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Seed desiccation responses in *Saraca asoca* (Roxb.) W.J.de Wilde

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Saraca asoca **is one among the 36 endangered medicinal plants of South India. As seeds are the main propagule with short viability, the present study has been carried out to assess the level of dehydration tolerance as a prerequisite to maintain extended viability. The viability and vigour of the seeds declined when their moisture content was reduced by different methods of desiccation. The critical moisture content (CMC) of the seeds was found to be 45–46%. Irrespective of the method of drying, dehydration of seeds resulted in the loss of viability, confirming their recalcitrant nature. Desiccation responses were investigated by exposing the seeds to five different conditions: (a) 30° ± 2°C, (b) silica gel, (c) 40° ± 2°C, (d) 20° ± 2°C and (e) 0° ± 2°C. The duration for reaching the critical moisture level was the longest in seeds kept in an air-conditioned room (20° ± 2°C) and minimum for those kept in a freezer (0° ± 2°C). The lowest critical moisture level (36.3%) was observed in silica gel and highest (49.2%) under freezer condition. Both the attainment and level of CMC showed marked variation under different desiccation treatments, which indicates the influence of storage temperature on CMC of *S. asoca* seeds.**

Keywords: Critical moisture content, desiccation, germination, *Saraca asoca.*

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SARACA ASOCA (Roxb.) W.J.de Wilde of subfamily Caesalpiniaceae is a globally vulnerable evergreen tree species existing as sporadic wild population in south and central Western Ghats[1]. Medicinal value of the species is evidenced from the annual bark requirement of about 10,724 tonnes with an annual growth rate of 15% by the Indian pharmaceutical industry for the year 2004–05 (ref. 2). The existing imbalance between species regeneration and removal rates could be overcome only by addressing the problems of seed as the main propagule. *S. asoca* seeds germinate as soon as they reach a humid floor; if not, they may become nonviable, because of their desiccation-sensitive nature, as in the case of many other recalcitrant seeds. Most recalcitrant seeds quickly lose their viability when moisture content falls below a critical level, making *ex situ* conservation problematic. Low seed set, poor natural regeneration and heavy seed pest infection[3] in the wild populations of the Western Ghats have attracted more studies in relation to seed water physiology. It is a fact that the storage of seeds is possible only with the reduction of moisture content to a minimum possible level[4]. The germination behaviour of *S. asoca* seeds has not been studied in detail so far. Therefore, the present study has been undertaken to assess the impact of desiccation on the germination and vigour of the seeds of *S. asoca*.

The seeds of *S. asoca* were collected during 2011–2014 from 10 accessions of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) campus, Thiruvananthapuram, located at an altitude of 150 m amsl on the foothills of the southern part of the Western Ghats (8°45′–8°47′N; 77°1′–77°4′E). Fully mature fruits were identified by their well-developed abscission zone and characteristic dark brown colour, and seeds were collected by shaking the branches.

The effect of desiccation on the loss of viability was studied by subjecting the seeds to different treatments as follows[5]: (a) In the laboratory (30° ± 2°C); (b) in a 4 l capacity desiccator containing 2 kg of silica gel; (c) in an oven (40° ± 2°C); (d) in an air-conditioned (AC) room (20° ± 2°C) and (e) in a freezer (0° ± 2°C).

Changes in seed moisture content during different methods of drying were determined according to the high constant temperature oven method, on fresh weight basis[6]. Five seed replicates for each sample were made and weighed before and 1 h after drying at 130° ± 2°C, and moisture content was calculated as the percentage of water on fresh weight basis.

The viability of seeds was determined on the basis of percentage of germinated seeds. The seed was scored as germinated when the radicle attained a length of 5 mm (ref. 7). Germination test was carried out in five replicates of ten seeds each, rolled in an acid-free germination paper kept in a seed germinator with no light (30° ± 2°C, 80% relative humidity). Seed germination associated parameters like speed of germination (SPG), mean daily germination (MDG), peak value (PV), germination value (GV)[8] and mean germination time (MGT)[9] were calculated.

Electrolyte conductivity of both control and desiccated seeds was measured according to the method of Bonner[10]. Samples of known weight from three randomly selected seeds were soaked in 40 ml of 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 microsiemen (μs).

All experiments were repeated five times and data were statistically analysed by one-way ANOVA method and values expressed as mean ± standard error. Significance of differences between mean values was tested by least significant difference (LSD) ($P < 0.05$).

