International Journal of Agricultural Technology 2015 Vol. 11(4):895-902
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Seed Dormancy and Germination in Two Wild Genotypes of *Sesbania* of the Southwest Mangroves in India

Shreelalitha, S. J.[1], Sridhar, K. R.[1*] and Sukesh, S.[2]

[1]Department of Biosciences, [2]Department of Applied Botany, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka, India.

Shreelalitha, S. J., Sridhar, K. R. and Sukesh, S. (2015). Seed dormancy and germination in two wild genotypes of *Sesbania* of the southwest mangroves in India. International Journal of Agricultural Technology 11(4):895-902.

Abstract Seed germination and vigour index of two wild legumes *Sebania bispinosa* and *S. speciosa* of the Nethravathi mangroves of Southwest India were evaluated. Seeds of *S. bispinosa* were more dormant compared to the seeds of *S. speciosa*. Among the physical treatments (soaking and elevation of temperature), soaking in distilled water (24 hr) resulted in highest seed germination as well as vigour index in both plant species. Among the chemical treatments (ethanol, sulfuric acid, thiourea, potassium nitrate and gibberelic acid), immersion in sulfuric acid (20 min) showed the highest seed germination and vigour index in *S. bispinosa*. In *S. speciosa* the highest germination and vigour index was seen by soaking in gibberellic acid (250 mg/l for 24 hr).

Keywords: Seed dormancy, seed germination, *Sesbania bispinosa*, *Sesbania speciosa*, mangroves, wild legumes

Introduction

The genus *Sesbania* consists of about 60 species encompassing annuals, perennials, herbs, shrubs and trees distributed in tropical and subtropical climates (Veasey *et al*., 1999). These C3 plants are more common in habitats with alternating wet and dry regimes than in those with evenly distributed rainfall. They are excellent nitrogen fixers and capable to grow rapidly in nitrogen deficient soils, thus possess high utility in agroforestry as intercrop, cover crop, green manure, mulch and fodder (Chotechaungmanirat, 2010). Morphological, agronomic and molecular diversity of 12 accessions of five *Sesbania* spp. representing diverse geographic regions have been investigated by Joshi-Saha and Gopalakrishna (2007).

Germination behaviour of seeds of *Sesbania* could be enhanced either by decreasing the dormancy period or by increasing the quality of seed lot. Esechie (1995) opined that the embryo viability of *Sesbania* seeds might be adversely affected by high temperatures and interferes with the active phytochrome. It is imperative to follow seed germination requirements of *Sesbania* in order to understand the possible role of various environmental factors for effective cultivation. Monteiro (1984) described five principal

[*] **Corresponding author:** Sridhar, K. R.; **Email**: kandikere@yahoo.com

characteristics in *Sesbania* seedlings: (i) epigeal germination with a long hypocotyl and short epicotyl, (ii) foliaceous cotyledons, very short petiolate and escaping from the testa, (iii) first eophyll simple and resembling the cotyledons, (iv) second eophyll paripinnate with fewer leaflets than the metaphylls, and (v) epicotyl longer than the internodes between the first metaphylls.

Despite the potential uses of *Sesbania*, meagre information is available on their agrobotanical traits including seed dormancy, germination and seedling properties. Therefore, the objective of the present study was to compare the seed germination and vigour index in two wild legumes *Sesbania bispinosa* and *S. speciosa* growing in a mangrove ecosystem of the Southwest India employing physical and chemical treatments.

Materials and methods

Two genotypes, *Sesbania bispinosa* (Jacq.) W. Wight and *Sebania speciosa* Taub. growing in Nethravathi mangroves of Southwest India (12 °50′N, 74 °51′E) were identified by the taxonomic key (Bhat, 2003). Dry pods of *Sesbania* were collected during January–February, 2011 in three lots using random sampling technique. They were separately thrashed and healthy seeds were selected by removing the debris and damaged seeds. Seeds were washed in distilled water, dried in sunlight for 2–3 days to attain constant moisture (<10%) and stored in airtight containers. Seeds showed considerable dormancy on incubation in distilled water (Fig. 1).

