



Seed pattern, germination and viability of some tree species seeds from Girnar Reserve Forest of Gujarat

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Abstract A study was carried out on seed characteristics of nine different tree species of six botanical families for germination and viability during three years, 2008–2009, 2009–2010 and 2010–2011. Average germination percentages after 5, 10 and 20 days were 27.22, 55.56 and 69.44 % for all nine species. Highest germination percentage was recorded in two species of bombacaceae, viz., *Bombax ceiba* (95 %), followed by *Ceiba pentandra* L (30 %). Rest of the species showed less than 30 % germination after 5 days of incubation. After 20 days, seedling length was highest in *B. ceiba*, followed by *Leucaena leucophala* with values of 16.20 and 16.05 cm, respectively. Average seedling vigor index (SVI) was 59.08 after 5 days, which increased regularly with values of 479.38 and 865.11 after 10 and 20 days. Highest SVI was recorded in *B. ceiba*, following by *Sterculia urens* at all the stages after 5, 10 and 20 days. Average viability was 91.22 % among all species, with highest value of 94 % for *Albizia lebbbeck*. Two species, viz., *C. pentandra* and *Delonix regia* exhibited lowest viability of 80 %. Root length/shoot length (RL/SL) ratio decreased with age from 9.86 at 5 days after germination to 0.75 and 0.52 at 10 and 20 days after germination, respectively. In general seeds were recalcitrant type. Knowledge on germination, pattern and viability is not only important for understanding community process but also for framing strategies for conservation of biodiversity and restoration of forests.

Keywords Germination percent · Tree species · Viability · Seed vigor Index · Seedling length

Introduction

Among farming community seeds have traditionally been conserved in every house hold to continue the cycle of food production even in years of scarcity (Seth and Mishra 1998). However, earlier the process was not well organized to improve germination. Seeds of some species are capable of germinating within few days following anthesis (Hume 1984). Malcom et al. (2003) defined germination as the process by which the dormant embryo grows out of the seed coat and establish itself as a seedling. According to Harrington (1972), seeds will die if allowed to dry out. Hyde (1954) had shown that seed coat permeability increases with the drying of seeds. Hence seeds of hard seeded species are collected before drying on mother plant, which ultimately leads to germination (Sidhu and Cavers 1977). Srivastava et al. (2001) worked on germination, ecology and seedling growth of different seeds and emphasized that germination studies need to be started earlier after collection, as seeds may undergo changes in their germination responses during storage. According to Chalm et al. (1967) main aim of seed testing is to get accurate and reproducible results regarding the purity, composition and the percentage of seeds that can be expected to produce normal seedling under favorable conditions. Ginwal et al. (2005) found considerable variability for seed source, germination, viability and seedling growth. Seeds develop particular germination characteristics to offset fluctuations in habitat survival (Fenner and Thomson 2005). Study on seed germination of forest seeds is of great interest among scientist as it is directly related with ecology of particular area. Seed biomass is one of the most

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fascinated trait, which has effect on germination (Leishman et al. 2000; Moles et al. 2005a, b). Relationship between seed size and germination percent is very important as smaller seeds show late germination compared to larger seeds, which have higher post dispersal predation than smaller seeds. Time for germination is also very important as it is correlated with survival of the particular species (Foster and Janson 1985). Germination study helps in identifying proper ecological conditions for particular species of any area. Normal seed contains an embryo or sometimes more than one, will develop into the seedling, and have a supply of reserve substances, which will be helpful in sustaining the seedling at the different embryo growth stages till it becomes self dependent (Black et al. 2006). Therefore, a dormant seed should have very low water content, which results in slow physiological processes.

