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## Comparative Analysis of Seed Development and Desiccation Aspects of *Saraca asoca* (Roxb.) W.J.De Wilde and *Caesalpinia pulcherrima* (L.) Sw.

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### ABSTRACT

*Saraca asoca* and *Caesalpinia pulcherrima* of the subfamily Caesalpiniaceae bear away by dry dehiscent pods. In spite of their similarity in mechanical dispersal, seeds of *S. asoca* are desiccation sensitive while that of *C. pulcherrima* are desiccation tolerant. For complete development, *S. asoca* seeds take 120 Days After Anthesis (DAA) whereas *C. pulcherrima* requires only 75 DAA. Mature seeds of *S. asoca* with 100% germination have high Moisture Content (MC) of 52.6%. On maturation drying, the MC of *C. pulcherrima* seeds become 9.5 with 83% germination. Seeds of *S. asoca* germinate from 60 DAA onwards but that of *C. pulcherrima* germinate only after seed maturation drying of 75 DAA. Presence of oligosaccharide like raffinose along with sucrose in *C. pulcherrima* also hints an orthodox seed lineage against the recalcitrant nature of *S. asoca* seeds. Aspects like maturation drying, germination, desiccation response, leachate conductivity and sugar composition were studied for finding facts of species specific distinct seed lineage.

**Key words:** Fruit/seed maturation, germination, sugar profile

### INTRODUCTION

*Saraca asoca* and *Caesalpinia pulcherrima* are flowering members of the subfamily Caesalpiniaceae beholding characteristic medicinal and aesthetic properties. Low seed set along with heavy seed predation might have been a factor for fragmented *S. asoca* populations as an endangered species (IUCN., 2014). Short viability and desiccation sensitiveness of *S. asoca* seeds against the extendable seed viability on desiccation of *C. pulcherrima* of same subfamily prompted a comparative study. An understanding of seed development of such species that differ in their water relations may further improve the scope of *ex situ* conservation. Many species have their own survival strategies with variable seed desiccation sensitiveness as one such adaptive feature (Pammenter and

Berjak, 2000). In relatively moist aseasonal habitat, desiccation sensitivity is more frequent wherein the proportion of species with recalcitrant seeds decline as seasonality increases in both temperate and tropical habitats (Tweddle *et al.*, 2003; Berjak and Pammenter, 2008). Orthodox seeds are shed with advanced desiccation while recalcitrant seeds are desiccation sensitive and lose viability at higher water content (Roberts, 1973; King and Roberts, 1979). Maturation drying significantly influence the metabolism of orthodox seed development and germination. These changes are not observed in recalcitrant seed due to several factors including hormonal balance, metabolite distribution of soluble carbohydrates in particular and physical properties of water (Kermode, 1990). Even though seeds of both species are similar in their initial development and dispersal events,

distinct switch over mechanism of physiological and biochemical stature occur in due course of their maturation phase. Such dissimilar desiccation responses of *S. asoca* and *C. pulcherrima* instigated to conduct a study on the role of soluble carbohydrates for delimiting seeds into recalcitrant and orthodox types. Reports on desiccation tolerance and integration of soluble carbohydrates in seeds by several authors as Koster and Leopold (1988) and Hoekstra *et al.* (1994, 2001) for extending seed viability on the basis of sugar signals also serve up the genesis of present study.

## MATERIALS AND METHODS

**Fruit/seed development and collection:** Fruit/seed development of *Saraca asoca* and *Caesalpinia pulcherrima* were traced out from selected accessions of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) campus at an altitude of 150 m, amongst the outskirts of southern Western Ghats (Lat-8°45' and 8°47'N; Long-77°1' and 77°4'E) during three consecutive years (2011-2014). Fully opened flowers were tagged at 6.00 am from the day of anthesis onwards and sequential growth of fruits/seeds were recorded as per Days After Anthesis (DAA). Fully matured fruits were identified by their well-developed abscission zone and characteristic dark brown colour. Generally, fruit samples from ten trees of each species were collected and pooled together out of which *S. asoca* seeds of 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 DAA and *C. pulcherrima* seeds of 5, 10, 15, 20, 25, 35, 45, 55, 65 and 75 DAA were collected in particular. Mature seeds of both species were subjected to open desiccation at laboratory condition (28±2°C, 65% RH) and sampled at the intervals of 48, 96 and 144 h.

**Moisture content evaluation:** Moisture Content (MC) of developing fruit walls and seeds of both species sampled in triplicates during the course of development and desiccation were outlined as per the high constant temperature oven method on fresh weight basis (ISTA., 1993a).

