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Effect of Seed Priming on Germination and Nursery Establishment of *Woodfordia fruticosa* (L.) Kurz (*Dhawai*)

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Abstract: *Woodfordia fruticosa* (L.) Kurz, (*Dhawai*) is a traditional medicinal shrub widely present in India and South East Asian countries. The natural population of this species is very sporadic because of its lesser germination in normal conditions and is now further declining alarmingly due to unscientific harvesting, which requires urgent conservation action for diversifying agro ecosystem and doubling farmers income. Hence, ensuring quality planting materials is must before introducing into farming systems. The viability of the freshly collected seeds of *Dhawai* was 100 % which decreased with an increase in storage duration. All the priming agents had a positive effect on the enhancement of germination and its parameters. Among all the priming treatments, 500 ppm of GA_3gave the highest germination (94.0 %) with the highest SVI and SVI-mass values of 132.54 and 16.17, respectively. Priming the seeds of *Dhawai* with different priming treatments improved seedling growth and survival. With 500 ppm GA3, seedlings had the longest shoot length (4.48 cm), the longest root length (2.16 cm), the widest collar diameter (0.23 mm), and the heaviest dry weight (6.60 mg). Seeds primed with 500 ppm GA_3 and 0.25 % Proline produced seedlings with the highest number of leaves (6.60). Priming with 4 mM glutamine resulted in the highest survival of the *Dhawai* seedlings. Though there is a high germination and nursery establishment were recorded in both GA_3 and Glutamine seed priming treatments, 24 hours refrigeration treatment can be recommended for easy, effective and eco-friendly mode of quality planting material production in Terai region of West Bengal. In contrast, priming treatments namely longer duration of hot air ovenation, higher concentration of acids, chemicals, hormones and other botanical priming agents deteriorated germination and nursery establishment compared to non-primed seeds.

Keywords: *Dhawai*, Medicinal plant, Seed priming, Germination, Nursery establishment

Woodfordia fruticosa (L.) Kurz, commonly known as *Dhawai* or Fire Flamed Bush (family Lythraceae), is a traditional medicinal shrub widely present in India and South East Asian countries, growing gregariously at higher altitudes of about 1500 m (Uday et al 2014). It is frost-hardy, a good coppicer, and is not grazed, so it grows naturally in large numbers in disturbed and other open areas; it is also a soil binder and an effective nurse for tree species such as *Shorea robusta* (Dinesha et al 2021a). The plant, especially the flowers, possess valuable pharmacological properties because of isolated compounds like tannins, flavonoids, glycosides, sterols, and polyphenols, which have high global commercial demand (Uday et al 2014, Mathew et al 2018). It is also a dye and gum-yielding species used in the perfume, leather, and textile industries and believed to be superior for woollen and silk fabrics (Dinesha et al 2021b). Furthermore, because of its ability to adapt to low-fertile, degraded, and disturbed soils, this species has been identified as a potentially valuable plant genetic resource for improving the marginal smallholder farming systems of tropical regions through soil and moisture conservation. The viability of the stored seeds at normal room conditions declines rapidly from 100 percent to a meagre one percent within a year, while heavy rains and other climatic conditions also affect the viability of seeds (Dinesha et al 2021a). Moreover, seedling survival of the species is also very low under normal conditions. Unfortunately, there have been very few studies on the species' germination and nursery establishment. In sterilized coir-pith compost medium, seeds germinated in 15–20 days with 70% survival (Mathew et al 2018). Transplanting seedlings into polybags containing a 4:1 potting mixture of soil and coir-pith compost improves their chances of survival, growth, development, and field survival (Mathew et al 2018). Seed germination can also be achieved when the seeds are sown in sandy soil during June and October, with 73.33 % survival in the field with polybag seedlings (Shankar and Rawat 2013). The natural population of this species is very sporadic and is now further declining alarmingly, which requires urgent conservation action, especially through introducing them into farming systems through mass cultivation (Mathew et al 2018). However, to achieve this objective, ensuring quality planting materials is a

must, which will not only conserve these species *in situ* but also aid in farm diversification while providing an added source of income enhancement for the growers due to their high pharmaceutical demands. Thus, to achieve the objective of conserving this species through mass cultivation, rapid establishment of the species is necessary by ensuring the availability of quality planting materials with rapid and uniform seedling growth, which is possible through seed priming (Farooq et al 2006). Seed priming is a seed treatment that involves controlled hydration to allow metabolic events before germination but is insufficient to allow radical protrusion through the seed coat (Farooq et al 2006). Seed priming treatments such as hydro-priming, chemo-priming, hormonal-priming, amino acid-priming, thermo-priming, botanical-priming, and bio-priming have been used to accelerate uniform germination, disease free seedling growth, better establishment, and improved yield in most of the crops under both normal and stress conditions (Devkota et al 2013, Ghadge 2018). Therefore, the current study was carried out to standardize the seed priming protocol of *Dhawai* for enhancing germination, seedling vigor, and nursery establishment in the Terai zone of West Bengal.

