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Research Article

Effect of seed moisture content, temperature and storage period on seed germination of *Saraca asoca* - An endangered medicinal plant

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ABSTRACT

An investigation was carried out during 2009-10 with an objective to determine the effects of seed storage and temperature on seed germination of Asoca (*Saraca asoca* (Roxb.) de Wilde). As there is a great demand for its bark coupled with shortage in supply, this plant is thus highly exploited and threatened. In this study a correlation between seed moisture content and seed germination was established. It was observed that the rate of moisture loss in seeds is indirectly proportional to the seed germination. Highest germination percentage (83.7%) was recorded at open nursery at 4 weeks after harvest followed by 25°C and 30°C at 3 weeks after harvest. The seed germinated immediately after harvest in open nursery whereas; at 25°C and 30°C, germination commenced only at two weeks after harvest. Under open nursery conditions, seeds failed to germinate eight weeks after harvest when the seed moisture content was below 22.8%. However, at 30°C, the seed germination continued up to nine weeks after harvest where the seed moisture content was <20%. The experimental results revealed that the seeds of *S. asoca* could not tolerate drying to lower moisture content of 20-23% and might be considered as recalcitrant.

Keywords: Seed moisture content, Recalcitrant seed, Seed germination, Temperature, Pre-treatment, *Saraca asoca*

INTRODUCTION

Asoka (*Saraca asoca* (Roxb.) de Wilde, Fam.: Fabaceae) is a legendary and sacred tree which is both ornamental and medicinal. Its bark contains tannin, catechol, flavanoides, sterol, glucosides and other alkaloids, which is also famous as 'female tonic'. Because of these medicinal properties there is a great demand for its bark and other plant parts from both national and international market. In India, during the year 2004-05 the annual estimated consumption of asoka was 2041 MT (NMPB, 2008), but due to shortage in supply it is adulterated with bark of *Polyalthia longifolia*. This plant is thus highly exploited and has become threatened in its natural habitat (IUCN, 2011).

In nature, its seeds shed at high moisture content levels (45-50%), and germinate under the tree when covered with soil or humus. However, in exposed condition, seeds do not readily germinate and the seedlings soon perish due to the drying of the radical, besides, it is short lived and are desiccation sensitive (Morton, 1987; Chanko and Pillai, 1997). Seeds of many tropical and temperate plant species do not survive dehydration (Chin and Roberts, 1980; Joet *et al.*, 2013). When seed moisture is at or below the levels considered lethal, there is a total loss of viability (Pritchard, 2004). Seed desiccation sensitivity may limit the *ex-situ* conservation of plant species and therefore, the first challenge for seed conservation is to determine seed response to desiccation (Gold and Hay, 2007). Hence, knowledge of the critical and lethal moisture levels of a species is indispensable for planning and execution of seed drying and storage (Martins *et al.*, 2003). Apart from this most of the seeds will germinate only between certain ranges of

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temperature. In the light of above facts, this study was carried out with an objective to determine the effects of seed storage and temperature on germination of *S. asoca* seeds.

MATERIAL AND METHODS

Experiment site

The experiment was conducted at ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat during 2009-2010. The seeds were collected from eight years old trees from Anupam Mission, Anand which is located at 22°35' N latitude, 72° 55' E longitudes and at an altitude of about 45.1 m above MSL.

Seed collection and storage

Fresh seeds were picked at full maturity just before the pods were about to dehisce. The collected seeds were shade dried under room conditions for a day and stored in an air tight polythene bags under room conditions. Seed samples were drawn for moisture content determination and for germination studies at weekly interval.

Seed treatment

Before the initiation of the experiment the collected seeds were first dipped in water and floated, chaffy seeds (around 30-35%) were discarded. The sunken seeds were used for the experiment. The seeds were treated with 0.2% Mercuric Chloride solution to prevent any fungal infections.

Moisture content determination

Seed moisture content (SMC) was determined immediately after harvest and at weekly interval, by gravimetric determination of moisture content in an oven at 103°C±2°C for 17 h (ISTA, 1993), in three replicates of fifteen seeds each. The moisture percentage of seed was calculated by using the formula Moisture % = Fresh weight – Dry weight/ Fresh weight X 100.