**Germination test:** Seed was scored germinated when the radicle come out to a length of 5 mm (ISTA., 1993b). Germination percentage was calculated out of six replicates of ten seeds each, rolled in an acid free germination paper kept in a seed germinator without light (30±2°C, 80% RH).

**Leakage conductivity measurements:** Electrolyte conductivity of both control and desiccated seeds was carried out according to the method of Bonner (1996). Known weight of samples from six randomly selected seeds were soaked in 40 mL deionized distilled water and kept in a closed container for 24 h at 27°C in the laboratory. The conductivity of the solution was measured with a dip cell conductivity meter (Systronics, DDR, type 306). Conductivities were expressed as decisiemens per meter (dS m<sup>-1</sup>).

**Sugar profiling by HPTLC:** Seeds of different developmental stages of *S. asoca* (40, 60, 80, 100 and 120 DAA) and *C. pulcherrima* (15, 45 and 75 DAA) were collected for sugar analysis. In addition to development stages, desiccated seeds of *S. asoca* (48, 96 and 144 h) and *C. pulcherrima* (144 h) were compared with control seeds for sugars by using a HPTLC system (CAMAG, Switzerland) made up of Linomat V sample applicator, CAMAG twin-trough plate development chamber, CAMAG TLC Scanner 3 and WinCATS Software 4.03 (Reich and Schibli, 2006). The period of experimentation was selected as 144 h due to the fact that *S. asoca* seeds remained viable only up to 144 h of natural desiccation compared to prolonged viability of *C. pulcherrima*.

**Statistical analysis:** All experiments were repeated a minimum of 5 times and the data were statistically analyzed by one way ANOVA and the values are expressed as Mean±Standard error. Significance of differences between means were tested by LSD (p<0.05).

## RESULTS

**Fruit/seed development:** Mature dry dehiscent pods of *S. asoca* split mechanically to disperse seeds like that of *C. pulcherrima*. Pinkish young fruits of *S. asoca* turn green, then brown on maturity by 120 DAA (Fig. 1a). *C. pulcherrima* takes 75 DAA (Fig. 1b) to attain maturity as the fruit colour turns from green to brown. In *S. asoca*, the characteristic fruit wall moisture content variations occurs after 110 DAA, towards the last phase of development from 43.46-27.78% (Fig. 2a) while in *C. pulcherrima* quick shifting of moisture content was noticed after 55 DAA from 48.94-15.34% (Fig. 2b) with stabilized seed dry weight of 0.146 g/seed. The maximum seed size in *S. asoca* at 100 DAA and of *C. pulcherrima* at 45 DAA decreases subsequently with the progress of development (Fig. 1a-b). In *C. pulcherrima*, seed diameter was significantly (p<0.05) changed throughout the entire developmental phase while *S. asoca* showed a significant change only up to 60 DAA, later on remained stable due to the lack of maturation drying.

**Moisture content and germination:** During development, the 85.02% moisture content of immature *S. asoca* seeds were decreased to 52.55% on maturity (Fig. 2a). Immature *C. pulcherrima* seeds also possess 88.35% MC but being orthodox the MC was drastically reduced to 9.51% on maturation drying. As part of the development after 45 DAA, moisture content of *C. pulcherrima* seeds was rapidly decreased from 63.23-29.01% (Fig. 2b) in contrary to the stable MC of *S. asoca* seeds. Desiccation sensitive seeds of *S. asoca* harvested at 40-50 DAA lack the property of germination due to physiological immaturity but germination percentage started to increase gradually from 37% of 60 DAA



and to the maximum of 100% at 120 DAA. Further natural drying between 48-144 h of mature *S. asoca* seeds results in the reduction of germination capacity to 36.67%. In *C. pulcherrima* seeds, 83.33% germinability of fully mature brown seeds of 75 DAA becomes 70% after 144 h of desiccation. Reduction of MC in *S. asoca* during desiccation resulted in viability decline but orthodox seeds of *C. pulcherrima* retained minimal moisture content that sustained viability.