MATERIAL AND METHODS

Experimental site: The present study was carried out in both the laboratory and nursery at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India from May 2019 to May 2021. The geographical location of the study area is 26° 23' 45.8" north latitude and 89° 23' 16.7" east longitude at 43 m above mean sea level, which falls under the Terai region of West Bengal. The Terai region is a heavy rainfall area with an annual average of 2000-3500 mm and average temperatures varies between 18°C (January) and 33°C (August). The relative humidity of the region ranges from 55 % to 90 % and overall, the area is warm and humid. The soil of the nursery/experiment site was moist and sandy to sandy loam with an acidic reaction, low in organic carbon, medium in available nitrogen and phosphorus, and high in available potash (Dinesha et al 2021a).

Freshly harvested seeds of *Dhawai* were collected from the mother stock maintained in the nursery, Department of Forestry, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar. The seeds were cleaned and shade dried to an ideal moisture level, and then used for the study. The characteristic features of the seed material selected for the study are documented (Plate 1). This gregarious shrub flowers profusely during the summer months, and tiny brown seeds are produced abundantly (Shankar and Rawat 2013).

Seed viability (Tetrazolium chloride test): Seeds collected 3, 6, 12, 24 and 36 months earlier stored in air tight glass bottles at room temperature were subjected to tetrazolium chloride (TTZ) test to assess the seed viability (Plate 1).

Control treatment: Seeds not primed with any priming agents, i.e., normal seeds, were taken as controls and were sown directly in the growing medium. All of the experiments in this study used Soilrite®, a commercial germination medium made up of 75 % peat moss and 25 % perlite. A separate control was taken if the duration between the priming treatments was one month or more, or else the same control was considered.

Priming treatments: The experiment was laid out in a completely randomized design (CRD) and replicated three times, with 50 seeds in each replication. The seeds in every treatment were tested for standard germination by keeping the seeds in petri plates over soil rite at room temperature (~25°C). Untreated seeds were taken as control. After the standardization of hydro-priming, the best hydro-priming duration was used for all other priming treatments. The different priming procedures with varying treatments (exposures/concentrations) were considered a sub-experiment, from which the significantly better treatment(s) were finally compared with each other along with the control. The primed seeds were kept in petri dishes with germination media for one month under normal laboratory conditions. The treatment details are given below.

Hydro-priming: *Dhawai* seeds were not directly soaked in water due to their tininess, but were instead kept in muslin cloth bags and then soaked in normal water (the volume of water was five times the seeds' weight) for 6, 12, 24, and 36 hours. The soaked seeds were then shade dried separately to gain their original weight (Plate 2).

Chemo-priming: The chemo-primers namely KNO_3, $CaCl_2$, H_3BO_3, and H_2SO_4 were used to prepare desired concentrations of 0.5, 1.0, and 2.0 %.

Hormonal priming: Hormonal concentrations of 100, 200 and 300 ppm salicylic acid (SA) and 100, 250 and 500 ppm gibberellic acid (GA_3) were prepared (Xie et al 2006).

Amino acid priming: Ascorbate 250 mg and 500 mg were dissolved separately in one litre of distilled water. L-glutamine at 2 mM and 4 mM concentrations were prepared separately by dissolving 29.2 mg and 58.4 mg, respectively, in 100 ml of distilled water (Ali et al 2013). Proline at 0.25 % and 0.50 % concentrations were prepared separately by dissolving 250 mg and 500 mg in 100 ml of distilled water.

Thermo-priming: The seeds were thermo-primed for desired duration as per above treatments. In low temperature priming (3-5°C), the moist seeds were placed in a refrigerator for 6-, 12- and 24-hours duration. In high temperature priming (40°C), the seeds were soaked in a beaker with water for 1, 3 and 6 hours.

