Short Communication

Range Mgmt. & Agroforestry 37 (1) : 104-107, 2016
ISSN 0971-2070

Effect of pre-sowing treatments on *Prosopis pallida* seed germination attributes

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Received: 28th December, 2015 Accepted: 9th May, 2016

Abstract

Synchronized and rapid seed germination is advantageous for tree species management and promotion of their cultivation in agro-forestry. Early and synchronized seed germination in largely accepted agroforestry tree species is important to enhance mass production and distribution of seedlings in short rain spell areas. Thus, *Prosopis pallida* seeds were subjected to sulphuric acid (H_2SO_4) scarification treatments at 20%, 30% and 40% concentration for 10 minutes along with soaking in boiled water (80°C) for ten minutes and in cow-dung slurry for 24 hours. Scarification with 30% H_2SO_4 resulted in synchronized germination with 66% germinability, 6.4 day of mean germination time ($\bar{t}$) and mean germination rate ($\overline{v}$) of 0.16 day^{-1} whereas soaking in boiled water recorded lower germinability.

Keywords: Germinability, *Prosopis pallida*, Seed treatments, Synchrony of germination.

Environment, energy and food security are the key instruments guiding global policies. All governments across the globe are working towards green economy. Thus all available resources including wastelands have been pressed to produce biomass to meet energy demand (Ramesha *et al*., 2014). *Prosopis* species are economically and ecologically important tree species in arid and semi-arid zones of the world (Vilela and Ravetta, 2001) and extensively deployed for rehabilitation of various kind of wastelands (Pasiecznik *et al*., 2001). Considerable literature highlights its utilities like supply of fuel wood, fodder and nectar (Pasiecznik *et al*., 2001; Tewari *et al*., 2013). *P. juliflora* and *P. pallida* are known to tolerate saline sites such as lowland flats and coastal dunes and in such conditions they can often dominate. They can survive with annual rainfall in the range of 50-250 mm. *P. pallida per se* potential enough to thrive on salt affected soils in arid and semi-arid lands (Pasiecznik *et al*., 2001; Velarde *et al*., 2003). Approximately 7 Mha of soils in India are salt-affected (Sharma *et al*., 2004) and which could be rehabilitated by planting with multipurpose trees (Bhojvaid *et al*., 1996). Development of forests and cropping system, for the restoration of such degraded sodic lands, are important in India for mitigation of adverse effect of climate change (Pandey *et al*., 2011; Singh *et al*., 2012). *P. pallida* and its natural hybrids are gaining importance for cultivation on farmlands in India because of their thorn-less trait (Ratha Krishnan *et al*., 2012). *P. pallida*, a suitable leaf and pod fodder species of arid environment and survive due to the adaptation and suitability to grow in agroforestry systems of resource scare regions. *P. pallida* pods and seed are nutritious fodder. The pods contain 9 per cent protein and seeds 34 per cent, one of the highest levels for any legume.

Natural regeneration of the species through seeds is good enough to colonize new sites however seeds that are passed through the digestive system of herbivores germinate effectively (Lynes and Campbell, 2000). Even though silvicultural practices and species acceptance studies on thorn-less sweet pod *Prosopis* were carried (Ratha Krishnan *et al*., 2012), attempts for obtaining synchronized germination at nursery are sparse. Early and synchronized seed germination is important in this species which is critical to enhance regeneration of it in short rain spell areas. In general, time to emergence can have an effect on early growth and biomass production of perennials. Study of germination parameters is vital for physiologists, seed technologists and ecologists to predict degree of success of the species (Ranal and Santana, 2006). Thus, an attempt was made to identify the level of precision that could be achieved through different seed treatments.