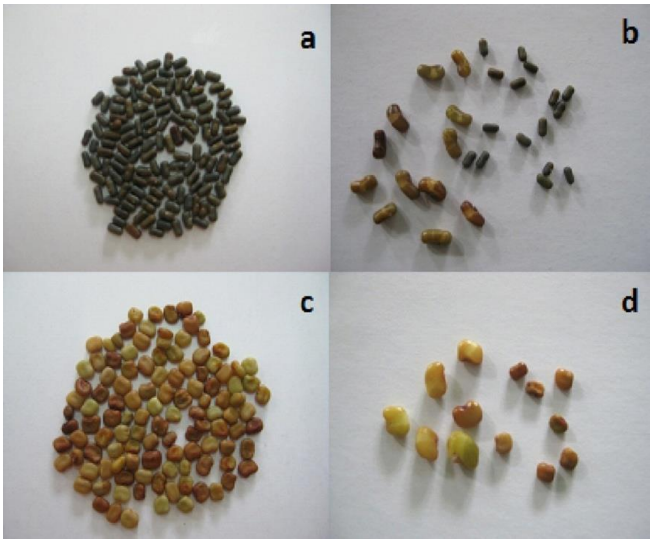

Figure 1. Dry (a) and imbibed/dormant soaked seeds (b) of *Sesbania bispinosa*; dry (c) and imbibed/dormant soaked seeds (d) of *Sesbania speciosa*.

Three replicates of 50 seeds each drawn from different lots, were spread uniformly on a layer of moistened cotton bed (distilled water, 5 ml) in standard glass Petri plates and incubated at 27±2°C with 12 hr photoperiod up to 10 days. Cotton beds were moistened periodically with distilled water to maintain humidity. Seeds were considered germinated based on the emergence of at least 2 mm radical from the seed coat, and germinated seeds were enumerated on each day. The germination response of seeds against various treatments (control, physical and chemical) in triplicate was assessed by randomized design. After 10 days, shoot length, root length, leaflet length were measured to estimate the vigour index. Seeds were rinsed in distilled water without any pre-treatment and placed on wet cotton bed and incubated for germination. Seeds were soaked in 10 ml distilled water up to 24 hr, followed by incubation on wet cotton bed as described above.

For temperature treatment seeds were pre-heated in Petri plates up to 1 hr in an oven at 45, 55 and 65 ℃. Ethanol treatment was given by soaking seeds in ethyl alcohol (50% and 100%) for 5 min, rinsed in distilled water. For sulfuric acid-treatment seeds were scarified with concentrated sulfuric acid (purity, 98%; Merck, India) for different duration (5, 10, 15 and 20 min). After acid-treatment, acid was poured off and the seeds were thoroughly rinsed in distilled water to ensure complete removal of acid. Seed soaking with different concentration of thiourea (purity, 99%; Sigma-Aldrich, India) (0.2, 0.4 and 0.6%) and potassium nitrate (purity, 99%; Sigma-Adlrich, India) (0.2, 0.4 and 0.6%) was provided for 24 hr. Salt-treated seeds were rinsed in distilled water. For hormone treatment, seeds were soaked with different concentration of gibberellic acid (GA3, G7645, Sigma-Aldrich, Germany) (250, 500 and 750 mg/l) for 24 hr. After hormone treatment, seeds were rinsed in distilled water.

Seeds subjected to above treatments were allowed to germinate on wet cotton bed. Cumulative germination (CG) percentage of seeds calculated daily up to 10 days (Bewley and Black, 1994): CG (%) = $(\Sigma n \div N) \times 100$ (where, n is the number of seeds germinated at each day and N is the total number of seeds sown). Vigour index (VI) was calculated based on the percentage seed germination on day 10 and the mean length of shoot and root (Bewley and Black, 1994): VI = (Mean shoot length + Mean root length) × Germination (%). **S**eed germination, seedling dimensions and vigour index in control and physical scarification were estimated.

Results and discussion

In spite of dormancy of *Sesbania* seeds, soaking in distilled water resulted in the highest germination, seedling growth and vigour index (Table 1). The highest germination was attained in both seeds on treatment

with temperature at 55°C. The vigour index of both seeds was also highest at 55°C. Although germination as well as vigour index of both seeds

Table 1. Percentage germination, average length (shoot, root and leaflet) and vigor index of untreated and treated seeds of *Sesbania bispinosa* and *S. speciosa* (n=3, mean).