Germination test

Seed germination tests were performed in the polybags of size 5x3 inches containing potting mixture of soil:sand:FYM in 1:1:1 ratio. The seeds were placed in the media during July, 2009. Seeds were kept for germination immediately after harvest and at every week interval at different temperatures (25 ± 1° and 30 ± 1° C) in a germination chamber and in open nursery. For each experiment, there were three replicates of fifteen seeds each arranged in a Factorial CRD with days after harvest (D) and temperature of germination (T) as two factors, and the germination was defined as the radical emergence by at least 2 mm (ISTA, 1993). Final germination was recorded until no further germination was observed for at least 1 month. The germination data were calculated each day and expressed as germination percentage of seeds.

Statistical analysis

Data were subjected to statistical analysis using statistical software SAS 9.2 (SAS, 2008) and comparison was made at 5% probability level.

RESULTS AND DISCUSSION

The asoca seeds are bold, ovoid to ellipsoid in shape, flat, shiny, hairless and covered with a brown thin seed coat with two to eight seeds per pod (Figure 2). On an average one kg of seeds contained around 160 seeds. Test weight of hundred seeds ranged from 633-969 g. Single seed weight was highly variable which ranged from 2.06 to 11.56 g. Average length and breadth of the seeds varied from 2.8 to 5.8 cm and 1.6 to 4.1 cm, respectively (Table 1).

The rate of water loss (Figure 1) in asoka seeds could be best described by a three phase reduction, wherein, the decrease in seed moisture was steady and at a very slow rate at the beginning *i.e* from harvest to four weeks after storage

Table 1: Physical characteristics of *Saraca asoca* seeds

Physical parameters	Value
Colour	Brown
Shape	Ovoid to ellipsoidal
Average length (range)	2.8±0.14 to 5.8±0.14 cm
Average breadth (range)	1.6±0.07 to 4.1±0.07 cm
Individual seed weight (range)	2.06±0.11 to 11.56±0.11 g
Test weight (100 seeds)	633±0.03 to 969±0.03 g

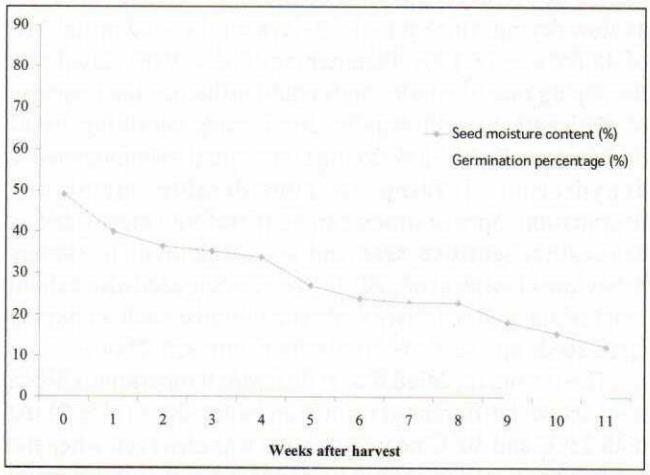

Figure 1: Relationship between days after harvest and seed moisture content on seed germination of *Saraca asoca* under open nursery conditions

Figure 2: Asoca (*Saraca asoca*) mature pods and seed germination; a. Developing Pod; b. Mature pod with seeds; c. Seedling in a polythene; d. Seeds showing germination

period, then a rapid decrease during the middle stage, then decreased slowly once it reached the moisture content (MC) of 22.9%. The drying process adopted here can be classified as slow drying, since it took 63 days for the seed initial MC of 48.86% to 18.19%. Pammenter *et al.* (1998) stated that the drying rate of whole seeds could influence the response of desiccation, with rapidly dried seeds surviving lower moisture contents. Slow drying contributed to homogeneous dehydration, leading to considerable membrane degradation. *Saraca asoca* can be therefore categorized as desiccation sensitive seed and are recalcitrant in storage behaviour (Joseph *et al.*, 2011). Besides, the seed also exhibit most of the characteristics of recalcitrance such as having large seeds and seeds being fleshy (Thomsen, 2000).