Seeds were obtained from ripe pods collected during winter month (January) from natural stand at Central Arid Zone Research Institute, Jodhpur (26° 25' N, 73° 00' E and 233 m elevation). Germination study was conducted at Indian Institute of Soil and Water Conservation, Res-

Table 1. Influence of pre-sowing seed treatment on germination parameters

Treatments	Germination %	$\bar{t}$ (day)	*CV* (%)	$\bar{v}$ (day^{-1})	*U* (bit)	*Z*
T_1	63.33 ± 0.44^{a}	7.24 ± 1.71^{bc}	44.86 ± 0.59	0.14 ± 1.92^{abc}	2.03 ± 1.87^{abc}	0.25 ± 1.92
T_2	65.56 ± 0.05^{a}	6.39 ± 1.80^{bc}	45.13 ± 0.95	0.16 ± 1.99^{ab}	1.43 ± 1.95^{bc}	0.48 ± 1.98
T_3	24.45 ± 0.27^{b}	5.71 ± 0.57^{c}	16.01 ± 0.29	0.18 ± 0.74^{a}	1.25 ± 0.7^{c}	0.28 ± 0.74
T_4	13.34 ± 0.29^{b}	9.67 ± 0.12^{a}	26.14 ± 0.39	0.11 ± 0.40^{c}	1.25 ± 0.37^{c}	0.08 ± 0.40
T_5	56.67 ± 0.29^{a}	6.72 ± 1.52^{bc}	32.66 ± 0.80	0.15 ± 1.72^{ab}	2.08 ± 1.66$_{ab}$	0.27 ± 1.72
T_6	58.89 ± 0.41^{a}	8.35 ± 1.54^{ab}	47.55 ± 0.58	0.12 ± 1.79^{bc}	2.45 ± 1.72^{a}	0.18 ± 1.79
CD (P<0.05)	22.58	2.38	NS	0.04	0.79	NS

-earch Centre, Ballari. Manually graded, good quality seeds were subjected to the pre-sowing treatments *viz*., soaking in different concentration of H_2SO_4, at 20% (T_1), 30% (T_2) and 40% (T_3) for 10 minutes along with soaking in boiled water for ten minutes (T_4) and in cow-dung slurry for 24 hours (T_5) (slurry was prepared from fresh dung). Untreated seeds were sown as control (T_6). Acid scarified seeds were washed in running tap water for three times in two minutes and soaked in water for 10 minutes. In a completely randomized block design, treatments were replicated thrice and thirty treated seeds were sown in polybags (4"×6" size; 150 gauge) filled with mixture of soil, sand and vermicompost in ratio of 3:2:1. The germination process was observed from 4th August to 5th September 2014 with emergence of cotyledon-leaves above the surface was counted as germinated.

Germination parameters *viz*., germinability, mean germination time, mean germination rate, uncertainty and synchrony are important for predicting the success of species in the plant succession (Ranal and Santana, 2006; Ranal *et al*., 2009). Therefore, germination parameters were estimated as per the procedures provided by the Ranal *et al.* (2009). Germinability is the ratio of number of seeds germinated to the number of seeds sown in the germination trial and expressed in per cent.

$$G = \frac{\sum_{i=1}^{k} n_i}{N} \times 100$$

where, n_i is summation of number of seeds germinated, N is the total number of seeds sown.

Mean germination time ($\bar{t}$) is calculated as per the procedure provided by the Ranal *et al.* (2009).

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Where, t_i is the time from the start of the experiment to the i^{th} observation (day), n_i number of seeds germinated in the i^{th} time (number correspondent to the i^{th} observation) and *k* is the time elapsed in germination.

Variance of germination time (s_t^2) is computed for calculation of coefficient of variance of germination time (CV_t).

$$s_t^2 = \frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\sum_{i=1}^{k} n_i - 1}$$

$$CV_t = \frac{s_t}{\bar{t}} \times 100$$

where. s_t is the standard deviation of the germination time ($\sqrt{s_t^2}$).