Treatment		Germination (%)	Mean length (cm)			Vigor index
			Shoot	Root	Leaflet	
Sesbania bispinosa						
Control		14	3.8	1.8	0.5	78.4
Soaking	Distilled water	30	5.6	2.5	0.6	243.0
Temperature (°C)	45	20	2.7	1.0	0.4	74.0
	55	26	4.5	1.2	0.6	148.2
	65	12	2.6	1.0	0.4	43.2
Ethanol (%)	50	15	4.0	1.9	0.7	88.5
	100	25	4.5	2.1	0.8	165.0
Sulfuric acid (min)	5	31	2.6	1.7	0.4	133.3
	10	31	3.0	1.8	0.4	148.8
	15	33	3.3	1.7	0.5	165.0
	20	46	4.6	2.1	0.7	308.2
Thiourea (%)	0.2	31	3.9	2.0	0.6	182.9
	0.4	32	4.4	2.1	0.8	208.0
	0.6	28	2.6	1.4	0.7	112.0
Potassium nitrate (%)	0.2	28	3.7	2.1	0.6	162.4
	0.4	28	4.5	3.0	0.8	210.0
	0.6	27	4.4	2.4	0.7	183.6
Gibberelic acid (mg/l)	250	24	6.1	2.6	0.8	208.8
	500	21	5.7	2.5	0.6	172.2
	750	18	4.1	2.1	0.7	111.6
Sesbania speciosa						
Control		44	7.1	2.5	0.6	422.4
Soaking	Distilled water	53	6.9	2.7	0.7	508.8
Temperature (°C)	45	38	4.7	1.8	0.6	247.0
	55	41	5.7	2.4	0.6	332.1
	65	33	5.7	2.4	0.6	267.3
Ethanol (%)	50	40	7.0	3.0	0.7	400.0
	100	62	7.3	3.2	0.7	651.0
Sulfuric acid (min)	5	76	6.2	2.3	0.6	646.0
	10	81	6.2	2.4	0.6	696.6
	15	82	6.4	2.6	0.6	738.0
	20	83	6.4	2.7	0.7	755.3
Thiourea (%)	0.2	64	3.7	2.3	0.4	384.0
	0.4	79	4.3	2.3	0.6	521.4
	0.6	70	3.3	1.9	0.4	364.0
Potassium nitrate (%)	0.2	77	7.0	2.4	0.7	723.8
	0.4	77	8.0	3.2	0.7	862.4
	0.6	76	8.0	3.1	0.8	843.6
Gibberelic acid (mg/l)	250	88	9.2	2.8	0.8	1056.0
	500	83	8.8	2.7	0.8	954.5
	750	77	8.5	2.7	0.7	862.4

decreased at 65°C, impact of temperature on shoot, root and leaflet length was marginal. In Nethravathi mangroves, seeds of *Sesbania* undergo one or two hydration-dehydration cycles during summer prior to the onset of monsoon favoring seed germination and seedling growth. Untreated, soaked and temperature-treated seeds of *S. speciosa* showed higher germination than seeds of *S. bispinosa*. The highest germination as well as vigour index in both seeds was achieved at 55°C. During summer in mangroves, the seed bank of *Sesbania* in soil seems to be influenced by raised temperature resulting in better germination on the onset of monsoon.

Scarification has a vital role amongst the different methods applied to eliminate dormancy in legume seeds (Hartmann *et al*., 1997). The most commonly employed scarification methods include rubbing with sand paper, heat treatment and acid-treatment. Treating seeds with chemicals those are not growth regulators could promote metabolic activity and induce germination. Scarification of seeds using ethanol (100% for 5 min), concentrated sulfuric acid (20 min), salts (0.4% for 5 min) and gibberellic acid (250 mg/l for 24 hr) increased seed germination, seedling dimension and vigour index in both seeds. Besides, the cell wall polysaccharides, galactomannans in *Sesbania* seeds are broken down during germination and their products support the growth of embryo (Potomati and Buckeridge, 2002). The seedling stage is a critical phase in the life cycle of a plant useful in studying the ecological and evolutionary history of higher plants (Duke and Polhill, 1981).

Ethanol treatment of seeds (100% for 5 min) showed good germination, seedling growth and vigour index in both seeds. However, soaking in water was superior to ethanol treatment in *S. bispinosa*, but it was opposite in *S. speciosa.* Treatment of seeds of *Sesbania* in sulphuric acid up to 20 min resulted in enhanced germination as well as vigour index, which was higher compared to control, soaking, temperature and ethanol treatments. Acid-treatment helps in permeability of seed coat of dormant seeds especially *S. bispinosa* to achieve better germination as well as vigour index. However, the duration required for acid-treatment to increase germination and vigour index was less in *S. speciosa* than *S. bispinosa* indicating hardseededness of the latter.