Botanical priming: Priming with moringa (*Moringa oleifera*), neem (*Azadirachta indica*), papaya (*Carica Papaya*) and pongamia (*Pongamia pinnata*) was done with their extracts (Ghadge 2018). In addition to these primers, mixture of flowers, stem and root extract of *Dhawai* in equal proportion was also used. The fresh leaves/flowers/stem/roots of the concerned species were collected separately and dried under shade. The shade dried plant parts were powdered separately using electric grinder and one, three and five grams of the powder was dissolved in 100 ml of distilled water to make the desired concentration of one, three and five per cent separately (Ghadge 2018).

Bio-priming: The liquid biofertilizers such as *Azotobacter* (AZB) and Phosphobacteria (PSB) were collected from the Plant Pathology Department of the University. The concentrations (10, 15 and 20 %) of these biofertilizers were prepared (Gowthamy et al 2017).

Evaluation of nursery performance of seedlings of *Dhawai* after seed priming: Seedlings of significantly better performed protocols of hydro-, chemo-, hormonal, amino acid, thermo-, botanical and bio-priming treatments were compared with each other along with control (i.e., seedlings from no priming treatment) for their performance under nursery condition for two months. Germinated primed seeds after growing on the petri dishes with Soilrite (growing media) under normal laboratory conditions for one month were transferred to the nursery in the root trainers containing three parts soil and one-part FYM as growing media. Entirely fifteen treatments each with 20 seedlings including control were evaluated with CRD replicated thrice.

Observations: Germination per cent (GP) was calculated by using International Seed Testing Association guidelines (Anonymous 2010) as proportion of seeds germinated from the total number of seeds treated and expressed as per cent. Germination speed (GS) was estimated following formula given by Czabator (1962). $GS = n_1/d_1 + n_2/d_2 + n_3/d_3 + \ldots\ldots$; where, n = number of germinated seeds, d= number of days. Mean daily germination (MDG) was estimated as total number of germinated seeds divided by total number of days taken for germination. Peak value (PV) was estimated as highest number of seeds germinated in a given day divided by the number of days at this peak value. Germination value (GV) was estimated (Czabator 1962). GV = PV × MDG, where PV is peak value and MDG is mean daily germination. Seedling vigour index (SVI) was estimated by multiplying the standard germination (%) with an average sum of shoot length (cm) and root length (cm) on the 30th day of germination. SVI-mass was estimated by multiplying the standard germination (%) with mean seedling dry weight (mg) on the 30th day of germination. Survived seedlings were counted after two months of transplanting in the nursery and expressed as per cent of the total seedlings transplanted in the nursery. Collar diameter was measured by using digital varnier caliper. Number of leaves were counted and recorded. Shoot and root length were measured using a graded scale. Seedling dry weight (SDW) of randomly selected 10 seedlings from each treatment was quantified by using an electronic weighing balance after desiccating them in hot air oven at 60°C for 48 hours.

Statistics analysis: The IBM SPSS version 2020 was used to perform the statistical analysis using Duncan's multiple range test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed viability: Seed viability of *Dhawai* of different storage duration reveals gradual decline of seed viability with increase in storage duration. The viability of the freshly collected seeds of *Dhawai* was 100 % which decreased with time. Viability of *Dhawai* reduced drastically and was completely lost after six months of storage. Decline in the endogenous levels of gibberellins was considered as the limiting factor for the maintenance of viability and/or germination of seeds. Similarly, viability of *Dhawai* seeds stored at normal room conditions was found to lose completely within one year of storage (Bhagat et al 1992).

Effect of seed priming on *Dhawai* for enhancing germination and seedling vigour- Hydro-priming: Hydro-priming significantly improved germination of *Dhawai* and its various parameters over control (Table 1, Plate 2). Hydro-priming the *Dhawai* seeds for 24 hours gave the highest germination of 76 %, earliest initiation of germination (IG; five days) and completion of germination (CG; 17 days), highest germination speed (GS; 3.90), mean daily germination (MDG; 2.25), peak value (PV; 0.5), germination value (GV; 1.13) and maximum growth of the seedlings as indicated by total seedling length (TSL; 1.35 cm), total seedling weight (TSW; 0.17 mg), seedling vigour index (SVI; 102.21) and seedling vigour index-mass (SVI-mass; 12.9). Therefore, all other priming treatments in the present study was done soaking the seeds in the respective solution of the primer for 24 hours. The germination of control (non-primed) seeds was least with only 21 % and so least were the IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass also. Increasing the duration of hydro-priming from six hours to 24 hours significantly increased the germination but further increasing the duration of priming over 24 hours significantly decreased germination. Germination of hydro-primed seeds for 6-24 hours increased by 45-55 % compared to control while, hydro-priming for 36 hours increased germination by only 18 % compared to control. Similarly, IG, GS, MDG, PV, GV, TSL,