**Leachate conductivity:** Figure 3a-b shows the relation between leachate conductivity and germination percentage during different periods of seed desiccation in *S. asoca* and *C. pulcherrima*. Seeds of *S. asoca* showed significant increase of leachate conductivity like other recalcitrant species with negative correlation ( $r = -0.97$ ,  $p < 0.05$ ) on viability. In *C. pulcherrima*, the germinability was maintained as 70 against the 83.3% of control after 144 h of desiccation without any significant increase of electrolyte conductivity. Both seed moisture content and germination percentage were reduced significantly during desiccation in *S. asoca* which

showed a positive correlation ( $r = 0.99$ ,  $p < 0.05$ ) with leachate outflow. Loss of seed water content was very negligible and non-significant with the orthodox seeds of *C. pulcherrima* after dehydration.

**Metabolism of carbohydrates:** Sugar changes through seed development in both species as showed in Fig. 4a-b which mainly includes glucose, fructose and sucrose. Synchronization of sugar accumulation at various levels of development are not correlated in both cases, thus their level of accumulation are independent. During the initial seed development, fructose act as a prominent soluble reserve compared to glucose and sucrose in both seed types. Generally, glucose as a hexose sugar which decreased with later stages of development except some slight enhancement met with seeds of 80 and 120 DAA in *S. asoca*. From 80 DAA the sucrose which was accumulated in *S. asoca* seeds was found to be declined from 35.5-12.6 mg g<sup>-1</sup>. Desiccation tolerant *C. pulcherrima* seeds gather more sucrose up to the end of maturation phase.

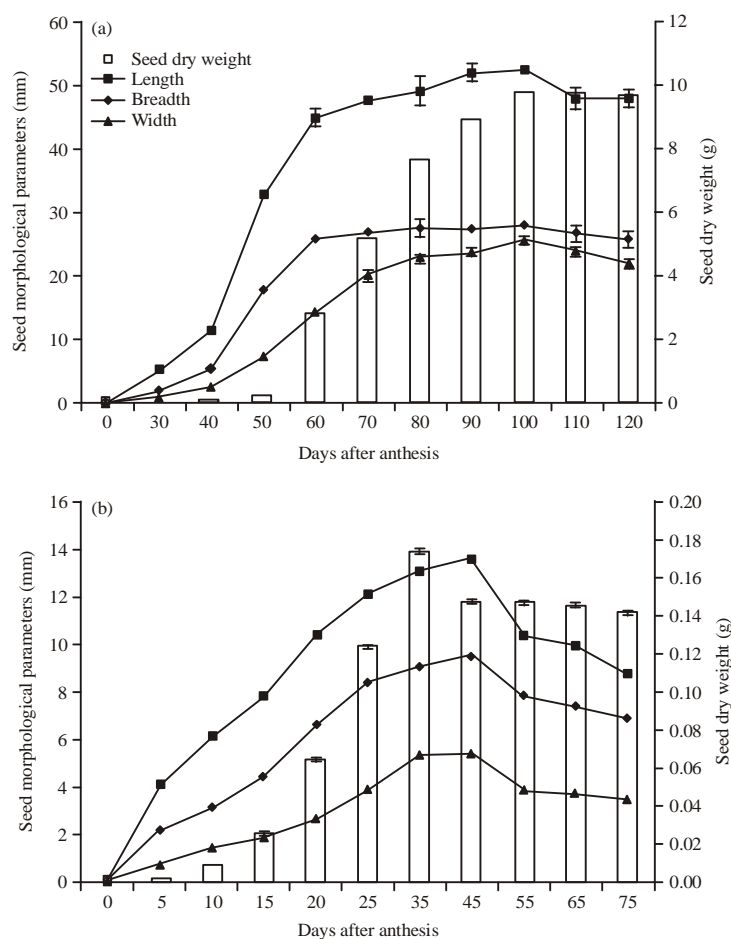


Fig. 1(a-b): Seed dry weight and morphological changes of developing seed in (a) *Saraca asoca* and (b) *Caesalpinia pulcherrima*. Bar denotes standard error

