilled water. Twenty microlitres of the eluate was injected into the HPLC system consisting of Waters u Bondapak–NH2 column, Waters 600 pump and Rheodyne 7725 injector. The mobile phase was acetonitrile-water (70:30) at a flow rate of 1.0 ml/minute. The sugars were detected and quantified using Waters 2414 refractive index detector.

RESULTS AND DISCUSSION

In conformation with the views of King and Roberts (1982) the seeds of *Theobroma cacao* were found to be highly recalcitrant since significant reduction in moisture content and seed viability being completely lost within 120 hours (6 days) of storage at room temperature (Fig. 1).

The significant increase in leachate conductivity in cocoa seeds indicated the loss of membrane integrity during desiccation resulting in an irreversible solute leakage (Fig. 2). A negative correlation was observed between electrolyte leakage and seed viability.

One of the earlier events associated with the loss of viability in recalcitrant seeds is the loss of membrane integrity as indicated by an enhanced concentration of solutes in seed leachates (Nautiyal and Purohit, 1985). Biochemical consequences of membrane deterioration include loss of solute control and the dispersal of the highly ordered system of membrane - associated enzymes (Stewart and Bewley, 1980).

A negative correlation has been drawn between the seed moisture content and the efflux of electrolytes from the seeds (Koster and Leopold, 1988; Leprince *et al*., 1993). According to Parrish and Leopold (1978) and Senaratna and McKersie (1983), the increase in electrolyte leakage is associated with a more permeable membrane system, the direct result of which can be the loss of metabolites, inability to maintain electrical, chemical or pH gradients and a mixing of the normally separated cellular constituents and the indirect result would be the loss of vigour and viability.

Of the various cellular components desiccation injury affects the membrane system most severely. The membranes lose their liquid crystal structure during dehydration. According to the 'water replacement theory' of Caffrey *et al*. (1988) a sugar such as sucrose formed a substitute for water at the membrane surface during desiccation affording protection against desiccation damage. The hydroxyl groups of sucrose may replace water by hydrogen-binding to the phospholipid head groups of the membrane.

A rapid increase in sucrose content was observed in cocoa seeds dehydrated at room temperature (Fig. 3A). The increase in sucrose content in the seeds is envisaged to occur
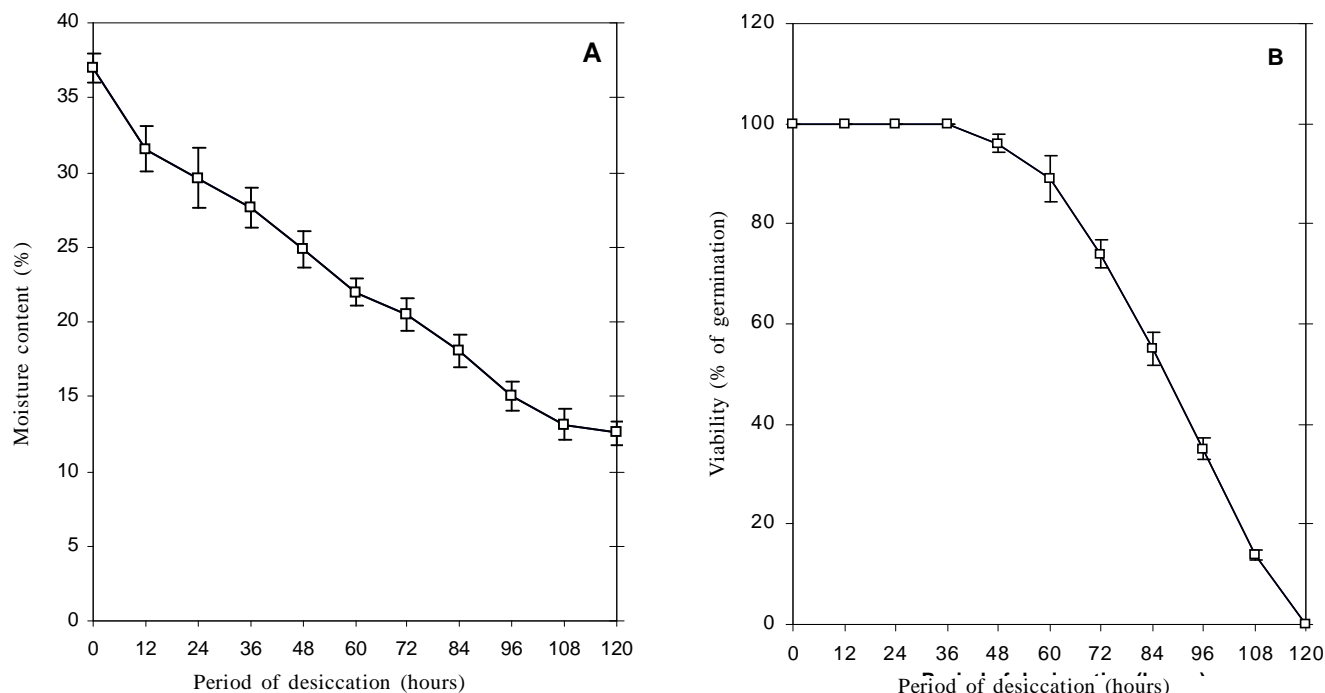
Fig. 1 : Effect of dehydration at room temperature on cocoa seeds (A) decrease in seed moisture content and (B) decrease in seed viability expressed in terms of percentage of germination
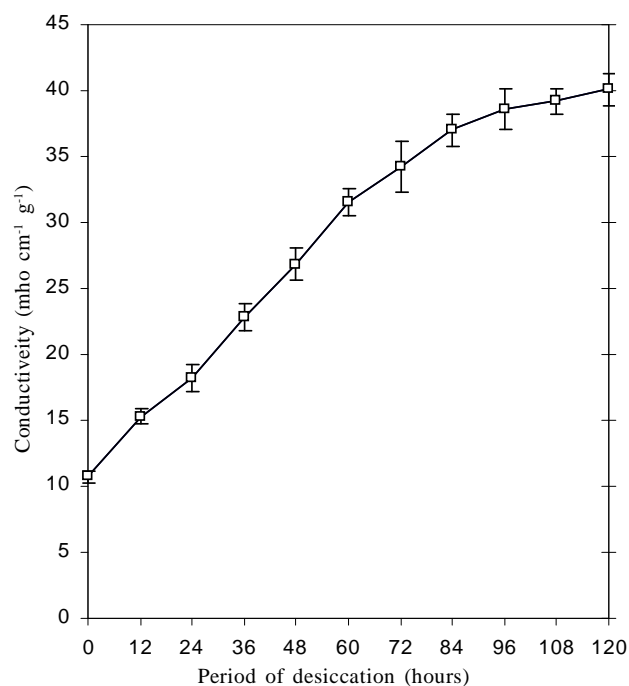