TSW, SVI and SVI-mass of the hydro-primed seeds also improved significantly over the non-primed seeds but CG of primed and non-primed seeds was statistically similar though the duration was relatively longer for non-primed seeds (Table 1). The IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass also gradually improved though not always significantly with increasing duration of hydro-priming from six hours to 24 hours. However, further increase of priming duration to 36 hours decreased (not always significantly) these parameters but still remaining better than control.

Priming the seed with water loosens the seed coat resulting in better imbibition of water and oxygen which increased the metabolic activities resulting into quicker, higher and uniform germination including faster seedling growth (Moghanibashi et al 2012, Dastanpoor et al 2013, Ghadge 2018). Hydro-priming treatments not only improved the germination rate and time but also enhanced the seedling vigour as indicated by higher values of germination, seedling length and weight. The present results were consistent with earlier studies where improved germination and its parameters were also observed following hydro-priming of 24 hours (Moghanibashi et al 2012, Dastanpoor et al 2013). Extending priming duration over 24 hours to 36 hours deteriorated germination and its parameters which was probably due to saturation of the seeds with water reducing oxygen thus restricting growth of emerging radical and plumule (Assefa 2008). Hydro-priming involve soaking the seeds followed by drying back before radical emergence and extending soaking time up to 36 hours might had initiated radical emergence in the soaked seeds. Drying back the seeds after radical emergence might have killed the radical reducing germination (Assefa 2008).

Chemo-priming: All the chemo-primers with varied concentration (0.5-2.0 %) significantly increased germination and improved various germination parameters of *Dhawai* over control except sulphuric acid at 1.0 or 2.0 % (Table 2). Sulphuric acid however, at 0.5 % concentration significantly increased germination (38.0 %) over control. Although potassium nitrate, calcium chloride and boric acid at the given concentrations including 0.5 % sulphuric acid significantly increased germination over control but couldn't improve germination and various germination parameters (Table 2) similar to 6-24 hours of hydro-priming (66-76 %, Table 1). Further, it was observed that increasing concentration of these chemo-primers over 0.5 % significantly decreased germination and the various germination parameters. This indicates corrosive and toxic effect of the chemo-primers at concentration over 0.5 % which might have destroyed the emerging radical and plumule, reducing germination and thus also reducing the entire studied germination parameters (Noor-un-Nisa et al 2013). The chemo-primers at various concentrations were also used in earlier studies on field crops including medicinal plants also gave comparable results to this present study (Farooq et al 2006). Among all the chemo-priming treatments, 0.5 % of H_3BO_3 and KNO_3 were found best. Similarly, 0.2-3.0 % KNO_3 in earlier studies was reported to increase the activity of total amylase and proteases in germinating seeds resulting improvement in proteins, free amino acid and soluble sugars during germination which significantly improved germination and its parameters (Noor-un-Nisa et al 2013, Vineeta et al 2018). Priming with 0.01-2.0 % H_3BO_3 was reported to enhance seed performances due to its role in rejuvenation and the buildup of nucleic acid and membranes, enhanced synthesis of protein, and improved antioxidant system (Noor-un-Nisa et al 2013).

Hormonal priming: Hormonal-priming significantly increased germination and improved various germination parameters of *Dhawai* over control (Table 3; Plate 3). However, salicylic acid (SA) was not that effective like GA_3, instead reduced germination and germination parameters when its concentration was increased from 100 to 300 ppm in contrast to increasing concentration of GA_3 (100-500 ppm). SA increased germination by 2-10 % whereas, GA_3 increased