According to Hartmann *et al*. (1997), thiourea breaks the dormancy especially seed coat inhibiting effect in deep embryo dormant seeds. Seed treatment with thiourea and potassium nitrate at 0.4% showed better germination and vigour index than other concentrations (0.2 and 0.6%). Although potassium nitrate and thiourea are commonly used in seed treatment, their role in breaking seed dormancy is not clearly known. Stimulation of seed germination by thiourea could be attributed to decrease of preventive effect of seed coat and cytokinin activity. Exogenous application of gibberellins induces germination due to metabolic changes in seeds (Mehanna *et al*., 1985). Gibberellic acid-treatment (250 mg/l for 24 hr)

showed better vigour index than germination in *S. bispinosa*, while germination as well as vigour index was highest in *S. speciosa* compared to physical and chemical scarifications. As depicted by Chakrabarti and Mukherji (2003), gibberellins are more effective in the regulation of radical and plumule elongation of *Sesbania* seeds in our study. According to Weyers *et al*. (1987), changes in the sensitivity of seeds to gibberellins could be attributed to the changes in the number of receptors, affinity of the receptor and the signal-transduction pathways.

In and around Nethravathi mangroves, three species of *Sesbania* are prevalent (*S. bispinosa, S. grandiflora* and *S. speciosa*) with abundance of *S. speciosa* (Bhat, 2003). A rough estimate of seed yield of *S. speciosa* per plant in Nethravathi mangroves is double than *S. bispinosa* (300–400 vs. 150–200 g). As the seeds of *S. bispinosa* are dormant, its seed bank in soil helps survival and perpetuation in mangrove habitats. Dormancy is a condition that seeds do not germinate even in favourable the environmental conditions (e.g. water, temperature and aeration) (Hartmann *et al*., 1997). Seed dormancy is the ability to increase the rate of survival by optimizing the distribution of germination in time or space. Seeds of *S. speciosa* are less dormant than *S. bispinosa* in our study corroborates earlier observations by Pollard *et al*. (2011) that pea-shaped seeds (e.g. *S. drummondii, S. formosa, S. grandiflora, S. speciosa* and *S. tripettii*) germinates better compared to cylinder-shaped seeds (e.g. *S. aculeata, S. cannabina, S. egyptica, S. exaltata, S. javanica, S. rostrata* and *S. sesban*).

Thick seed coat inhibits seed germination by prevention of gas exchange, water uptake, light penetration and escape of inhibitors from the embryo (Taylorson and Hendricks, 1977). Inhibitors like phenolics present in seed coat also capable to block the seed germination (Thompson *et al*., 2001). The total phenolics in whole seeds of *S. speciosa* is higher than those of *S. bispinosa* (2.1 vs. 1.9%) (K.R. Sridhar, unpub. obs.). However, germination of *S. speciosa* seeds was higher than *S. bispinosa* in our study indicating total phenolics at 1.9% may not be responsible for seed dormancy of *S. bispinosa* and could be due to the seed coat hardness. Interestingly, catechin in the seed coat of *Sesbania virgata* showed allelopathic effect by inhibiting seed germination and seedling growth of surrounding weeds qualifying its use in weed control (Simões *et al*., 2008).

Environmental factors such as temperature, pH, salinity, low oxygen concentration, flooding and sediment accumulation affect seed germination in wetlands (Shonjani, 2002). *Sesbania bispinosa* is known to grow well in waterlogged or unirrigated conditions, tolerant to high temperature (36–44 ℃), high soil alkalinity (pH 10) and establishes during rainy season in different soils (e.g. loamy, clayey and sandy soils) (Prasad, 1993). In our study, treatment of seeds at 55 ℃ for 1 hr showed moderate (*S. bispinosa*) to high (*S. speciosa*) germination rates and high vigour index indicating their adaptation to the tropical coastal habitats. Ipor and Oyen (1997) also

reported growth of *S. bispinosa* in wide pH range (5.6–9.3) in Indian soils. In addition, *S. bispinosa* also tolerates high concentration of exchangeable sodium (≥ 50% soil CEC) under irrigated conditions. For instance, percentage of seed germination of *Sesbania sesban* was not affected at 5.8 mg/l NaCl (Dan and Brix, 2007). Among the salts used in our study, 0.4% showed good seed germination as well as vigour index in both seeds. Thus, it is necessary to understand the impact of NaCl on seed germination as the salinity in Nethravathi mangroves elevates during the summer due to inflow of salt water.

In summary, the wild genotypes *S. bispinosa* and *S. speciosa* have adapted to the saline conditions in mangroves and sand dunes of Southwest India and established seed banks. Mere soaking in distilled water, seeds of both plants showed significant germination as well as vigour index. The highest seed germination and vigour index were also achieved in *S. bispinosa* on treatment with concentrated sulfuric acid followed by soaking in distilled water and gibberrellic acid. Seeds of *S. speciosa* showed the highest germination as well as vigour index on treatment with gibberrellic acid. The species complex of *Sesbania* with multiple uses (nutritional, medicinal, agricultural and industrial) is underutilized in Southwest India needs special attention to follow agrobotanical features, agricultural potential and germplasm collection.