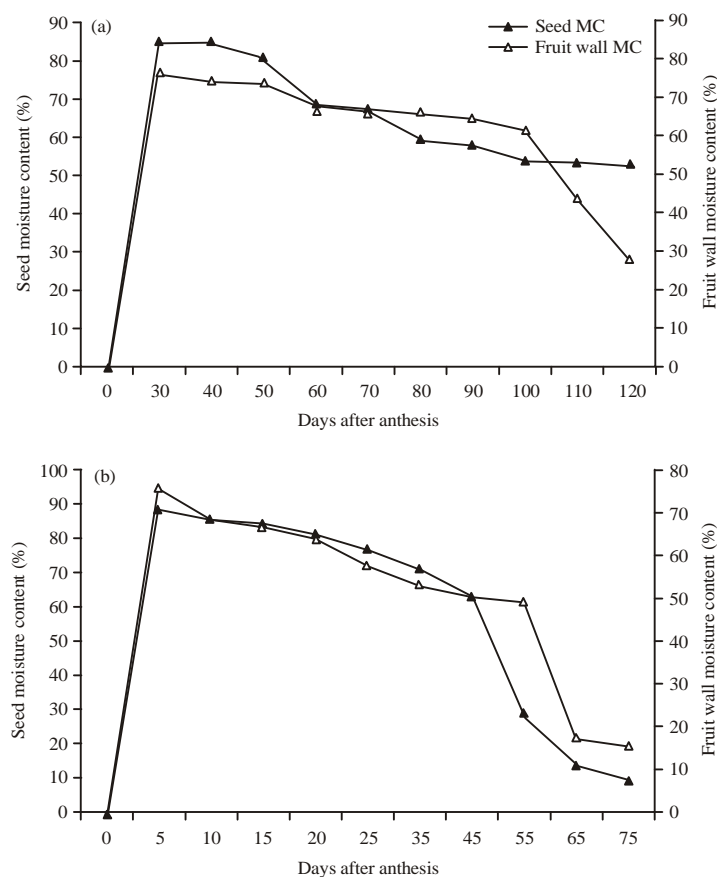


Fig. 2(a-b): Changes of fruit wall and seed moisture contents of (a) *Saraca asoca* and (b) *Caesalpinia pulcherrima* during development

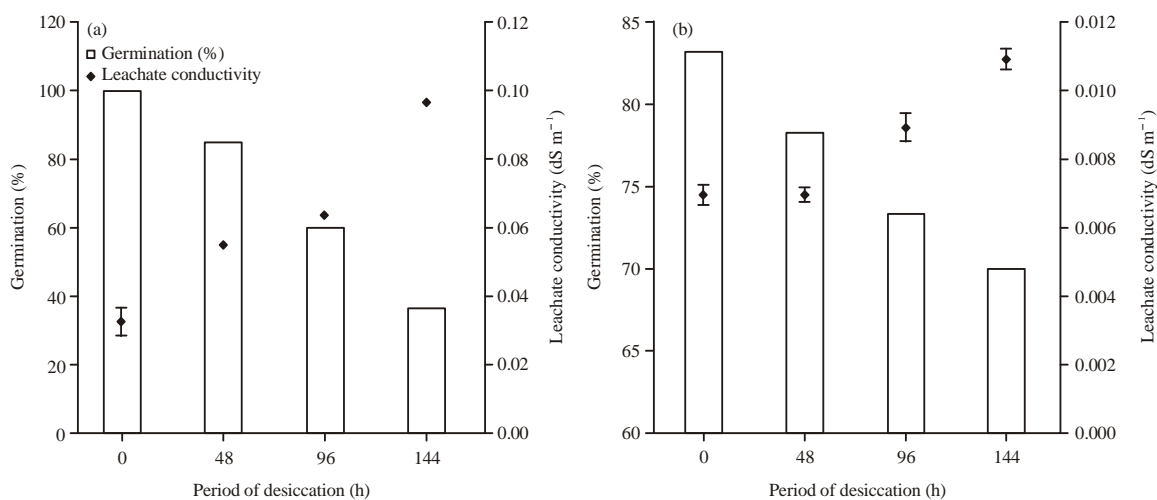


Fig. 3(a-b): Effect of desiccation on germination and leachate conductivity in (a) *Saraca asoca* and (b) *Caesalpinia pulcherrima*. Bar denotes standard error

*Saraca asoca* seeds after 144 h of open drying eventually lost all the three sugars (Fig. 5a) while in *C. pulcherrima*, glucose content remained stable as 0.2 mg g<sup>-1</sup> and fructose content get reduced from 1.2-0.4 mg g<sup>-1</sup>. On contrary with recalcitrant seeds of *S. asoca*, *C. pulcherrima* seeds with

6.7 mg g<sup>-1</sup> sucrose was significantly increased to 13.61 mg g<sup>-1</sup> on 144 h of desiccation and concomitantly the raffinose level also become detectably enhanced to 1.18 mg g<sup>-1</sup> which depicted the desiccation tolerance of *C. pulcherrima* (Fig. 5b).

