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Viability Test of *Sesbania rostrata* Seed with Hot Water Treatment

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Abstract
The study was conducted at the Laboratory of the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, during March to August, 2008 to examine the effect of hot water treatment at different temperatures with varying immersion periods on the viability of *Sesbania rostrata* seeds. Effect of dipping seeds in hot water at temperature of 78, 79, 80 and 81^{0}C for 24, 25, 26, 27 and 28 min were evaluated for this purpose. The lowest seed viability and seed germination (0.5%) was obtained at 81 ^{0}C for 28 min immersion period. The lowest hard seed (3.0%) and minimum (0.63%) pathogenic infection was found at same temperature and immersion period. Germination and tetrazolium tests were used throughout the experiment for estimating seed viability. Extreme hot water treatment deteriorated the viability of *Sesbania rostrata* seeds.

***Keyword**: Sesbania rostrata,* hot water treatment, viability, immersion period.

1. Introduction

Sesbania rostrata, a green manuring crop could play significant roles to improve soil health by addition of organic matter to our soils (Singh *et al*., 1994). *Sesbania rostrata* can supply nearly 200 kg N ha^{-1} when incorporated at 50 days of sowing (Rinaudo *et al*., 1983).

Inspite of these advantages, farmers are not interested in growing *Sesbania* as green manuring crops in the rice based cropping systems because of its poor germination ability. Poor germination of *S. rostrata* seed has been reported from on farm as well as on station researches. Moreover, it was reported that seeds of *Sesbania aculeata* germinated up to 70-80% but that of *Sesbania rostrata* germinated only up to 30-35% (Bhuiya and Bari, 1989). Depending upon maturity and storage, *S. rostrata* may have up to 95% hard seeds (Amin, 1987). *Sesbania* has some hardseeded species, such as *S. arabica, S. canadian, S. exaseratar, S. sesban, and S. rostrata* though they are the members of Fabaceae family (Ghai *et al*., 1985). Such seeds normally exhibit delayed germination because of their thick seed coats which is impermeable to water (Rolston, 1978).

To the most seed technologists and commercial seedsmen, viability means that a seed is capable of germination and producing a "normal" seedling. Therefore, it is used synonymously with germination capacity. In another sense viability denotes the degree to which a seed is alive, metabolically active, and possesses enzymes capable of catalyzing metabolic reactions needed for germination and seedling growth. In this context, a given seed may contain both live and dead tissues, and may or may not be capable of germination. Numerous tests exist for determining seed viability (Copeland and McDonald, 2005).

However, it is the common expectation that seeds must germinate at favourable situation of germination. But it might be prevented by various dormancy mechanisms (Quinlivan, 1971). Different types of treatment such as alternating temperature, sand paper scarification, acid treatment, steeping seeds in hot water are

normally used for breaking seed dormancy. However, during those treatments viability of seeds may deteriorate. Convenient results were observed at higher chemical or temperature, with the longer soaking period, or in cases in which degree of dormancy of tested-seeds become weaker. When the activity of the chemical was too high, most of the seeds lost viability. Emulsion of 1-thiocyanato-2, 3-dibromopropane (U-1), 1, 3-dithiocyanato-2-bromopropane (U-2), 1-isothiocyanato-2-bromopropene (U-3) and 1-thiocyanato-2-bromopopene (U-4) showed remarkable activity in breaking dormancy. The seeds lost viability when the activity of these chemicals was too high (Hideo *et al.*, 2005). Without viability seeds are not capable for germination. Until to-day, most studies have been conducted on hardseededness and are mainly on forest species and pasture legumes, where hardseededness is an acute problem. A few investigations have been conducted to evaluate hardseededness and viability of a dormant seed. A study was, therefore, undertaken to determine the effect of hot water treatment and immersion period on the viability of *Sesbania rostrata* seeds as well as the declining rates of insect infestation and pathogen infection.