Mean germination rate ($\bar{v}$) was calculated as the reciprocal of the mean germination time.

$$\bar{v} = \frac{1}{\bar{t}}$$

Uncertainty of germination (U) process was calculated based on relative frequency of germination (f_i) and logarithm of the relative frequency of germination to the base 2($\log_2 f_i$) and its expression is given below.

$$U = \sum_{i=1}^{k} f_i \log_2 f_i$$

$$f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

Synchrony of germination (Z) was computed based on the combination of the seeds germinated at i^{th} time ($C_{n_i 2}$). Where is the quotient between the sum of the partial combination of the number of seeds germinated in each , two by two and the two by two combination of the total number of seeds germinated at the end of the experiment, assuming that all seeds that are germinated responded simultaneously.

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\sum n_i,2}}$$

$$C_{n_i,2} = n_i (n_i - 1)/2$$

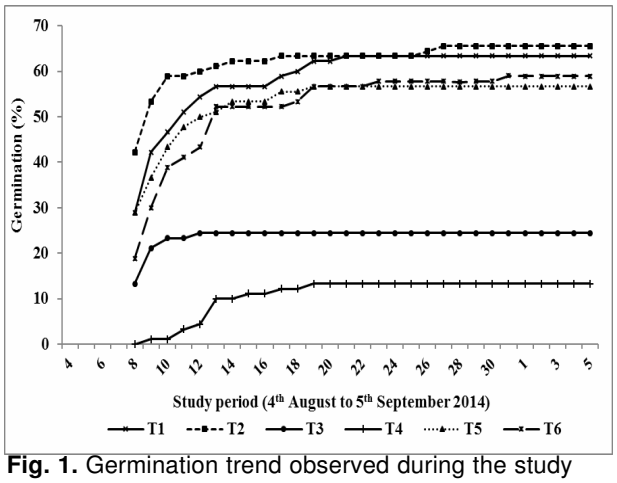

Fig. 1. Germination trend observed during the study

The data on germination and related attributes were statistically analyzed for determining variance by using procedure provided for complete randomized block design in Web Based Agricultural Statistics Software Package (WASP 2) of ICAR Research Complex for Goa (http://icargoa.res.in/wasp2.0/index.php). Analysis of variance (ANOVA) was used to express the statistical difference among the treatments. Critical difference (CD) at 0.05 level of significance was calculated and comparisons of means were carried out by Duncan multiple range test.

Germinability of *P. pallida* seeds was about 63%, 65% and 24% in T_1, T_2 and T_3 acid scarification treatments, respectively. Germination in T_4, T_5 and T_6 was 13%, 57% and 59%, respectively (Table 1; Fig. 1). Reduction of germinability at 20% acid scarification was 3.8% whereas at 40% acid scarification was 70% compared to the T_2 (Fig. 1). It was observed that 40% acid scarification (T_3) for 10 minutes burned seed coat as seeds turned blackish and thereafter poor germinability was recorded. The immersion of seed in higher concentrated H_2SO_4 disrupts the seed coat, triggers protein synthesis (McDonald and Khan, 1983) and germination (Aliero, 2004). In case of *Parkia biglobosa* seeds higher percentage of germination was recorded in 98% concentrated H_2SO_4 scarification in short period compared to 90%, 70% and 50% (Aliero, 2004). However, prolonged immersion was injurious to the seeds as the acid might rapture vital parts of the embryo (Aliero, 2004). In case of *P. pallida*, treating seeds at 40% concentration of H_2SO_4 for 10 minutes time period burned the seed coat and damaged vital parts of the embryo. In T_4 reduction in germination was 89% compared to T_2 thus indicating that *P. pallida* seeds were also intolerant to thermal treatment as *P. velutina* and *P. pubescens* seeds' germination decreased to 97% and 92%, respectively when treated with boiling water in comparison with chemical scarification (Vilela and Ravetta, 2001). Aliero (2004) inferred that *Parkia biglobosa* seeds germination decreased when seeds were soaked more than 4 seconds in boiling water and concluded that embryo gets destroyed.