Viability is the capacity of seed being alive. Viability studies help to select proper genotype or species, as it provide a measurement of germinability. 2,3,5-Tri phenyl tetrazolium chloride is mostly used as an alternative of the germination test for all types of seeds. This test was first time described by Kuhn and Jherel (1941). According to Lakon (1942), if seed is viable, then it is going to germinate and TTZ is useful for topographical evaluation of the viability of the seeds as it uses staining of the embryo. The test can evaluate the viability of seeds rapidly, also when in a state of dormancy (Gaspar and Nagy 1981). Evaluation of monocot as well as dicot seeds can be easily done (Khare and Bhale 2000). Proportion of species producing dormant seeds increases with the gradient of dryness and unpredictable rainfall. Seed viability remains higher for dry tropical compared to wet tropical species. High temperature and moist environments have adopted traits for reproductive and dispersal mechanism in rainforest. Cyclic perturbation due to seasonality allows germination seasonally. On the basis of viability seeds are classified into two different types, viz, orthodox and recalcitrant seeds, orthodox seeds usually remain dormant but retain their viability for longer time period, whereas recalcitrant seeds show very less dormancy (Khurana and Singh 2001). The aims of the present study were (1) to record germination percent of each of nine forest tree species collected from Girnar Reserve Forest area, and study variations among them, (2) to check viability among all species and to compare them with germination value, and (3) to study associations among seed morphological parameters and germination traits using statistical analysis.

Materials and methods

About 100 to 150 seeds of each of the nine tree species were collected for three years during 2008–2009, 2009–2010 and 2010–2011 from Girnar Reserve Forest and

surrounding areas near Junagadh (70°28'–70°27'N longitude and 21°30'–21°26'E latitude) just after fruiting season, and were taken to laboratory. Appropriate time period for seeds collection was decided on the basis of phenological studies. Morphological characters were studied using Gunn (1979) and Gavit and Parabia (1989). Seeds were measured for characters like length, width, thickness, volume, surface, shape, hilum shape and color. Two lots of seeds were prepared for germination and viability test each. Petri-dish with 20 cm diameter was lined with brown colored three layered moistened germination papers for germination experiment. For studying germination, three sets of petri-dish, comprising three replications, each having ten seeds for each tree species were kept at room temperature (30 ± 1 °C). All related physiological parameters such as root length, shoot length, seedling length, seed vigor index, number of lateral roots, root length shoot length ratio, germination percent and germination values were observed at regular intervals of 5, 10 and 20 days. For germination percent regular count was done for each species. Germination value was calculated using formula given by Djavanshir and Pourbeik (1976):

$$GV = (3DGs/N) \times (GP/10),$$

where DGs: daily germination speed; N: means number of seeds; GP: germination percentage.

Seedling fresh weight was measured using digital balance with sensitivity of 0.0001 g. For dry weight, seedlings were kept at 60 °C in oven for 48 h, and then measured on digital balance.

Seed viability was measured using 2,3,5-tetrazolium chloride test (ISTA 1983). 2,3,5-Tetrazolium chloride (0.1 %) was prepared in the phosphate buffer solution of 7.0 pH. Seeds of all species were soaked in water at room temperature (30 ± 1 °C) for 6 h. Seeds were de-coated with the help of forceps after imbibitions. De-coated seeds were kept in 0.1 % tetrazolium solution in beakers in darkness at room temperature for 20 h. Assessment of seed viability was done using method of Khare and Bhale (2000) after 20 days. Seeds were classified as orthodox or recalcitrant on the basis of their viability. Statistical analysis for regression and correlation was done wherever possible. Simple statistical analysis parameters like average, minimum, maximum, standard deviation along with one way ANOVA was calculated using methods given by Gomez and Gomez (1984).