The results revealed that at different temperatures asoca seed showed different germination behaviour (Table 2). At both 25° C and 30° C no germination was observed when the seeds were kept for germination immediately after harvest up to two weeks after harvest (WAH). Here germination commenced from second week onwards and recorded a germination percentage of 40.73% and 60.87% at 25° C and 30° C, respectively. The highest germination percentage of 81.03% and 80.67%, respectively was recorded at 25° C and 30° C at three WAH, whereas, in open nursery condition germination was recorded 4 WAH (83.67%). At 25° C germination could not be achieved after seventh week when the SMC was 22.97%, whereas, at 30° C, germination was recorded till ninth week until the seed moisture content dropped to 20.27% (Table 3).

Under open nursery condition, seed germination commenced immediately after harvest at high SMC (48.86%) and continued till eight weeks after harvest when SMC was 22.75%. Here, immediately after harvest to one week after harvest 78% germination was achieved which increased gradually and reached a highest germination of 83.67% at four weeks after harvest (Table 3). Thereafter, germination gradually decreased with decrease in SMC and reached a final germination of 11.6% at eight WAH. Our results are in conformity with Pammenter *et al.* (Pammenter *et al.*, 1998; Pammenter *et al.*, 2000) wherein he described two types of damage that could occur in desiccation sensitive seeds on drying: strict desiccation damage due to the loss of macro-molecular structures caused by removal of water; and aqueous-based oxidative damage that occurred at intermediate water contents.

Results further revealed that mean germination time (MGT) followed the same trend as with the seed germination. MGT was not recorded at 25° and 30° C at harvest and 1 WAH as there was no germination. MGT also commenced and reached 84.4 at 2 WAH. However, least MGT was recorded at 25° C (32.4) followed by 30° C (48.0) and open nursery condition (72.2). Maximum MGT of 110 was recorded in open nursery condition at 2 WAH and least at 30° C (31.3 at 6 WAH) (Table 3). MGT gradually decreased under open nursery condition from 105.0 to 59.2.

From the results in Table 2 it is revealed that at both the temperatures (25° and 30° C) the germination commenced at 2 WAH and first germination was recorded as 55.3 and 34.2 days at 25° and 30°C, respectively, whereas, in open nursery it was 39.7 days. However, at 10 WAH there was no germination and hence, the parameter was not recorded. Days for 50 % germination were also recorded at 2 WAH at 30° C and under open nursery condition, whereas, it did not occur at 25° C in all the seeds at any harvest time except at 3 WAH (Table 2). Days for 50% germination varied between 103 - 113 days at 30° C and 93-147 days under open nursery conditions. In the same condition germination started immediately after harvest (147.5 days) and continued upto 6 WAH (104.9 days) and thereafter there was less than 50 % germination.

Recalcitrant seed sensitivity to dehydration depends on species, drying rate and drying temperature. Critical moisture

Table 2: Effect of different temperatures and days after harvest on seed germination of *Saraca asoca*

Treatments	Days taken for first germination			Days for 50% germination			Days taken for completion of germination		
	Temperature (T)								
	25° C	30° C	Open nursery	25° C	30° C	Open nursery	25° C	30° C	Open nursery
D_1- Immediately after harvest	0	0	29.4	0	0	147.5	0	0	154.1
D_2- 1 week after harvest	0	0	34.8	0	0	129.8	0	0	149.7
D_3- 2 weeks after harvest	55.3	34.2	39.7	0	113.4	107.5	55.3	113.4	133.4
D_4- 3 weeks after harvest	43.9	30.0	46.3	48.7	103.4	101.8	50.6	114.8	121.7
D_5- 4 weeks after harvest	21.1	36.6	33.8	0	0	96.6	21.1	100.6	100.5
D_6- 5 weeks after harvest	84.1	32.7	37.4	0	0	93.1	90.2	90.7	117.0
D_7- 6 weeks after harvest	39.2	31.1	44.9	0	0	104.9	83.3	31.1	111.8
D_8- 7 weeks after harvest	35.6	55.8	54.6	0	0	0	75.3	75.7	55.6
D_9- 8 weeks after harvest	0	63.7	59.4	0	0	0	0	63.7	59.3
D_{10}- 9 weeks after harvest	0	61.9	0	0	0	0	0	62.6	0
Mean	25.4	31.5	34.6	4.4	19.7	71.2	34.2	59.4	91.2
	D	**T**	**DxT**	**D**	**T**	**DxT**	**D**	**T**	**DxT**
SEm±	0.9	0.5	1.7	0.8	0.4	1.4	0.9	0.5	1.6
CD (5 %)	2.8	1.5	4.9	NS	NS	4.1	2.6	1.4	4.6