Fresh seeds of *S. asoca* possess 51.6% moisture content with 100% germination capacity. Irrespective of the methods and means of desiccation adopted, both seed moisture content (MC) and germination percentage (GP) concurrently became significantly reduced. The time required to attain the critical moisture levels in the seeds differed with the different methods of drying. It was maximum (240 h) for seeds in the AC room (20° ± 2°C) and minimum (8 h) for seeds kept in the freezer (0° ± 2°C). For the seeds maintained at 40°C in the oven, the duration was 24 h. It was 96 and 144 h respectively, for the seeds kept in silica gel and under laboratory (30° ± 2°C) conditions (Table 1). Seeds kept in the freezer had comparatively higher critical moisture content (CMC), i.e. 49.2% with 52% germination, whereas those maintained in silica gel showed 36.3% CMC, which is considered as minimum among all the treatments. Seeds dehydrated at 40°C, 30°C and 20°C conditions, showed more or less an average range of CMC of 45–46%. Irrespective of the drying method, leachate conductivity increased considerably with the onset of desiccation (Figure 1 *c*) and showed strong negative correlation with MC, GP, MDG, SPG, PV and GV. MGT of the desiccated seeds displayed negative correlation with MC, GP, MDG, SPG, PV and GV in all drying methods, except under 20°C condition (Figure 1 *h*). Germination aspects like SPG, PV, MDG and GV (Figure 1 *d–g*) are maximum in seeds with high moisture content (Figure 1 *a*), while they are reduced considerably during desiccation, irrespective of the drying method and showed high positive correlation with germination percentage (Figure 1 *b*).

Tweddle *et al.*[11] studied the desiccation-sensitive seeds which are most common in tropical rainforests. The moisture content of *S. asoca* seeds at the time of shedding is 51–52%, which is in the upper distributional range between 36% and 90% of recalcitrant seeds; non-endospermous seeds of *S. asoca* hold two cotyledons and an embryonic axis covered with thin seed coat, wherein the cotyledons act as the sole food storage organs covering 95.2% of the whole seed with hypogeal germination[12]. According to Daws *et al.*[13], minimal seed coat ratio with

Table 1. Time required for reaching the critical moisture level in *Saraca asoca* seeds subjected to desiccation by different methods

Treatment	Time (h)	MC	LC	GP	MDG	SPG	MGT	PV	GV
Control	0	51.55^{d}	8.72^{a}	100^{b}	0.34^{c}	0.51^{b}	20.3^{a}	0.16^{b}	0.06^{b}
Freezer (0°C)	8	49.18^{d}	79.59^{d}	52.00^{a}	0.24^{b}	0.17^{a}	33.6^{c}	0.05^{a}	0.02^{a}
Oven (40°C)	24	45.47^{bc}	48.03^{c}	52.00^{a}	0.14^{a}	0.20^{a}	28.2^{bc}	0.07^{a}	0.01^{a}
Silica gel	96	36.31^{a}	45.34^{c}	54.00^{a}	0.14^{a}	0.19^{a}	34.3^{c}	0.08^{a}	0.01^{a}
Laboratory (30°C)	144	46.89^{c}	31.87^{b}	46.00^{a}	0.14^{a}	0.20^{a}	26.3^{ab}	0.06^{a}	0.01^{a}
Air-conditioned room (20°C)	240	45.11^{b}	26.44^{b}	44.00^{a}	0.13^{a}	0.19^{a}	25.1^{ab}	0.08^{a}	0.01^{a}

Mean values followed by the same letter do not differ according to Tukey's test $P < 0.05$.
MC, Moisture content; LC, Leachate conductivity; GP, Germination percentage; MDG, Mean daily germination; SPG, Speed of germination; MGT, Mean germination time; PV, Peak value and GV, Germination value.

respect to whole seed mass (2.3%) in *S. asoca* could be treated as indicating a thin seed coat, which may be the possible reason for quick seed germination. In *S. asoca*, maximum seed shedding coincides with the monsoon period, which could be considered as a general ecological adaptation of recalcitrant species for minimizing the risk of seed desiccation in natural conditions.

Seed desiccation in *S. asoca* was evaluated by changes in fresh weight and moisture levels affecting the viability which followed a pattern typical of recalcitrant seeds, i.e. seeds could not be dehydrated below a relatively higher moisture content without loss of viability[14]. The longevity of the seeds was mainly affected by the reduction in moisture content below a critical value, which may vary considerably among different species[15]. The term 'critical moisture content' or 'lowest safe moisture content' denotes moisture content below which seeds die; it may vary with the method adopted for desiccation[16]. Therefore, it may not be possible to define unambiguously a CMC for viability loss in recalcitrant species[16].