Results and discussion

Results obtained for 2008–2009, 2009–2010 and 2010–2011 showed variability for most of the characters studied, which are described and discussed under three

Table 1 Seed morphology—quantitative and qualitative parameters

A. Qualitative parameters						
No.	Species	Colour	Surface	Shape	Hilum shape	
1.	<i>Aegle marmelos</i>	Yellow, whitish light yellow	Rugose, glabrous	Sub ovate, ovate, broad elliptic	Circular	
2.	<i>Albizia lebbbeck</i>	Yellowish brown, U shaped black line	Rough, glabrous	Ellipsoidal, subellipsoidal	Elliptic	
3.	<i>Bombax ceiba</i>	Reddish to blackish brown	Glabrous, granular, rough	Ovate, sub ovate, ellipsoidal	Circular, narrow elliptic	
4.	<i>Cassia siamea</i>	Brownish black	Rough, shining	Ovate, sub ovate, ellipsoidal	Circular	
5.	<i>Ceiba pentandra</i>	Black, light black	Rough, granular	Sub reniform, sub ovate, oblong	Circular	
6.	<i>Delonix regia</i>	Brown with yellow spots	Smooth, transversely septed	Elliptic, oblong, suboblong	Circular	
7.	<i>Leucaena leucophala</i>	Blackish brown	Smooth, shining	Ellipsoidal, subellipsoidal	Circular	
8.	<i>Peltophorum pterocarpum</i>	Blackish brown	Smooth, shining	Oblong, ovate, sub-ovate, sub-reniform	Linear, narrow ovate	
9.	<i>Sterculia urens</i>	Blackish to light brown	Smooth, shining	Broad narrow elliptic	Circular	
B. Quantitative parameters						
No.	Species	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)	Volume (cm ³)
1.	<i>Aegle marmelos</i>	0.78 ± 0.06	0.57 ± 0.05	0.22 ± 0.02	0.07 ± 0.03	0.10 ± 0.02
2.	<i>Albizia lebbbeck</i>	1.23 ± 0.02	0.57 ± 0.10	0.21 ± 0.05	0.21 ± 0.01	0.15 ± 0.02
3.	<i>Bombax ceiba</i>	0.28 ± 0.06	0.17 ± 0.12	0.20 ± 0.24	0.09 ± 0.03	0.17 ± 0.15
4.	<i>Cassia siamea</i>	0.42 ± 0.01	0.36 ± 0.04	0.38 ± 0.04	0.02 ± 0.02	0.06 ± 0.01
5.	<i>Ceiba pentandra</i>	0.77 ± 0.02	0.47 ± 0.05	0.24 ± 0.04	0.07 ± 0.02	0.07 ± 0.02
6.	<i>Delonix regia</i>	1.61 ± 0.01	0.76 ± 0.03	0.52 ± 0.02	1.24 ± 0.27	0.64 ± 0.05
7.	<i>Leucaena leucophala</i>	0.66 ± 0.05	0.58 ± 0.06	0.16 ± 0.06	0.05 ± 0.01	0.06 ± 0.03
8.	<i>Peltophorum pterocarpum</i>	1.10 ± 0.02	0.54 ± 0.05	0.28 ± 0.04	0.62 ± 0.10	0.17 ± 0.02
9.	<i>Sterculia urens</i>	1.20 ± 0.04	0.80 ± 0.05	0.54 ± 0.03	0.19 ± 0.17	0.52 ± 0.07
	Average ± SD	0.89 ± 0.03	0.54 ± 0.06	0.31 ± 0.06	0.28 ± 0.07	0.21 ± 0.05

Parameters values have been expressed as mean ± SD

heads, viz., seed morphology, seed germination and seed viability.

Seed morphology

Seeds of nine tree species showed different colors, such as yellow, brown, light brown, blackish brown and brownish black (Table 1A). Seeds of *Delonix regia* showed some yellow-colored spots, while seeds of *Albizia lebbbeck* showed U-shaped lining along with brown color. Seeds showed different kinds of surfaces like rugose, glabrous, smooth, shining and transversely septed. Ovate, sub-ovate, elliptical, sub-elliptical, broad narrow elliptic, reniform, subreniform were some of the shapes for different seeds. Hilum shape varied from elliptical to linear, ovate and circular. Most of the species showed circular shape of hilum. Length, width, thickness and volume were highest in *D. regia* and lowest length, width and thickness were observed in *Bombax ceiba*, but the lowest seed volume was

in *Cassia siamea*. Length, width, thickness and volume showed average values of 0.89, 0.54, 0.31, 0.21 cm, respectively (Table 1B). Positive significant correlations were observed between seed length and seed weight (0.767**), and seed weight and number of lateral roots (0.811**). Srivastava et al. (2001) also obtained significant positive association between seed size and seed weight. Working on forest species *Artocarpus heterophyllus* in North eastern India, Khan (2004) reported that germination time is significantly correlated with that of seed mass. According to Augustine et al. (2001) variations in the qualitative and quantitative traits and morphology of seed serves as source of systematic characterization of species.