Acknowledgements

Authors are grateful to Mangalore University for permition to carry out this study in the Department of Biosciences. SJS acknowledges the University Grants Commission, New Delhi, India for the award of fellowship under the scheme Research Fellowship in Sciences for Meritorious Students. KRS acknowledges the award of UGC-BSR Faculty Fellowship by the University Grants Commission, New Delhi.

References

Bewley, J. D. and Black, M. (1994). Seeds: physiology of development and germination. USA: Plenum Press. 297 pp.

Bhat, K. G. (2003). Flora of Udupi. Udupi, Karnataka, India: Indian Naturalist. 597 pp.

Chakrabarti, N. and Mukherji, S. (2003). Effect of phytochrome pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. Biologia Plantarum 46:63-66.

Chotechaungmanirat, S. (2010). Potential of three tropical legumes for rotation of corn-based cropping system in Thailand. Kasetsart Journal 44:1004-1009.

Dan, T. H. and Brix, B. (2007). The influence of temperature, light, salinity and seed pre-treatment on the germination of *Sesbania sesban* seeds. African Journal of Biotechnology 6:2231-2235.

Duke, J. A. and Polhill, R. M. (1981). Seedlings of leguminosae. In Polhill, R.M. and Raven, P.H. (Eds.), Advances in Legume Systematic - Part II. Kew: Royal Botanic Gardens. pp. 941-949.

Esechie, H. A. (1995). Partitioning of chloride ion in the germinating seed of two forage legumes under varied salinity and temperature regimes. Communications in Soil Science and Plant Analysis 26:3357-3370.

Ipor, I. B. and Oyen, L. P. A. (1997). Sesbania adanson. In Hanum, F. and Van der Maesen, L. J. G (Eds.), Plant Resources of South-East Asia. Leiden, The Netherlands: Backhuys Publishers. pp. 236-240.

Joshi-Saha, A. and Gopalakrishna, T. (2007). Agromorphological and molecular variability in the genus *Sesbania*. Genetic Resources and Crop Evolution 54:1727-1736.

Mehanna, H. T., Martin, G. C. and Nishijima, C. (1985). Effect of temperature, chemical treatment and endogenous hormone content on peach seed germination and subsequent seedling growth. Scientia Horticulturae 27:63-73.

Monteiro, R. (1984). Taxonomic studies on brazilian legumes with forage potential: Sesbania, Lupinus. (Master's thesis). University of St. Andrews, St. Andrews, UK. 445 pp.

Pollard, M. A., Fischer, P. and Windhab, E. J. (2011). Characterization of galactomannans derived from legume endosperms of genus *Sesbania* (Faboideae). Carbohydrate Polymers 84:550-559.

Potomati, A. and Buckeridge, M. S. (2002). Effect of abscisic acid on the mobilisation of galactomannan and embryo development of *Sesbania virgata* (Cav.) Pers. (Leguminosae - Faboideae). Revista Brasileira de Botanica 25:303-310.

Prasad, M. N. V. (1993). Bioresource potential of *Sesbania bispinosa* (Jacq.) W.F. wight. Bioresource Technology 44:251-254.

Shonjani, S. (2002). Salt sensitivity of rice, maize, sugar beet and cotton during germination and early vegetative growth. (Master's thesis). Giessen Institute of Plant Nutrition, Justus Liebig University, Germany. 139 pp.

Simões, K., Du, J., Kretzschmar, F. S., Broeckling, C. D., Stermitz, F. S., Vivanco, J. M. and Braga, M. R. (2008). Phytotoxic catechin leached by seeds of the tropical weed *Sesbania virgata*. Journal of Chemical Ecology 34:681-687.

Taylorson, R. B. and Hendricks, S. B. (1977). Dormancy in seeds. Annual Review of Plant Physiology 28:331-354.

Thompson, D. I., Edwards, T. J. and Stader, J. V. (2001). *In vitro* germination of several Sought African summer rainfall *Disa* (Orchidaceae) species: is seed testa structure a function of habitat and a determinant of germinability?. Systematics and Geography of Plants 71:597-606.

Veasey, E. A., Schammass, E. A., Vencovsky, R., Martins, P. S. and Bandel, G. (1999). Morphological and agronomic characterization and estimates of genetic parameters of *Sesbania* Scop. (Leguminosae) accessions. Genetics and Molecular Biology 22:81-93.

Weyers, J. D. B., Patterson, N. W. and A'Brook, R. (1987). Towards a quantitative definition of plant hormone sensitivity. Plant, Cell and Environment 10:1-10.

(Received: 19 March 2015, accepted: 30 April 2015)