Seeds of *S. asoca* maintained in the freezer showed high CMC and viability extended only up to 24 h (Figure 1 *b*), which can be explained by the formation of ice crystals in moist seeds[17]. Protein denaturation[18], membrane instability[19] and lack of considerable proportion of unsaturated fatty acids[20] are the possible reasons behind the deleterious effects of subambient temperature. Compared to other drying methods, seeds maintained in the freezer reached comparatively much higher level of mean leachate conductivity (Figure 1 *c*) within a short period of desiccation, which indicates membrane damage susceptibility at low temperature. Loss of *S. asoca* seed viability in the freezer may be independent of water content, as also observed in desiccation-sensitive seeds of *Inga vera*[21].

Slowly desiccated seeds of *S. asoca* lose viability at relatively high moisture content than rapidly dried seeds (Table 1), because slow drying contributes to homogeneous dehydration and seed tissues spend a prolonged period with intermediate water content, which leads to considerable membrane damage[22]. Degradative processes appear to be aqueous based and oxidative in nature[16]. It has been suggested that metabolic functions of cells are altered in seeds with water content that is intermediate between the fully hydrated state and the lower threshold of tolerance to desiccation[23]. Thus, reducing the time of exposure to intermediate levels of hydration should minimize the damage associated with desiccation. Fast drying has distinct advantages over freezer drying, because lower water content can be achieved using silica gel[24].

In *S. asoca*, though CMC was attained after 24 h of dehydration at 40°C (Figure 1 *a*), such drying is not generally suitable for recalcitrant behaviour as tested in *Quercus nigra*, because of considerable protein detrimental degradation[10]. The bulky cotyledons of the seed may act as a protective factor for preventing severe water loss from the embryonic axis, which might be the reason for this viability extension at a certain level in *S. asoca* as against *Q. nigra* under higher temperature drying. Seeds maintained at 20°C achieved CMC with maximum days of dehydration compared to other desiccation methods (Table 1), because lower drying temperature is more favourable to moist seeds avoiding thermal damage[25]. The different seed drying conditions show marked influence on drying rate of non-orthodox species, which may contribute to high variability in desiccation tolerance[26]. Water serves as a protectant of macromolecular structure and controls the level of metabolic activity in plants. Thus, loss of water will strongly affect the nature of physical and biochemical reactions[22].

Detailed analysis of soluble sugars by HPTLC in *S. asoca* seeds indicated sucrose as the major contributor to increase in total soluble carbohydrates, as observed in recalcitrant seeds of *Inga vera* as well[21]. This supports the hypothesis of decreased respiratory rates. Absence of raffinose family of oligosaccharides with major reserve of sucrose highlighted the desiccation-sensitive nature of *S. asoca*[27]. Studies on seed desiccation tolerance in selected tropical tree species pointed out that freezing and artificial seed desiccation methods cannot induce protective mechanisms against dehydration with special reference to sugar metabolism[21]. During desiccation, seeds of *S. asoca* discharged more electrolyte leachates by the effect of damaged cell membranes, unlike in orthodox