Seed germination

Significant variations were observed among germination parameters during all the years. Highest root length of 3.03 cm after 5 days was observed in *Sterculia urens*,

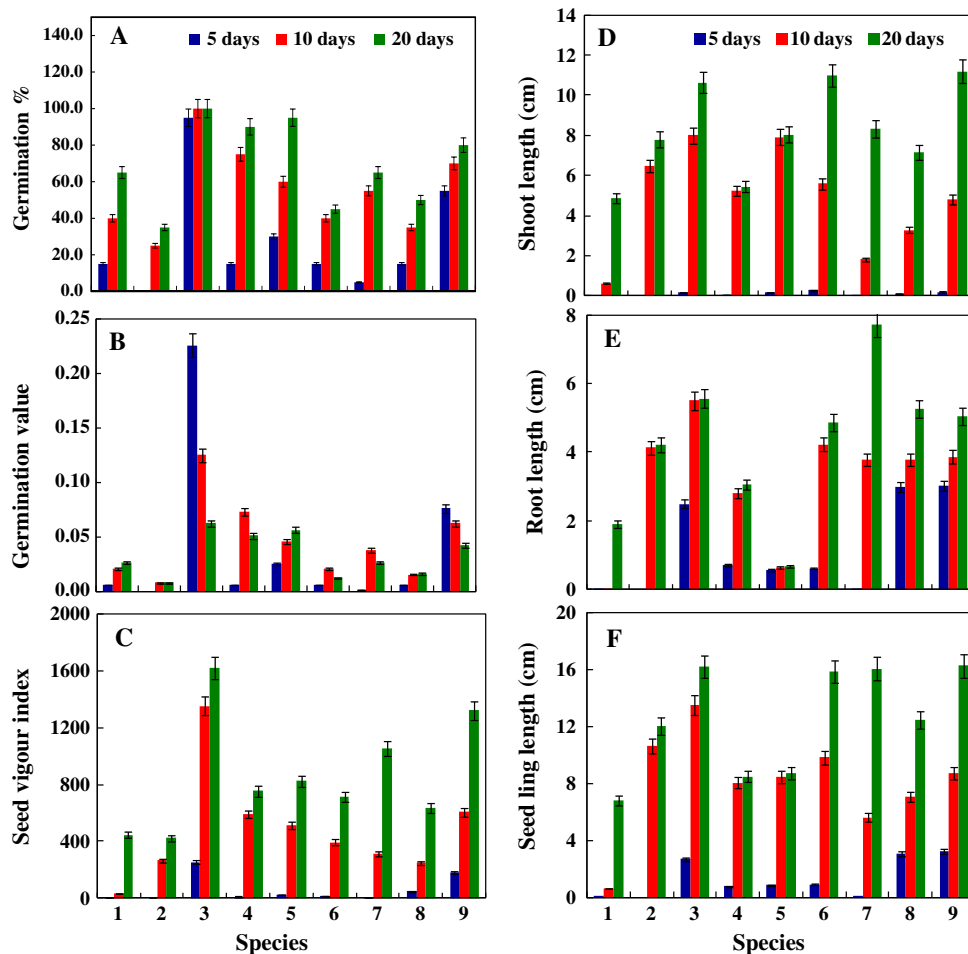


Fig. 1 Average values of some important germination parameters after 5, 10 and 20 days after experiment (*1 Aegle marmelos* (L.) Corr., *2 Albizia lebbeck* (L.) Bth., *3 Bombax ceiba* L., *4 Cassia siamea* Lam., *5 Ceiba pentandra* L., *6 Delonix regia* (Boj.) Raf., *7 Leucaena leucocphala* (Lam) de Wit, *8 Peltophorum pterocarpum* (DC) Backer ex Heyne, *9 Sterculia urens* Roxb. **A, B** and **C** are expressed in cm while **D** and **E** are shown in g. Figure explains that seed vigour index is highest in *Bombax ceiba* L. after 20 days, while seedling dry weight was highest in *Sterculia urens* Roxb. **A, B** and **C** are in cm, while **D** and **E** are in g)

while after 10 and 20 days higher root lengths was observed in *B. ceiba* with values of 5.51 and 5.57 cm, respectively. Total seedling length after 5 days was found to be highest in *S. urens* with values of 3.22 cm, while after 10 and 20 days it was higher in *B. ceiba* and *S. urens* with values of 13.52 and 16.24 cm, respectively. Although after 20 days *B. ceiba* and *Leucaena leucocphala* also showed good increase with value of 16.20 and 16.05 cm (Fig. 1a). *B. ceiba* showed highest germination percentage (100 %) after 10 days, with all seeds germinated. Niroula et al. (2012) reported that germination in *B. ceiba* can be enhanced up to 63 % in control condition by addition of continuous water. Agboola (1998) observed 97.3 and 93.7 % germination for *Ceiba pentandra* and *L. leucocphala*, respectively after 2 days, while 95 and 65 % germination was observed in the same species in this study after 20 days. *A. lebbeck* showed lowest germination percentage after 20 days with value of only 35 %. Among all nine