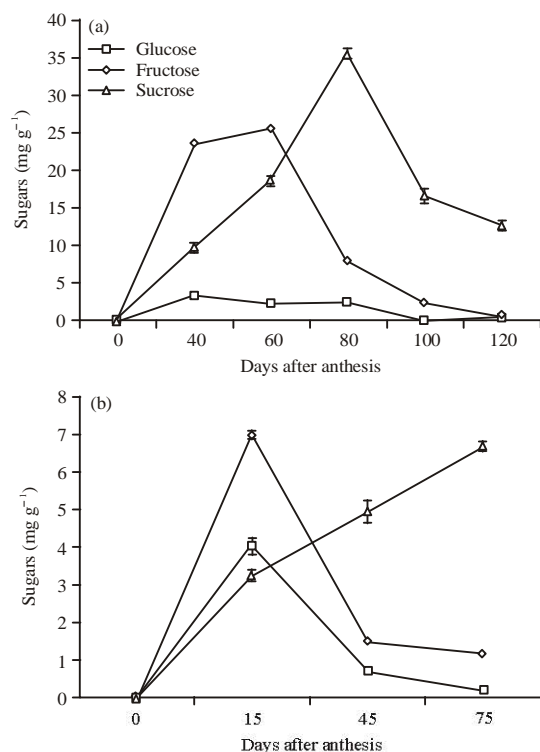


Fig. 4(a-b): Sugar changes during seed development in (a) *Saraca asoca* and (b) *Caesalpinia pulcherrima*. Bar denotes standard error

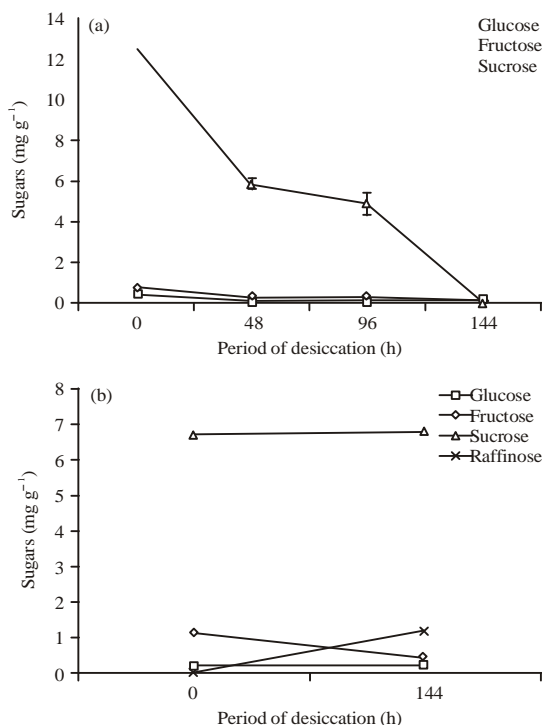


Fig. 5(a-b): Effect of desiccation on sugar composition in mature seeds of (a) *Saraca asoca* and (b) *Caesalpinia pulcherrima*

## DISCUSSION

*Saraca asoca* is a tropical rain forest species of India (Begum *et al.*, 2014) while *Caesalpinia pulcherrima* is a tropical American (Nayar *et al.*, 2014) species naturalized at Indian homesteads. Irrespective to their similarity in fruit type and seed dispersal mode, both species have distinct physiological and biochemical mechanisms over pre and post shedding phases. Desiccation tolerant seeds of *C. pulcherrima* show determinate developmental pattern which involves reserves accumulation up to maturation drying. On contrary, the indeterminate and continuous reserve accumulation until seed senescence is the characteristic feature of *S. asoca* seeds. The seed size of *C. pulcherrima* reaches a plateau after 45 DAA and reported to be stabilized by the completion of maturation drying (Carvalho and Nakagawa, 2000). Mature seeds of *S. asoca* possess 52.55% moisture content as an essential requirement for recalcitrant seeds to remain viable (Purohit *et al.*, 1982). In *S. asoca*, viability decline occurs even at comparatively higher moisture content of 46.95% (Anilkumar *et al.*, 2011). Maximum seed dry weight of *C. pulcherrima* (0.174 g/seed) was attained at 35 DAA, when the green fruits became slightly brown stage of physiological independency. Concomitantly a decline in seed dry weight was noticed which may be also due to the respiration of seeds with high MC (70.85%). By the end of maturation process, seeds with moisture inside the fruit tend to respire more and the consumption of accumulated reserves causes some reduction of dry matter (Krony and Egli, 1997; Dias *et al.*, 2006). A reduction in seed dry weight at the end of the maturation process, due to respiration has also been reported in castor (Da Silva *et al.*, 2009) and sunflower (Sader and Silveira, 1988) seeds. In *S. asoca*, maximum seed dry weight of 9.76 g within 100 DAA was extended till the last phase of development without much reduction as reported in the recalcitrant seeds of *Machilus thunbergii* (Welbaum and Bradford, 1988).