Table 1. Effect of hydro-priming on germination and its parameters of *Dhawai*

D	IG	CG	GP	GS	MDG	PV	GV	TSL	TSW	SVI	SVIm
0	7.5^{a}	19.0^{a}	21.0^{d}	0.92^{d}	0.56^{d}	0.21^{b}	0.12^{b}	1.26^{c}	0.13^{b}	26.4^{d}	2.72^{d}
6	5.5^{b}	19.5^{a}	70.0^{ab}	3.34^{b}	1.80^{b}	0.48^{a}	0.86^{a}	1.32^{ab}	0.17^{a}	92.1^{ab}	11.60^{ab}
12	6.0^{b}	18.5^{a}	66.0^{b}	3.00^{b}	1.78^{b}	0.40^{ab}	0.72^{a}	1.31^{ab}	0.15^{ab}	86.1^{b}	9.90^{b}
24	5.0^{b}	17.0^{a}	76.0^{a}	3.90^{a}	2.25^{a}	0.50^{a}	1.13^{a}	1.35^{a}	0.17^{a}	102.2^{a}	12.90^{a}
36	6.0^{b}	18.5^{a}	39.0^{c}	1.84^{c}	1.05^{c}	0.27^{b}	0.28^{b}	1.28^{bc}	0.15^{ab}	49.8^{c}	5.64^{c}
M	6.00	18.5	54.40	2.60	1.49	0.37	0.62	1.30	0.15	71.32	8.54
Sig.	0.00^{*}	NS	0.00^{*}	0.00^{*}	0.00^{*}	0.04^{*}	0.01^{*}	0.02^{*}	0.05^{*}	0.00^{*}	0.00^{*}

D- duration in hours (zero hour is control); M- mean; IG- Initiation of germination; CG- Completion of germination; GP- Germination per cent; GS- Germination speed; MDG- Mean daily germination; PV- Peak value; GV- Germination value; TSL- Total seedling length (cm); TSW- Total seedling weight (mg); SVI- Seedling vigour Index; SVIm- Seedling vigour Index-mass; S- Significant (α= 0.05); NS-Non significant

germination by 59-72 % over control (22 %) with similar trend in increment of germination parameters. Hormonal treatments however, effectively reduced the initiation (4-5 days; Table 3) and completion of germination period 15-16 days as compared to 4.5-7.0 days for IG and 16-19.5 days for CG by hydro- and chemo-priming (Table 1 & 2). IG was earliest as 4.0 days with 100, 250 and 500 ppm of GA_3 priming while, extended up to 5.5 days with 100 ppm of Salicylic acid priming as compared to 7.5 days with control. Similarly, CG was recorded earliest at 15.0 days with 100, 200 and 300 ppm SA priming while, a bit extended up to 16.0 days with 100 and 250 ppm GA_3 priming as compared to 19 days with control. Maximum GS and MDG were recorded in 500 ppm of GA_3 which is at par with 100 ppm and 250 ppm GA_3 while, minimum values were in control. Both PV and GV also followed similar trend (Table 3). TSL varied from 1.41 cm to 1.26 cm with a mean of 1.33 cm. TSW varied from 0.14 mg to 0.18 mg with a mean of 0.16 mg. Similarly, both SVI and SVI-mass followed similar trend (Table 3).

Among the hormonal-priming, 500 ppm of GA_3 was found the best priming treatment for improving germination and its various parameters. This may be attributed to the key role of gibberellins in the break of dormancy and in the control of reserve hydrolysis on which the growing embryo depends (Lovegrove and Hooley 2000). The faster emergence of seeds primed with GA_3 might be due to its stimulation effect in the formation of enzymes during early phases of germination which helped for a fast radicle protrusion and hypocotyl elongation to penetrate the soil up (Salisbury and Ross 1992). In contrast, SA exhibited the reverse trend as it is a