D = Days after harvest; T = Temperature

Table 3: Effect of different temperatures and days after harvest on seed germination of *Saraca asoca*

Treatments	Germination percentage (%)			Mean germination time (MGT)		
	Temperature (T)					
	$T_1$25° C	$T_2$30° C	T_3Open nursery	$T_1$25° C	$T_2$30° C	T_3 Open nursery
Days after harvest (D)						
D_1–Immediately after harvest	0	0	78.03	0	0	96.9
D_2- 1 week after harvest	0	0	78.9	0	0	105.0
D_3- 2 weeks after harvest	40.7	60.9	82.4	56.3	86.7	110.3
D_4- 3 weeks after harvest	81.0	80.7	82.3	48.3	88.0	101.6
D_5- 4 weeks after harvest	20.9	40.5	83.7	45.8	68.2	90.9
D_6- 5 weeks after harvest	40.5	40.2	61.1	87.5	61.3	86.2
D_7- 6 weeks after harvest	39.9	20.5	60.3	61.5	31.3	89.7
D_8- 7 weeks after harvest	41.0	40.9	21.8	56.7	66.3	55.0
D_9- 8 weeks after harvest	0	21.7	11.6	0	63.0	59.0
D_{10}- 9 weeks after harvest	0	20.3	0	0	63.4	0
Mean	24.0	29.6	50.9	32.4	48.0	72.2
	D	**T**	**DxT**	**D**	**T**	**DxT**
SEm±	0.9	0.5	1.6	1.1	0.6	1.9
CD (5 %)	2.7	1.4	4.6	3.2	1.7	5.6

D = Days after harvest; T = Temperature

content (CMC) is considered to occur when a significant reduction in germination percentage is observed and lethal moisture content is determined by a complete lack of germination (Pritchard, 2004). CMC is the lowest-safe moisture content of the species and for asoca it was recorded to be between 48.86 to 23.97% and the lethal moisture content varied from and 23.97 to 18.22%. Knowledge of the critical and lethal moisture levels of a species is indispensable for planning and execution of seed drying and storage. The results from the present study are complementary to finding made by Becwar *et al.* (1982) on desiccation sensitive silver maple (*Acer saccharinum* L.) and areca palm (*Chrysalidocarpus lutescens* [Bory] Wendl.) and Joseph *et al.* (2011) on *Garcinia kola.* King and Roberts also suggested that seed death resulting from desiccation occurs at or below critical moisture and is caused by enzymatic breakdown (King and Roberts, 1980).

CONCLUSION

From this study it could be concluded that the seeds of asoca could not tolerate drying to lower SMC of 20-23% and might be described as recalcitrant. Moreover, recalcitrant seeds tend to be produced by trees, are relatively large in size, shed during the rainy season and germinate relatively rapidly, unhindered by the presence of a relatively thin seed coat (Pritchard, 2004). Recalcitrant seeds pose serious challenges in *ex-situ* germplasm storage as their seeds are intolerant to desiccation and sensitive to chilling (Roberts, 1973). Since this species is threatened, the future line of work should concentrate on effective *ex situ* conservation measures such as alternate method of propagation, cryopreservation or living collections to ensure their continued existence.

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