LATE MATURATION CHANGES IN SAL (*SHOREA ROBUSTA*) SEED AND THEIR EVALUATION AS INDICES FOR PROPER TIMING OF SEED COLLECTION

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PHARTYAL, S. S., THAPLIYAL, R. C., NAYAL, J. S. & RAWAT, M. M. S. 2002. **Late maturation changes in Sal (*Shorea robusta*) seed and their evaluation as indices for proper timing of seed collection.** Sal (*Shorea robusta*) seeds are best collected approximately 69 days after anthesis (DAA) when fruit coat colour changes from greenish yellow to dark brown. The high germination indicated that physiological maturity was reached around this time. At this stage of development fruit volume, weight, cotyledonary petiole length and germination value were at their maximum and electrical conductivity of seed leachates and mean germination time were at their minimum. In sal, the initial manifestation of germination is through elongation of cotyledonary petiole, which bursts through the pericarp and carries the radicle/ plumule axis to the ground. The petiole length may therefore serve as a potential vigour index for sal seed.

Key words: *Shorea robusta* - maturity indices - electrical conductivity - cotyledonary petiole - mean germination time - vigour index

PHARTYAL, S. S., THAPLIYAL, R. C., NAYAL, J. S. & RAWAT, M. M. S. 2002. **Perubahan kematangan lewat dalam biji benih sal (*Shorea robusta*) serta penilaiannya sebagai indeks untuk kutipan biji benih pada masa yang sesuai.** Biji benih Sal (*Shorea robusta*) paling baik dikutip kira-kira 69 hari selepas antesis (DAA) apabila warna kulit buah berubah daripada kuning kehijauan kepada coklat gelap. Percambahan yang tinggi menandakan bahawa kematangan fisiologi dicapai pada masa ini. Pada tahap ini isipadu, berat dan panjang petiol kotiledon buah serta nilai percambahan adalah maksimum. Sebaliknya kekonduksian elektrik bagi larut lesap biji benih dan min masa percambahan berada pada tahap minimum. Dalam sal, tanda awal percambahan adalah melalui pemanjangan petiol kotiledon yang pecah melalui perikarpa lalu membawa paksi radikel/plumul ke tanah. Panjang petiol dapat bertindak sebagai indeks kecergasan yang berpotensi bagi biji benih sal.

Introduction

The knowledge of the right stage and time of seed maturity is essential for collection of abundant quantity of healthy and vigorous seeds. Fully ripened seeds retain viability longer than seeds collected when immature (Harrington 1970, Stein *et al.* 1974). These aspects have been studied by several workers in tree seeds, for

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example, in *Quercus* spp. (Bonner 1974, 1976), *Q. robur* (Grange & Finch-Savage 1992), *Q. nigra* (Bonner 1996), *Shorea roxburghii* (Panochit *et al.* 1986), *Acer platanoides* (Hong & Ellis 1992), *Aesculus hippocastanum* (Tompsett & Pritchard 1993), *Michelia compressa* (Lin & Chen 1995), *M. kusanoi* (Chien & Lin 1997) and *Aesculus indica* (Uniyal & Nautiyal 1996).

High level of viability and vigour is attained when seeds reach desired levels of metabolic status. Nautiyal and Purohit (1985) conducted a series of studies on biochemical and physiological changes accompanying the growth and development of sal (*S. robusta*) seeds. The sal seed is typically recalcitrant, characterised by moisture content as high as around 50% at the time of dispersal, and is desiccation-intolerant and have a short viability of about 10 days under ambient conditions (Purohit *et al.* 1982, Tompsett 1985).

High mortality of sal seedlings and lack of natural regeneration have been widely discussed in India (Anonymous 1953). Several factors have been implicated in the lack of regeneration of *S. robusta*, the major one being the post-recruitment drought (Bhatnagar 1961), which leads to failure of establishment. However, one of the little noticed factors could be the inability of radicle to penetrate the soil resulting in the failure of normal development of seedlings. The orientation of seed and lack of proper directional growth of plumule and radical are major factors in the survival of sal seedlings (Sharma & Purohit 1980). Similar observations have been recorded for several dipterocarp species (Anonymous 1985).

The short viability of sal seed necessitates its immediate sowing. Therefore, it is necessary to collect seed only at a time when the viability and vigour of seed is at its maximum. An investigation was taken up to evaluate the vigour indices for the seed of this species. Vigour index could be of potential value to determine the proper timing of seed collection.