2. Materials and Methods

The laboratory experiment was conducted at the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, during March to August, 2008. The experiment was conducted with the following treatments, (a) 4 levels of temperature of water: (i) Control treatment (at room temperature), (ii) 78^0C, (iii) 79^0C, (iv) 80^0C and (v) 81^0C temperature; and (b) 5 levels of immersion period: (i) 24, (ii) 25, (iii) 26, (iv) 27 and (v) 28 minutes. The experiment was laid out in complete randomized design (CRD) with 4 replications.

In the present experiment, routine germination and Tetrazolium tests were followed to find out the appropriate temperature and immersion period at which the viability of the *Sesbania rostrata* seeds becomes zero (0) i.e., the point at which the embryo died due to high temperature. The seeds which were not able to imbibe water at the end of the prescribed test period and remained hard were treated as hard seeds and the seeds which carried pathogen inside or outside the seed with any part of seed were called pathogen infected seed. Stereomicroscope was used for pathogen identification.

Hot Water Seed Treating Device (Water bath) was used to apply the treatments. The seeds were immersed in boiling water at 78^0C for 24, 25, 26, 27 and 28 minutes after taking the seeds in clean thin cotton sac. After that, the seeds were taken out from water bath and dried in shade for 10-20 minutes. Then the treated four hundred (400) seeds were used for germination in four replications. After washing the petridish, it was rubbed out by spirit. Then two filter papers (soaked in water) were set in the petridish. One hundred (100) seeds were placed in each petridish. The petridishes were incubated at room temperature during the experiment period. The same procedure was followed for the next treatment. But for control treatment tap water at room temperature was used.

One hundred seeds were taken from each treatment for tetrazolium test. The seeds were cut equally with two halves with a sharp blade and immersed in 2, 3, 5-triphenyl tetrazolium chloride solution for 5 hours and finally, number of viable seeds were counted (The seeds which become pink after Tetrazolium test are called viable seeds).

Data on germination, viability, hard seeds and pathogen infected seeds were collected regularly upto 14 days according to the standard characterization procedure (ISTA, 2006).

The collected data were analyzed to assess their statistical significance and the mean separation was done by DMRT. For the analysis, angular transformation of data was made.

3. Results and Discussion

3.1. Seed germination

Seed germination decreased sharply with increasing temperature and immersion period and the lowest seed germination (0.50%) was obtained at 81^0C temperatures for 28 minutes immersion period (Fig. 1). The highest seed germination (50%) was recorded at 78^0C temperature of water when seed was immersed for 24 minutes (Fig. 1). Islam *et al.* (2005) found that hot water treatment was effective for breaking dormancy of *Sesbania rostrata* seed and the height germination of seeds (81.5%) was found at 75^0C temperature of water and 25 minute immersion period. However, seed germination was deteriorated at higher water temperature (more than 75^0C) and under higher immersion period. Francoise *et al.* (2002) observed that pre-treatment of seeds at 45^0C progressively reduced subsequent germination at the optimal temperature (25^0C). Seeds did not germinate at 45^0C and almost all of them were dead after 72 hours of soaking at this high temperature. This loss of seed viability was associated with a large increase in leakage of K_+ and total electrolytes into the incubation medium, and with production of malondialdehyde in the embryonic axis and cotyledons, suggesting a loss of membrane integrity probably due to lipid per-oxidation.

3.2. Seed viability

Effect of different temperatures of hot water and immersion periods had significant effect on seed viability (Table 1). Seed viability decreased gradually with increasing temperature and immersion period and the lowest seed viability (0.50%) was obtained at 81^0C temperature for 28 minutes immersion period (Table 1).