Table 2. Effect of chemo-priming on germination and its parameters of *Dhawai*

Treatments	Conc.	IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	21.0^{g}	1.26^{f}	0.13^{d}	2.72^{f}
KNO_3	0.5 %	5.0^{de}	16.0^{b}	0.17^{abc}	1.36^{ab}	0.17^{abc}	9.59^{a}
	1.0 %	5.5^{cde}	16.5^{b}	0.17^{abc}	1.30^{cdef}	0.17^{abc}	6.07^{de}
	2.0 %	6.0^{bcd}	17.5^{ab}	0.18^{a}	1.39^{a}	0.18^{a}	7.57^{c}
$CaCl_2$	0.5 %	5.0^{de}	17.0^{ab}	0.15^{bcd}	1.29^{def}	0.15^{bcd}	7.75^{bc}
	1.0 %	5.0^{de}	17.0^{ab}	0.17^{ab}	1.32^{bcde}	0.17^{ab}	7.03^{cd}
	2.0 %	4.5^{e}	17.0^{ab}	0.16^{abc}	1.33^{bcd}	0.16^{abc}	7.44^{c}
H_3BO_3	0.5 %	5.0^{de}	17.0^{ab}	0.15^{cd}	1.28^{def}	0.15^{cd}	8.70^{ab}
	1.0 %	5.5^{cde}	18.0^{ab}	0.15^{bcd}	1.31^{cdef}	0.15^{bcd}	7.20^{c}
	2.0 %	5.0^{de}	17.5^{ab}	0.17^{ab}	1.35^{abc}	0.17^{ab}	7.82^{bc}
H_2SO_4	0.5 %	5.5^{cde}	17.0^{ab}	0.15^{bcd}	1.28^{ef}	0.15^{bcd}	5.57^{e}
	1.0 %	7.0^{ab}	17.5^{ab}	0.13^{d}	1.27^{ef}	0.13^{d}	2.74^{f}
	2.0 %	6.5^{abc}	17.0^{ab}	0.16^{abc}	1.31^{bcdef}	0.16^{abc}	2.59^{f}
Mean		5.62	17.23	41.15	1.31	0.15	6.37
Sig.		0.00*	0.29	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details

Table 3. Effect of hormonal priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5^{a}	19.0^{b}	22.0^{d}	0.73^{e}	0.33^{d}	0.24^{d}	27.72^{d}	2.97^{d}
	100 ppm	5.5^{a}	15.0^{b}	32.0^{c}	1.07^{c}	0.32^{d}	0.34^{d}	41.64^{c}	4.97^{c}
SA	200 ppm	5.0^{a}	15.0^{b}	29.0^{c}	0.97^{cd}	0.36^{d}	0.34^{d}	39.72^{c}	4.83^{c}
	300 ppm	5.0^{a}	15.0^{b}	24.0^{d}	0.80^{de}	0.40^{d}	0.32^{d}	31.24^{cd}	3.76^{cd}
	100 ppm	4.0^{b}	16.0^{a}	85.0^{b}	2.66^{b}	1.31^{b}	3.48^{b}	118.14^{b}	14.45^{a}
GA_3	250 ppm	4.0^{b}	16.0^{a}	81.0^{b}	2.53^{b}	0.81^{c}	2.03^{c}	105.28^{c}	12.15^{b}
	500 ppm	4.0^{b}	15.5^{ab}	94.0^{a}	3.04^{a}	1.57^{a}	4.77^{a}	132.54^{a}	16.17^{a}
Mean		4.64	15.36	52.43	1.68	0.73	1.65	70.90	8.47
Sig.		0.00*	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details; SA- Salicylic acid; GA_3- Gibberellic acid

germination inhibitor and at high concentration is toxic to the germinating seeds (Zhang et al 2004, Xie et al 2006). During seed germination, GA is synthesized in embryos and secreted into aleurone layers (Lovegrove and Hooley 2000). The production of a-amylases in aleurone layers is believed to be essential for seed germination which is tightly regulated by gibberellic acid (GA), abscisic acid (ABA) and SA (Lovegrove and Hooley 2000, Zhang et al 2004, Xie et al 2006). In aleurone cells, GA is perceived by receptors that promote the expression of α- amylases but antagonizes with germination inhibitors like ABA and SA that block the production and expression of α-amylases and abscisic-acid-inducible WRKY gene suppressing seed germination (Lovegrove and Hooley 2000, Xie et al 2006). The germination was promoted due to exogenous application of GA as it increased the proportion of GA in the seed system