Materials and methods

Sal fruits are winged and serve as the dispersal unit. Throughout this study they are referred to as "seeds". Four trees were selected as replicates within the campus of Forest Research Institute, Dehra Dun, which is situated at 30° 20' 40" latitude, 77° 52'12" longitude and 640 m asl altitude. Flower buds appear in the last week of March and anthesis begins around the second week of April. The first harvest was made 48 days after anthesis (DAA) and subsequently at weekly intervals up to the sixth week, i.e. 83 DAA. A total of six collections were made. For each harvest, seeds were collected in the morning, brought to the laboratory and were de-winged immediately. Measurements were made on the length, width, volume, fresh and dry weight as well as the fruit colour. The volume of the seeds was determined using a measuring cylinder by water displacement method. The fresh weight values were determined for eight replicates of 100 seeds per sample (Anonymous 1993). Moisture content was measured for four replicates with 10 g of seed in each sample by the oven-dry method at 103 ± 2 °C for 17 ± 1 hours. Results are presented on a wet weight basis. Dry matter percentage was obtained after drying the seed at 60 °C for 72 hours in a hot air oven. The germination tests were carried out by placing four replicates of 50 seeds each on moist germination paper in Petri dishes at 30 ± 1°C in a laboratory seed germinator. The emergence of 1.0 cm cotyledonary petiole length was taken as the criterion of germination. The germination value (GV) was calculated according to Czabator (1962) while mean germination time (MGT) was calculated according to Bonner (1983). Length of cotyledonary petiole was measured after 14 days of sowing, using four replicates of 25 seedlings selected at random (except for the first harvest, where all seedlings were considered). Electrical conductivity of seed leachates (EC) was measured according to Bonner (1991), using four replicates of 10 seeds each. Seeds were weighed, rinsed in a strainer under running tap water for 15 seconds to remove surface dust and then steeped in 50 ml deionised water in 100 ml beakers. The seeds were stirred with a spatula to break the surface tension and ensure uniform seed-to-water contact. The beakers were covered and placed in an incubator at 20 ± 1°C for 24 hours. The electrical conductivity of the leachates was measured using a digital conductivity meter and expressed in mhos g^{-1} of seed at 20 °C. The data recorded under each parameter were subjected to one-way ANOVA. The critical differences (CD) were calculated by LSD method at 5 and 1% rejection level to see the significance of differences between different collection dates with respect to all the parameters.

Results

At the time of first harvest (48 DAA), seeds were greenish yellow with firmly attached yellow wings. The wings gradually turned brown as development progressed. At the time of final harvest they had turned dark brown and were easily detachable. The data on seed length, width and volume revealed significant differences ($p = 0.01$) between different harvests (Table 1). Seed length and width increased steadily and reached a maximum of 21.3 and 13.3 mm respectively at 76 DAA. Both the length and width declined slightly by 83 DAA, when the seed had reached the post maturity stage.

Moisture content declined concomitantly until preceding the last harvest (76 DAA) when it started to level off; actual values were 70.7% initially and 46.6% at 83 DAA. Dry matter increased from an initial value of 30.2 to 53.3% at 83 DAA. Seed fresh weight increased rapidly from 48 DAA (52.2 g) to 69 DAA (164.9 g) and then declined slightly by the last harvest.

The germination on different harvest dates varied from 18.5 to 99.0%. Highest germination rates of 98.5 and 99.0% were recorded between 69 and 76 DAA. Maximum germination was recorded when seed moisture content declined to 46.8%. The GV and MGT revealed significant differences ($p = 0.01$) between different collection days. Highest GV of 546.5 was recorded for seeds collected at 69 DAA followed by a GV of 458.1 at 76 DAA. At first harvest a minimum value of 3.69 was recorded. Seeds at 48 and 55 DAA took significantly more time to complete germination compared with seed at 69 DAA, which took a minimum time of 2.74 days.

Table 1 Chronological changes in morpho-physiological characteristics during late maturation phase of *Shorea robusta* (sal) seed

DAA	Length (mm)	Width (mm)	Volume (ml)	Fresh weight of 100 seed (g)	Dry matter %	Moisture content %	Germination %	Germination value (GV)	Mean germination time (MGT) (days)	Cotyledonary petiole length (cm)	Electrical conductivity of leachates (EC) (mhos g^{-1})
48	14.20	8.88	0.68	52.18	30.20	70.7	18.50 (25.46)	3.69	13.52	0.83	0.035
55	15.35	9.36	0.87	77.40	31.90	67.4	62.50 (52.25)	28.47	13.09	3.03	0.026
62	18.55	11.03	1.26	159.9	46.30	56.5	90.00 (71.60)	264.3	5.70	4.96	0.022
69	20.28	12.72	1.61	164.9	50.30	50.3	98.50 (83.90)	546.5	2.74	5.68	0.016
76	21.33	13.27	1.59	155.3	52.80	46.8	99.00 (85.93)	458.1	3.61	4.98	0.024
83	20.81	12.88	1.59	155.8	53.30	46.6	97.00 (80.16)	362.9	4.19	4.48	0.033
SE	± 0.41	± 0.14	± 0.15	± 0.61	± 1.31	± 0.85	± 2.08	± 5.55	± 0.424	± 0.128	± 0.0012
CD at 5 %	0.867	0.304	0.308	1.31	2.79	1.81	4.42	11.82	0.903	0.272	0.0025
CD at 1 %	1.199	0.421	0.426	1.81	3.86	2.51	6.11	16.34	1.250	0.376	0.0034

* Values in parentheses are arc sin transformations.