species average numbers of seeds germinated after 5, 10 and 20 days were 2.72, 5.56 and 6.94, respectively. De-ketay (1996) working on germination of 20 multipurpose leguminous tree species observed that germination remained faster and higher at 25–30 °C for all tree species and therefore, recommended 30 ± 2 °C as optimum temperature for germination for leguminous tree species. Mai-Hong et al. (2003) obtained 98 % germination soon after fruiting in *Peltophorum pterocarpum* with temperature range of 28–34 °C, whereas in this study same species showed 50 % germination. Moreover, Maithani (1990) advocated that increase in temperature leads to faster germination. Satyanarayana et al. (2011) in case of *S. urens* found that during early phase up to 6 days protein content and reducing sugars accumulated in high amount, which resulted in rapid germination. Hall and Swine (1981) and Whitemore (1978) concluded that rain forest seeds germinate faster because they have very short dormancy period.

Highest germination values of 0.23 and 0.13 were observed in *B. ceiba* after 5 and 10 days, respectively, however, after 20 days higher germination value of 0.06 was observed in two different species of family Bombacaceae viz., *B. ceiba* and *C. pentandra* (Fig. 1b). According to Swaminathan et al. (1992) germination value is a better physiological trait to measure performance of seed and quality; hence it is very important trait.

In our study average seed vigor index in case of *A. lebbeck* was 423 (Fig. 1c). However, Farooqui et al. (2009) observed a much higher seed vigour index value of 1,324.80 for *A. lebbeck*. It can be due to different room temperatures of 20 ± 2 °C compared to temperature of 30 ± 2 °C in this study along with different environmental conditions.

After 5 days pooled averages for root length, shoot length and seedling length were 1.17, 0.11, 1.28 cm, respectively for all tree species, which increased to 4.26, 8.27, 12.53 cm after 20 days, respectively (Fig. 1d–f). Results indicated non-significant variations for root length, shoot length and seedling length after 5 days; however, after 10 and 20 days the variability for these parameters was statistically significant. Significant positive correlations between root length and seedling length after 5 (0.99**), 10 (0.74**) and 20 days (0.84**) were observed at all periods. Significant positive correlations were also observed between germination percentage and seed vigor index at 5 (0.96**), 10 (0.89**) and 20 days (0.66*).