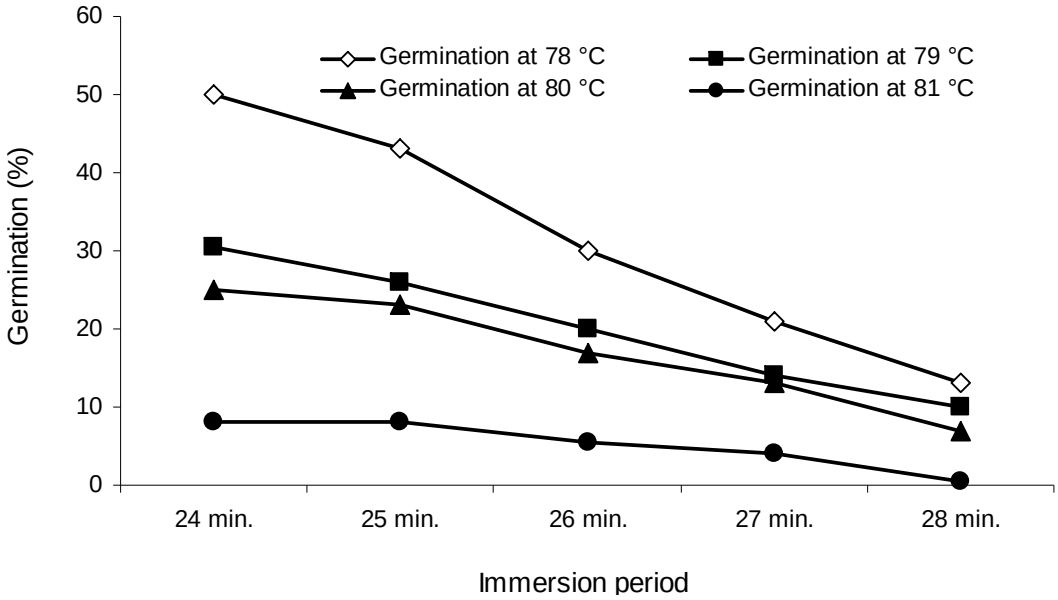

Fig. 1. Effect of different hot water temperatures and immersion periods on the germination of *Sesbania rostrata* seeds.

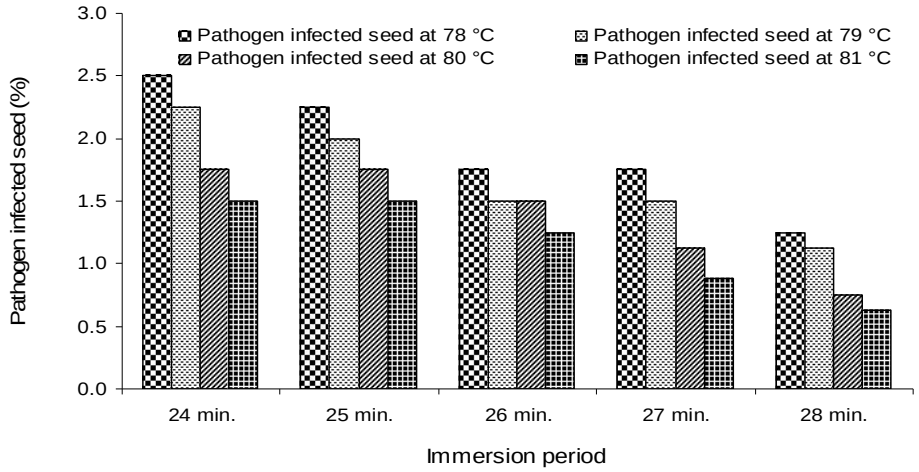

Fig. 2. Effect of different hot water temperatures and immersion periods on pathogen infection of *Sesbania rostrata* seeds.

The highest number of viable seed (56%) was recorded at 78^0C temperature of hot water when the seeds were immersed for 24 minutes (Table 1). Seed viability decreased sharply for 28 minutes immersion period with rise in temperature. Islam *et al.* (2005) found that hot water treatment was effective for breaking dormancy of *Sesbania rostrata* seed and height seed viability (95.5%) was found at control condition (without hot water treatment) and seed viability was decrease when hot water treatment was applied. Similarly, the maximum wheat seed viability at 5^0C after 12 months storage and minimum wheat seed viability at 50^0C temperature after 4 months storage was observed by Nasreen (1999).