The EC of seed leachates and cotyledonary petiole length for each harvest are also presented in Table 1. The maximum EC of 0.035 mhos g^{-1} was recorded for seed at 48 DAA followed by 83 DAA (0.033 mhos g^{-1}). However, these two values were not statistically different. The minimum EC of 0.016 mhos g^{-1} was recorded for seed at 69 DAA. There were significant differences ($p = 0.01$) between cotyledonary petiole lengths. The maximum length of 5.68 cm was recorded for 69 DAA seed, followed in descending order by 76 DAA (4.98 cm) and 62 DAA (4.96 cm) seeds. The minimum cotyledonary petiole length of 0.83 cm was recorded for seed collected at 48 DAA.

Simple correlation coefficients between important parameters of seed during maturation are presented in Table 2. The germination per cent showed significant positive correlation ($p = 0.01$) with seed length ($r = 0.96$), width ($r = 0.94$), volume ($r = 0.97$), fresh weight ($r = 0.95$), dry matter ($r = 0.94$) and cotyledonary petiole length ($r = 0.97$) but significant negative correlation with moisture content ($r = 0.96$). There was no significant correlation of germination percentage with EC during seed development.

Table 2 The simple correlation coefficient between important parameters of *Shorea robusta* (sal) seed during late maturation phase

Characters	Germination value (GV)	Mean germination time (MGT)	Cotyledonary petiole length	Germination (%)
Seed length	0.94**	- 0.97**	0.88*	0.96**
Seed width	0.96**	- 0.96**	0.85*	0.94**
Seed volume	0.96**	- 0.98**	0.90*	0.97**
Seed fresh weight	0.91*	- 0.97**	0.96**	0.95**
Seed dry matter (%)	0.93**	- 0.98**	0.88*	0.94**
Seed moisture content (%)	- 0.93**	0.96**	- 0.87*	- 0.96**
Electrical conductivity	- 0.62	0.54	- 0.76	- 0.61
Germination (%)	0.91*	- 0.94**	0.97**	-

* Significant at 5% rejection level ** Significant at 1% rejection level

Discussion

Natural seedfall in sal commences in the first week of June and continues until the last week of July or beginning of August. During this period, the seed enters into a phase of desiccation. This is obvious from Table 1 where moisture content decreased from 70.7 to 46.6% in the third week of July. Seed maturity reached its peak around 69 to 76 DAA, when the germination and GV reached its highest level while MGT reached its lowest level. The moisture content of seed at this time was between 50.3 and 46.8%. The moisture content further declined to 46.6% with the decline in germination and GV and the increase in MGT. A further decline down to 20% moisture content after harvesting results in complete loss of seed viability (Purohit *et al.* 1982, Tompsett 1985). Generally, in recalcitrant seeds maturity indices, measured as germination ability, is achieved during the last few weeks on the tree or, to some extent, after harvest (Tompsett 1987, Finch-Savage & Blake 1994). However, the effect of stage of maturity at harvest on seed storability is unclear (Chien & Lin 1997). Panochit *et al.* (1986) showed that seeds of *S. roxbughii* collected two weeks before natural seedfall has higher storability than those collected at natural seedfall. The onset of moisture loss triggers a decline in the physiological activity of maturing cotyledons as well as embryo before the sal seed falls from the branch (Nautiyal & Purohit 1985). Thus changes taking place in seeds by 76 DAA could be considered as part of senescence which eventually led to the loss of vigour. This is shown by the increasing trend of EC following the lowest value of EC at 69 DAA. It indicated disturbances in the integrity of tissue membranes at this stage when seed enters into a rapid desiccation phase. The relation between high germination and low EC of seed leachates has been reported by Yadav *et al.* (1987) in sal seed, while Sahlen and Gjelsvik (1993) correlated seed maturity with low EC of seed leachates in *Pinus sylvestris*. Tekrony & Egli (1997) reported low level of vigour, tested by EC of seed leachates, for immature seed while high level of vigour (low EC) for physiologically mature seed.

The seed had the best chance of recruitment around 69 DAA, when seed attained the maximum cotyledonary petiole length (Table 1). The length of cotyledonary petiole, therefore, could be the major determinant of high vigour of sal seed. Similar observations have been recorded for several other dipterocarp species (Anonymous 1985). Since sal seed became germinable quite early during ontogeny, the germination per cent was less indicative of the level of maturity reached by the seed as germination between 69 to 83 DAA did not vary significantly ($p = 0.05$). However, GV and cotyledonary petiole length was significantly ($p = 0.01$) high at 69 DAA. EC and MGT were at their lowest values at this collection time, indicating that the seed was at maximum vigour at this stage. Since the magnitude of correlation coefficient between cotyledonary petiole length and germination percentage and seed fresh weight was high, $r = 0.97$ and $r = 0.96$ respectively, the fresh seed weight could be of practical value for field foresters to judge the vigour of seed and to obtain physiologically mature and highly vigorous seeds.

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