Mean values for number of lateral roots were 2.07, 8.38 and 10.76 after 5, 10 and 20 days, respectively (Fig. 2a). Highest value was observed in *P. pterocarpum* with 24.8 lateral roots, while lowest value of only 2.8 numbers of lateral roots were recorded for *Aegle marmelos* after 20 days. Fresh weight (g) of seedling was highest for *P. pterocarpum* after 5 days, with value of 1.18 g (Fig. 2b), while dry weight of seedling was higher for *L. leucophala* with value of 0.09 g (Fig. 2c). However, after 20 days, *D. regia* and *S. urens* exhibited higher fresh (4.52 g) and dry weight of seedlings (1.07 g). Zhang and Deming (1998) in a study on 24 tree species from lower sub-tropical forest observed that heavy seeds germinate faster compared to low weight seeds. However, Norden et al. (2008), tested germination among 1038 different trees species from different regions and concluded that it is not necessary that only heavier seeds germinate faster. Correlations between seedling fresh weight and number of lateral roots was non-significant after 5 days (0.29), but became significantly positive after 10 (0.73**) and 20 days (0.89**), which means seeds adapted themselves with time during germination. Seed weight although did not show direct effect on germination after 20 days but it showed significant positive association with number of lateral roots.

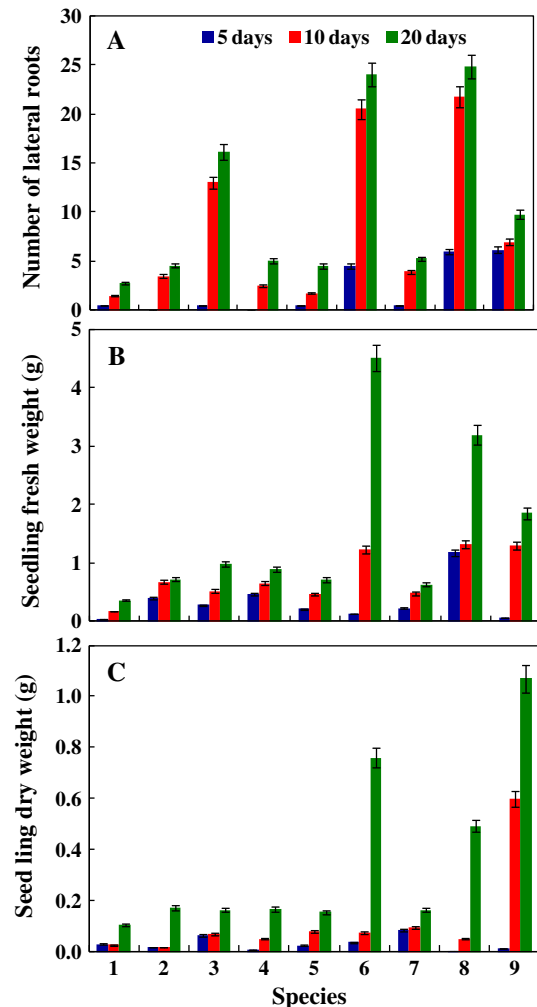


Fig. 2 Average values of lateral roots, and fresh and dry weights of seedlings after 5, 10 and 20 days after experiment (1 *Aegle marmelos* (L.) Corr., 2 *Albizia lebbeck* (L.) Bth., 3 *Bombax ceiba* L., 4 *Cassia siamea* Lam., 5 *Ceiba pentandra* L., 6 *Delonix regia* (Boj.) Raf., 7 *Leucaena leucophala* (Lam) de Wit, 8 *Peltophorum pterocarpum* (DC) Backer ex Heyne, 9 *Sterculia urens* Roxb., Y axis shows percent seeds tested. A to F are different physiological traits associated with viability test)

RL/SL ratio was highest in *P. pterocarpum* after 5 days, with value of 35.29, which decreased to 2.07 and 0.93 for after 10 and 20 days, respectively, suggesting decline in RL/SL ratio with time. Average values for most of the germination traits such as root length, shoot length, seedling length, seed vigor index and number of lateral roots showed constant increase after 5, 10 and 20 days, but RL/SL ratio showed constant decrease with time (Fig. 1).