Plate 1. Collection of *Dhawai* seed for seed viability test

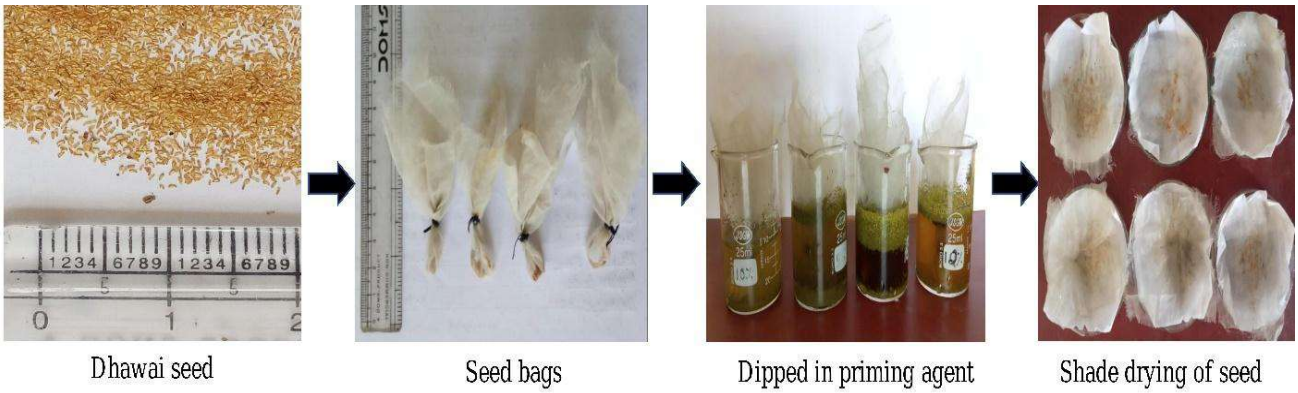

Plate 2. *Dhawai* seed priming process

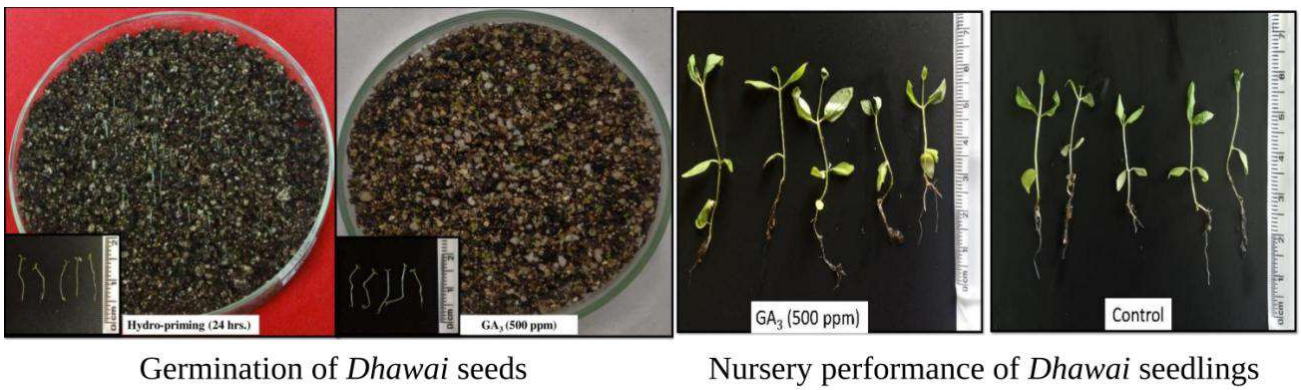

Plate 3. Germination of seeds and nursery performance of *Dhawai* seedlings

promoting germination. The exogenous application of SA even at low concentration decreased the proportion of endogenous GA with antagonizing effects of inhibiting α-amylase and WRKY gene suppressing seed germination.

Amino acid priming: Amino acid priming significantly improved the germination and germination parameters over control (Table 4). Priming with Ascorbate, Glutamine and Proline significantly improved germination by 25-29 %, 36-66 % and 34-58 %, respectively over control (22 %). Increasing the concentration of Glutamine from 2 mM to 4 mM significantly increased germination by 30 % but increasing Proline from 0.25 % to 0.50 % significantly decreased germination by 24 % while, increasing Ascorbate from 250 mg l^{-1} to 500 mg l^{-1} also decreased germination but the decrease was non-significant. Among the amino acid priming treatments, Glutamine 4 mM gave significantly higher germination (88.0 %) than all other amino acid priming treatments. Amino acid priming also significantly reduced the days to IG (3-4 days) and CG (13-15 days) over control by 3.5-4.5 days and 4-6 days, respectively (Table 4) which is two days and 4-4.5 days earlier, respectively than hydro-priming (Table 1); 1.5-3 days and three days earlier than chemo-priming (Table 2) and 1-1.5 days and 1-2 days, respectively than hormonal priming (Table 3). However, IG and CG exhibited a mixed influence on increasing concentration of amino acids. Similar trend on other germination parameter was also observed with amino acid priming as was observed with germination (Table 4). Maximum GS and MDG were recorded in 4 mM of Glutamine which is statistically at par with 0.25 % of Proline while, minimum values were in control (Table 4). Similarly other germination parameters also followed similar trend i.e. maximum values with 4 mM Glutamine priming while, minimum values with control (Table 4).