3.3. Hard seed

Effect of temperature of hot water and immersion period had significant effect on seed hardness. Hard seed percent was the lowest (3%) at 81^0C hot water treatment when seeds were immersed for 28 minutes. On the other hand, the highest percent of hard seed (21%) was recorded at 78^0C hot water treatment when seed was immersed for 24 minutes (Table 1). Number of hard seed decreased sharply with increasing immersion period. Similarly, the highest percentage of hard seeds (86.18%) of *Sesbania rostrata* under control condition and the lowest (2.35%) at 90^0C temperature for 60 second immersion period was reported by Anis (2000). Subburamu and Sridhar (1977) also found that the percentage of hard seeds decreased in *Phaseolus mungo (Vigna mungo*) cv. T.9 due to treating them with hot water at 50^0C for 15 and 30 minutes.

3.4. Pathogen infected seed

Percentage of pathogen infected seed decreased gradually with increasing temperature and immersion period (Fig. 2). The lowest number of pathogen infected seed (0.63%) was recorded at 81^0C for 28 minutes immersion in hot water treatment and the highest number of pathogen infected seed (2.50%) was recorded at 78^0C for 24 minutes immersion period (Fig. 2). In respect of seed borne fungal diseases infection, 10 minutes immersion in hot water of 52-54^0C temperature was found effective for wheat seeds (Khaleduzzaman, 1996). Winter *et al.* (1996) also found 52^0C and 5-10 minutes immersion to be effective for eliminating the infection of *Helminthosporium sativum* and *Drechslera teres* on barley seeds.

Table 1. Combined effect of different hot water temperatures and immersion periods on the viability and hardseedness of *Sesbania rostrata* seeds.

Interaction		Viability (%)	Hard seed (%)
Temperature (^{0}C)	Duration (minute)		
78	24	56.00a (48.00)	21.00a (42.73)
	25	52.00b (45.00)	18.50b (40.13)
	26	45.00c (36.41)	15.50c (38.36)
	27	35.00f (31.21)	13.00d (35.08)
	28	22.00i (25.32)	10.00ef (29.35)
79	24	45.00c (36.41)	18.75bc (40.27)
	25	41.00d (35.37)	14.25d (35.76)
	26	29.00g (26.57)	13.50d (35.38)
	27	22.00i (25.32)	12.00e (31.31)
	28	16.00j (22.34)	7.25hij (23.75)
80	24	40.00de (38.31)	11.25fg (26.72)
	25	38.00ef (33.77)	6.50ijk (22.37)
	26	25.00h (27.21)	5.25lm (18.33)
	27	21.00i (25.22)	5.50klm (18.85)
	28	12.00kl (18.31)	4.25mn (15.39)
81	24	13.00k (19.59)	8.75hi (24.89)
	25	13.00k (19.59)	6.00ijkl (21.96)
	26	10.40l (15.95)	5.75jkl (20.83)
	27	7.00m (12.25)	5.50klm (18.81)
	28	0.50n (4.06)	3.00n (13.99)

Level of significance	***	***
$S_{\bar{x}}$	0.75	0.91
CV (%)	3.53	3.02

Based in 400 seeds having 100 seeds in each replication
Figures within parentheses are the angular transformed values used for statistical analysis
In a column figures having similar letters do not differ significantly whereas figures having dissimilar letters differ significantly as per DMRT.
*** Significant at 0.001 levels of probability.
NS = Not significant

4. Conclusions

Hot water treatment is essential for breaking dormancy and better germination of different crop seeds. From the present study, hot water treatment was found effective for breaking dormancy of *Sesbania rostrata* seeds and seed germination was increased with increasing hot water temperature and immersion period. Seed germination and viability decreased gradually with increasing hot water temperature and immersion period. Maximum seed germination was recorded at 78 ^{0}C temperature of hot water under 25 minute immersion period. Consequently, seed germination was reduced to 0% at 81 ^{0}C temperature of hot water under 28 minute immersion period. It is therefore, suggested not to exceed the temperature of hot water and immersion period beyond 78 ^{0}C and 25 min. respectively for breaking dormancy of *Sesbania rostrata* seed.

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