Fig. 2 : Electrolyte leakage from cocoa seeds in response to desiccation at room temperature measured as electrical conductivity of the leachates

through the conversion of the oligosaccharides into sucrose or by the hydrolysis of starch into soluble sugars, a fact supported by the significant decline observed in starch content in desiccating cocoa seeds (Abis, 2007).

Involvement of sucrose as a factor contributing to desiccation tolerance has been widely reported (Bernal-Lugo and Leopold, 1992; Crowe *et al.,* 1998*;* Lin *et al.,* 1998; Buitink *et al.,* 2000). However, the high amount of sucrose does not always prevent the desiccation induced loss of viability in the seeds. The main limitation of sucrose is that it crystallizes upon slow drying and, therefore, the hydroxyls will be unavailable for water replacement in biomembranes (Brenac *et al.,* 1997). In cocoa seeds, the high amount of sucrose formed during desiccation (Fig. 3A) and was not found to prevent the loss of viability as dehydration advances, implying that sucrose alone is having only a limited role in conferring desiccation tolerance in seeds. According to Hoekstra *et al.* (1994) and Lin *et al.* (1998) sucrose as such might not be the key element in conferring desiccation tolerance, since ambiguous correlations exist between sucrose mass and desiccation sensitivity.

Raffinose is found to occur in low amount in cocoa seeds and the content was found to decline steadily throughout the period of desiccation (Fig. 3B). The decline in raffinose content was found to accompany a reduction in the percentage of seed germination.