Lower mean germination time ($\bar{t}$) was recorded in T_3 and higher in T_4 and both treatments recorded lower germination with different time lag. Sequential ascending order of mean time taken by the treatments was $T_3<T_2<T_5<T_1<T_6<T_4$ (Table 1). However, the T_2 attained germination peak early among germination treatments with better germinability (Table 1; Fig. 1). Mean germination time ranged between 5.71 to 9.67 days and lower mean germination was recorded with T_3 and it was 11% less than T_2. Treatments T_5, T_1, T_6 and T_4 taken 5, 13, 31 and 51% higher mean germination time compared to T_2 (Table 1). However, general germination timing in *Prosopis* species ranges from 8–10 days and no significant differences in germination timing were found among the *Prosopis* species evaluated by Vilela and Ravetta (2001).

The CV_t ranged from 16 to 47.6%. Variation in mean germination rate of treatments was found significant at 5% level of significance (0.039). The recorded $\bar{v}$ was higher in T_3 (0.18 day^{-1}), however its influence on overall germinability of seeds was poor as few seeds germinated early in T_3. Higher was also recorded in T_2 (0.16 day^{-1}) with higher germinability and closely followed by T_1 (0.14 day^{-1}) and T_5 (0.15 day^{-1}) (Table 1; Fig. 1). Mean germination rate was higher at 30 and 40% acid scarification compared to other treatments and it ranged between 0.11 to 0.18 seeds day^{-1} (Table 1). Lower mean germination rate was recorded with T_4 (0.11) due to intolerance to thermal treatment. Except T_4, all pre-sowing treatments T_1, T_2, T_3 and T_5 recorded higher mean germination rate compared to control (T_6). Thus it confirmed triggering effect of acid scarification on seed germination due to seed coat disruption and exposure of lumens of macrosclereids cells for imbibitions of water (Aliero, 2004). It was also found that acid scarification had profound effect on protein synthesis during germination of Indian rice grass (*Oryzopsis hymenoides*) seeds (McDonald and Khan, 1983). Thus result confirmed that acid scarification has influenced *P. pallida* seed germination.

The uncertainty of germination varied significantly among the treatments. Treatments T_3 and T_4 recorded less unc-

-ertainty (1.25) as these treatments failed to influence germination process. The measured uncertainty in T_3 and T_4 was not reflected in synchronization of germination. Treatment T_2 recorded low uncertainty which was reflected in synchronization of germination. Uncertainty of germination was significantly high in $T_6>T_5>T_1$ (Table 1; Fig. 1). Therefore, it indicated that 20% acid scarification was not sufficient to influence germination process of *P. Pallida.* Synchronization of germination process was higher in T_2 (0.48) compared to lower synchronization level in T_4 (0.08) and T_6 (0.18).

Though, T_6 recorded better germinability, however germination was poorly synchronized, which is most essential for commercial nursery production (Table 1). Estimated uncertainty was less in T_3 and T_4 however these two treatments were poor in germinability. Concept of uncertainty was applied to seed germination and conventionally it is interpreted that low values indicate more synchronized germination (Ranal *et al*., 2009). Influence of 30% acid scarification (T_2) was positive on seed germination and it was also reflected in synchrony of germination process. Germination in T_2 was 167% better synchronized over control T_6 (Fig. 1; Table 1). Thus treating *P. pallida* seeds at 30% H_2SO_4 was advantageous for obtaining synchronized germination with less uncertainty.

We concluded that seed coat scarification is required for quick and synchronized germination in *Prosopis pallida*. Acid scarification at 30% H_2SO_4 was found effective in influencing germination process and recorded higher germinability and synchrony of germination with lower uncertainty. For achieving better precision in germination treatments further trials on time periods of acid scarification treatment (*i.e.*, immersion time) at different concentration, preferably around ± 30% H_2SO_4 are essential.

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