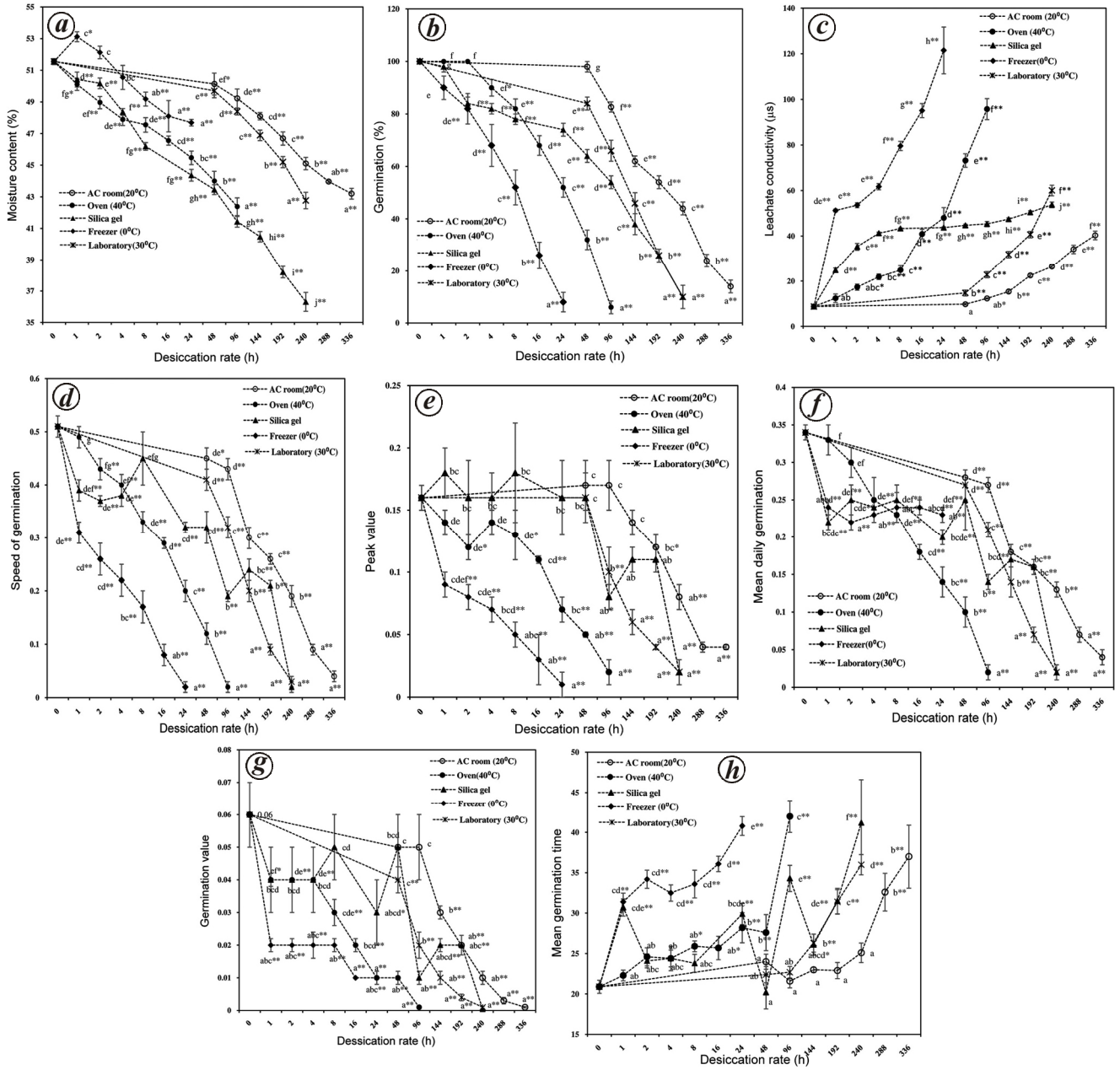

Figure 1. Changes in (***a***) moisture content, (***b***) germination percentage, (***c***) leachate conductivity, (***d***) speed of germination, (***e***) peak value, (***f***) mean daily germination, (***g***) germination value and (***h***) mean germination time of *Saraca asoca* seeds subjected to desiccation by different methods. ANOVA done separately. *Significant at $P = 0.01$ level, **Significant at $P = 0.05$ level. Vertical bars are standard errors of the respective means. Mean values of each treatment having the same letters without asterisk are not significant at 1% and 5% P level based on LSD (Duncan's multiple range test).

seeds of *Caesalpinia pulcherrima*. High-vigour seeds usually possess well-organized cellular membrane, which results in low electrical conductivity values[28].

Irrespective of the dehydration method used, the inverse proportionality between MDG and MGT values signifies adverse effect of deterioration on *S. asoca* seeds during desiccation. Slower water absorption capacity and smaller surface area to mass ratio are the possible factors of longer MGT in large-seeded species[29]. Desiccation-sensitive seeds of *S. asoca* also possess high MGT (Figure 1 *h*). SPG is a reflection of germination energy and SPG values were significantly reduced (Figure 1 *d*) along with PV and GV (Figure 1 *e* and *g*), during seed desiccation. The values of seed leachate conductivity and MGT increased as dehydration progressed; thus both showed a negative correlation with other germination parameters. An average hold of MGT values near the control sample up to 10 days of dehydration at 20°C condition (Figure

1 *h*), suggests non-significant correlation between MGT and GV. Also, GV is an index of combining speed and completeness of seed germination, which is closely related to the survival of seedlings.

The viability of *S. asoca* seeds was adversely affected below 45–46% moisture content in 20°C, 30°C and 40°C drying treatments; thus it was assigned as the CMC of species, though seeds exhibit critical moisture levels in the range 36.3% (minimum)–49.2% (maximum), as seen in silica gel and freezer conditions respectively (Table 1). Desiccation response of *S. asoca* is similar to that of acorns, because seeds usually shed at MC of 48–53% and are usually dried to 40–45% MC before storage. CMC of *S. asoca* seeds is similar to that of *Hopea helferi* (47%) and *Hopea vervosa* (43–50%). The understanding of CMC of seeds using different drying methods in *S. asoca* species has practical importance for optimization of seed storage condition, as a part of effective germplasm preservation with special attention given to seed water content.

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