Seed viability

In TTC test seeds of all species were viable with highest mean value of 94 % in *L. leucophala* and lowest mean

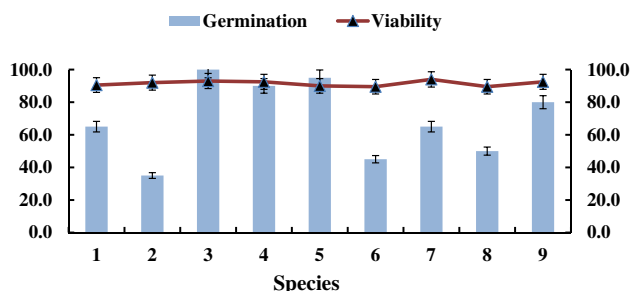
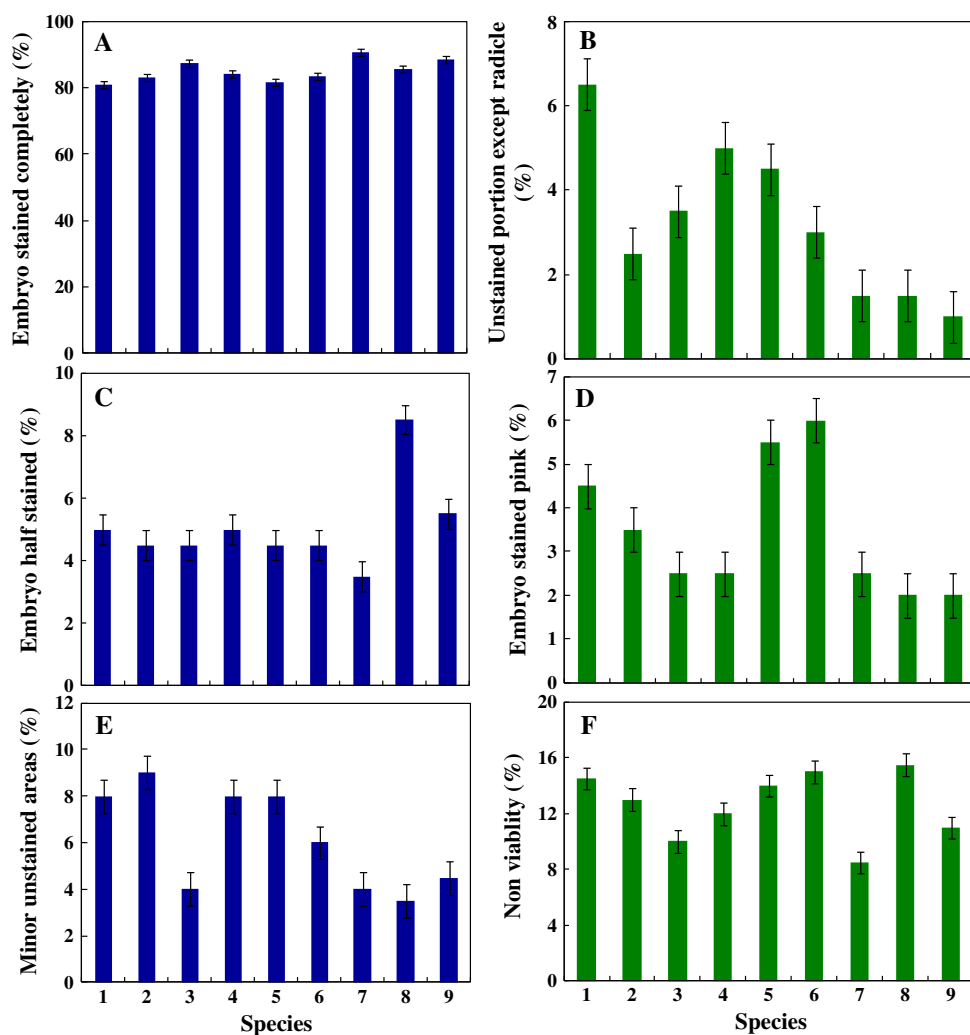