The oligosaccharides such as raffinose are consistently present during the tolerant stage and the desiccation tolerance declines as the oligosaccharides are decreased to a low level. A great decrease in the mole ratio of oligosaccharides/sucrose from the original value is noticed with the disappearance of
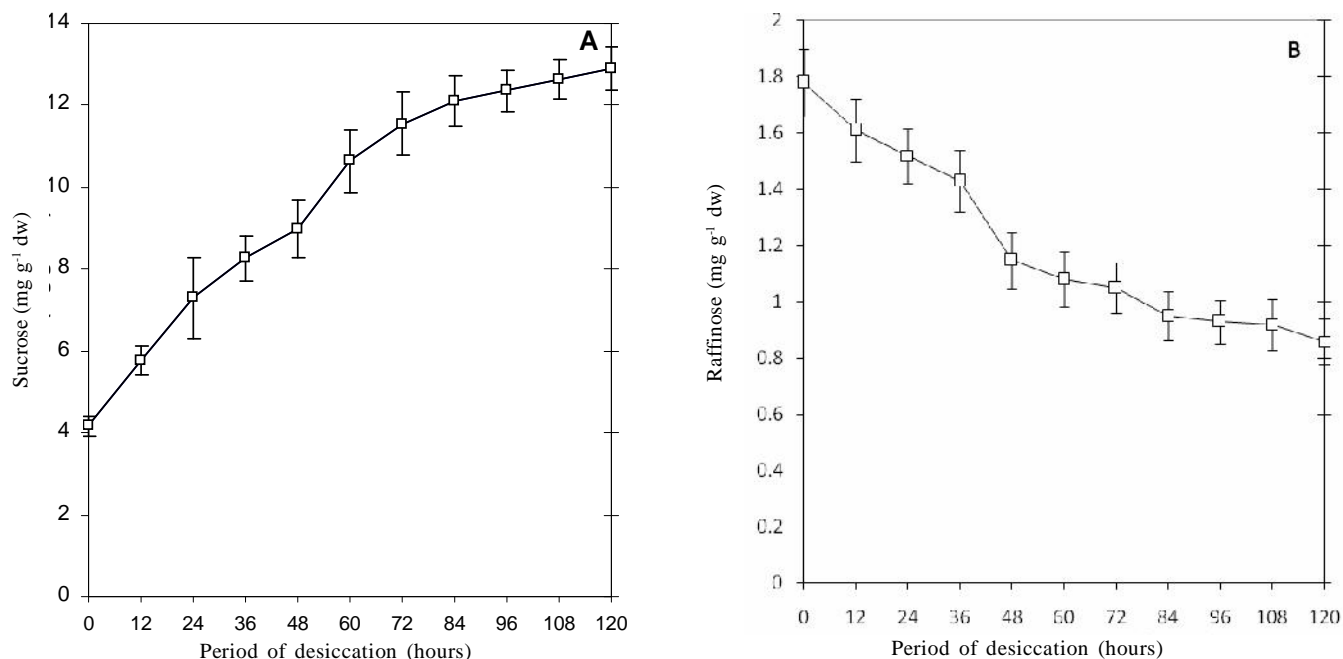
Fig. 3 : Variation in the levels of sucrose and raffinose in cocoa seeds during desiccation at room temperature (A) Increase in the level of sucrose during successive stages of desiccation (B) Decrease in the level of raffinose during desiccation. Both are expressed in terms of mg per gram dry weight

desiccation tolerance (Lin *et al.,* 1998).

Leprince *et al.* (1993) and Buitink *et al.* (2000) found that oligosaccharides like raffinose prevent crystallization of sucrose during desiccation, thus, contributing to desiccation tolerance in angiosperm seeds. The ability of sucrose to provide protection to membranes upon drying and the enhancement of this effect in the presence of raffinose, led the authors to suggest an interaction between phospholipids and sugars with a direct relevance to the viability of dry seeds. Bochicchio *et al.* (1994) implied that abrupt loss of water in maize seeds as a result of rapid drying might have impaired enzyme reactions including those responsible for an increase in raffinose concentration. The declining raffinose content is attributed partly to its hydrolysis into monosaccharides.

In general, orthodox and recalcitrant seeds have tissues with sucrosyl-oligosaccharide: sucrose mass ratio 1:7 and. 1:12, respectively and hence, the ratio of sucrosyl-oligosaccharide to sucrose in seed tissues is a good indicator of seed storage category (Steadman *et al.*, 1996).

The cocoa seeds in the present study exhibited 100 per cent germination when the sucrose to raffinose ratio was less than 8:1. An increase in the ratio resulted in a decrease in seed viability. Total loss of viability was found to occur when the ratio exceeded about 15:1. It is thus, obvious that seed viability in cocoa is maintained in the initial stages of desiccation by the sucrose fraction in conjunction with the raffinose content. As the raffinose content has declined below a critical value and the optimal sucrose: raffinose ratio could not be maintained, the deleterious effects of desiccation set

in, culminating in the loss of vigour and viability.

It is also suggested that sugars function as protectants of protein and membranes in dehydrating seeds and sucrose and raffinose impart desiccation tolerance through their involvement in vitrification the formation of high viscosity, super saturated solutions called amorphous glass during drying (Bruni and Leopold, 1991 and Buitink and Leprince, 2004). Because of its high viscosity, the glassy state of cytoplasm is found to impose a stasis on intracellular activity, reducing the deleterious effects of deranged metabolism, including damaging free radical reactions and protecting macromolecules against denaturation. The decline in the viability of cocoa seeds is correlated with a decrease in raffinose content. This implies that, it is the sucrose to raffinose ratio that is responsible for the maintenance of viability as a result of vitrification in the initial stages of desiccation in cocoa seeds. This is in accordance with the views of Wolkers *et al.* (1999) that it is the ratio of sucrose to oligosaccharide important in imparting desiccation tolerance than their absolute amounts.

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