Desiccation tolerance has been defined as the ability of seeds to germinate following drying (Hay and Probert, 1995) and drying methods however appear to affect viability of desiccated seeds over a period of time. Unlike *C. pulcherrima*, desiccation of recalcitrant *S. asoca* seeds caused the discharge of more electrolyte leachate by the effect of damaged cell membranes (Sorensen *et al.*, 1996). Similar observations have been reported in desiccation sensitive seeds of *Machilus thunbergii* (Lin and Chen, 1995) and *Theobroma cacao* (Li and Sun, 1999). Rapid decrease in germination percentage in accordance with moisture loss is an established fact in viability loss of recalcitrant seeds. The ionic leakage was caused by cell membrane rupture and outflow of water during dehydration (Zheng, 1991). The loss viability of desiccation sensitive *S. asoca* seeds during desiccation could be correlated with irreversible solute leakage (Becwar *et al.*, 1982; Berjak *et al.*, 1989; Fu *et al.*, 1990). Sensitivity to desiccation that resulted in cell death, solute leakage and leachate conductivity of *S. asoca* seeds was in consonance with the

view of Mirdad *et al.* (2006) owing to its recalcitrant nature. In addition to their ecological adaptations, observations regarding seed development and desiccation aspects of *S. asoca* and *C. pulcherrima* also support their recalcitrant and orthodox nature respectively (Prajith and Anilkumar, 2012; Prajith *et al.*, 2013).

Decrease of glucose and fructose during seed development phase in *C. pulcherrima* is a commonly observed feature of orthodox seeds (Chen and Burris, 1990; Leprince *et al.*, 1990; Ooms *et al.*, 1993). Nevertheless, during seed development of *S. asoca*, sucrose translocation from the site of photosynthesis increases up to 80 DAA followed by a significant reduction which indicates the synthesis of starch as the abundant storage material in mature seeds (Bewley and Black, 1994). Reduction of fructose during seed maturation also is confirmatory to the above unit because starch synthesis involves maximum mobilization of fructose as one of the hydrolytic products of sucrose. Lack of any changes in the distribution of glucose reveals its mobilization for respiration since *S. asoca* seeds are recalcitrant and hence metabolically active. But in *C. pulcherrima* sucrose is increased continuously during seed development presumably due to the reduction of starch synthesis after 45 DAA whereas reduction of both glucose and fructose indicates reduced rate of sucrose hydrolysis and utilization of glucose and fructose for respiration and starch synthesis, respectively. Accumulation of sucrose is a prerequisite for desiccation tolerance in orthodox seeds of *C. pulcherrima* because involvement of sucrose as a factor contributing to desiccation tolerance has been widely reported (Koster and Leopold, 1988; Crowe *et al.*, 1998; Buitink *et al.*, 2000; Halperin and Koster, 2006). *Saraca asoca* seeds contain sucrose reserve and lack any form of oligosaccharides substantiate the desiccation sensitive nature of species as similarly reported in the case of *Camellia sinensis* (Devey *et al.*, 1986). But orthodox seeds of *C. pulcherrima* possess oligosaccharide raffinose in addition to sucrose content which remain unchanged during desiccation and hence are tolerant to desiccation since their viability is retained after six days of desiccation. The interaction of raffinose restricts the crystallization of sucrose, thus enabling maximal desiccation tolerance in orthodox seeds (Koster and Leopold, 1988). The water replacement hypothesis suggests that during dehydration, sucrose replaces water on the macromolecular surfaces and enable stabilization of membranes in the desiccated state. High level of sugars (particularly sucrose, in conjugation with raffinose and stachyose) in maturing seeds has been suggested to afford such a mechanism in desiccation tolerant seeds (Leopold and Vertucci, 1986; Koster and Leopold, 1988; Chen and Burris, 1990; Leprince *et al.*, 1990). Desiccation sensitivity in recalcitrant seeds at water content higher than 30% may involve crystallization of sucrose which is the main limitation to water replacement concept. Thus the presence or absence of oligosaccharides like raffinose possibly is one marker to segregate the orthodox seeds of *C. pulcherrima* from the recalcitrant ones of *S. asoca* in

addition to other seed physiological parameters like maturation drying, moisture content, germination, storability and leachate conductivity.

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