Fig. 3 Comparison of germination and viability percentage after 20 days (1 *Aegle marmelos* (L.) Corr., 2 *Albizia lebbek* (L.) Bth., 3 *Bombax ceiba* L., 4 *Cassia siamea* Lam., 5 *Ceiba pentandra* L., 6 *Delonix regia* (Boj.) Raf., 7 *Leucaena leucocphala* (Lam) de Wit, 8 *Peltophorum pterocarpum* (DC) Backer ex Heyne, 9 *Sterculia urens* Roxb. Overall viability was highest in all of the species compared to germination percent only two *Bombacacea* members viz. *Bombax ceiba* L. and *Ceiba pentandra* exhibited more germination percentage than that of viability. Although in rest of the species germination percentage was lowest compared to viability)

value of 89.5 % in *D. regia* and *P. pterocarpum*. Average viability for three years after 20 days was 91.5 %, which itself shows that all seeds were capable for germination at

Fig. 4 Average values of important seed viability parameters obtained using TTZ test (1 *Aegle marmelos* (L.) Corr., 2 *Albizia lebbek* (L.) Bth., 3 *Bombax ceiba* L., 4 *Cassia siamea* Lam., 5 *Ceiba pentandra* L., 6 *Delonix regia* (Boj.) Raf., 7 *Leucaena leucocphala* (Lam) de Wit, 8 *Peltophorum pterocarpum* (DC) Backer ex Heyne, 9 *Sterculia urens* Roxb.)



that time (Fig. 3). Viability was low in *P. pterocarpum* with value of 15.5 %. In our study, viability percentage was high compared to germination percentage in most of the species, however, two species *B. ceiba* and *C. pentandra* showed higher value of germination percentage than viability percentage. Averages values for different categories of viability test, viz., embryo stained completely (ESC), embryo half stained (EHS), minor unstained on cotyledons opposite the radical (MUS), unstained portion except radical (UPER) and embryo stained light pink (ESLP) were, 85, 5.06, 6.11, 3.22 and 3.44 %, respectively (Fig. 4a–f). Highest value for ESC and EHS were, 90.5 and 8.5 % for *L. leucocphala* and *P. pterocarpum*, respectively; whereas, highest values for MUS, UPER and ESLP tests were 9, 6.5 and 6 %, respectively for *A. lebbek*, *A. marmelos* and *D. regia*, respectively. Species *A. marmelos* and *L. leucocphala* showed lowest average values of 81 and 3.5 % for EHS and MUS, respectively, however, *S. urens* exhibited lowest value of 1 % for UPER. Species, *S. urens* and *P. pterocarpum* exhibited lowest values of 2 % in case of ESLP. Murali (1997) reported in a

study with 99 species of Western Ghat, Karnataka that small seeds germinate faster compared to large seeds. Furthermore, he added that seeds collected during rainy season showed less viability period compared to seeds collected during dry season. These results are in line with our study as all seeds were found viable after collection during dry seasons. In a study on tree species of Victoria Range Forest at Bhavanagar, Gohil (2005) found high germination and viability percentage soon after collection during dry season, which corroborate our results.

Conclusion

It is concluded that seed morphological parameters are directly associated with germination. This association indicated that if seed vigor index is high, germination will be speedy. In addition, all the seeds collected were recalcitrant after short period of collection. The work can be useful in selection of region specific species. Further, if seeds are sown within a month after their dispersal or ripening, germination will be high among the selected nine tree species, as all species showed good germination with lowest value of 35 % in *A. lebbeck* and highest 100 % in *B. ceiba* after 20 days. Species like *B. ceiba*, *S. urens* and *C. pentandra* showed more than 30 % germination within 5 days, which indicated that these tree species are useful for social forestry programmes.

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