Among the amino acid priming, 4 mM Glutamine priming was found the best primer for improving germination and its various parameters. Providing Glutamine exogenously might have supplemented the Glutamine synthetase activity, aiding nitrogen metabolism and acted as substrate for protein synthesis. Thus, increasing Glutamine increased germination by increasing the availability of nitrogen and glutamate leads to ammonium release in an oxidative deamination reaction catalyzed by glutamate dehydrogenase (Mifflin and Habash 2002). Proline increased germination over control in very less concentration of 0.25 % which was reported due to its influence on production of protein and sugar in the germinating seeds (Ali et al 2013). Increasing the concentration of Proline and Ascorbate significantly decreased the germination due to toxic effects of these primers at higher concentrations to the germinating seeds (Yang et al 2012). Earlier studies with field crops exhibited excellent germination with amino acid priming both under stress and normal conditions which was attributed to their influence on production of protein, seed sugar, oil, fiber content, moisture content, and ash (Yang et al 2012, Ali et al 2013). Increased germination especially during stress was found with amino acid priming due to increased cell division, cell wall expansion, and other developmental processes. This is because amino acids are key substance in the network of plant antioxidants, including glutathione and enzymatic antioxidants that detoxify H_2O_2 to counteract oxygen radicals produced by the Mehler reaction and photorespiration during stress (Ali et al 2013). *Dhawai* seeds being inherently micro-biotic in nature with very fine seed coat were reported with very less germination under normal natural and control conditions (Mathew et al 2018, Dinesha et al 2021a). The micro-biotic nature with very fine seed coat might normally render the seeds to stress under normal germinating conditions restricting the seeds to germinate. Priming with amino acids might have played a role of stress busters for the *Dhawai* seed aiding its germination.

Thermo-priming: Priming *Dhawai* seeds with varying

Table 4. Effect of amino acid priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5^{a}	19.0^{a}	22.0^{e}	1.52^{e}	0.33^{b}	0.51^{d}	27.72^{e}	2.86^{e}
Ascorbate	250 mg l^{-1}	4.0^{b}	13.0^{c}	51.0^{cd}	3.93^{c}	1.07^{a}	4.17^{b}	68.32^{cd}	7.90^{cd}
	500 mg l^{-1}	4.0^{b}	14.5^{b}	47.0^{d}	3.14^{d}	0.94^{a}	2.94^{c}	65.08^{d}	7.64^{d}
Glutamine	2 mM	3.0^{c}	13.5^{bc}	58.0^{c}	4.31^{c}	0.93^{a}	4.03^{b}	79.70^{c}	8.83^{c}
	4 mM	3.0^{c}	14.0^{bc}	88.0^{a}	6.29^{a}	1.00^{a}	6.29^{a}	125.37^{a}	14.74^{a}
Proline	0.25 %	3.0^{c}	15.0^{b}	80.0^{b}	5.34^{b}	0.41^{b}	2.20^{c}	105.34^{b}	12.07^{b}
	0.50 %	3.5^{bc}	13.5^{bc}	56.0^{c}	4.00^{c}	0.86^{a}	3.45^{c}	78.44^{c}	8.99^{c}
Mean		3.64	14.07	57.43	4.07	0.79	3.37	78.57	9.00
Sig.		0.00*	0.02*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

See Table 1 for germination parameter details

duration of high and low temperature significantly influenced germination, IG, CG, GS, MDG, PV, GV, TSL, TSW, SVI and SVI-mass (Table 5). Refrigerating (3-5ºC) the seeds increased germination by 52-67 % over control (22 %). Increasing the duration of refrigeration also increased germination. Germination recorded with six hours of refrigeration was 74 % which increased significantly by 11 % with increase in duration to 12 hours. Further increase in duration of refrigeration to 24 hours non-significantly increased germination by 4 %. Hot air ovenating the seeds for one hour also increased germination by 46 % over control (22 %). In contrast to refrigeration, increasing the duration of hot air priming (40ºC) by keeping the seeds in hot air oven from one hour to three hours significantly reduced the germination by 31 % and further increasing the duration to six hours reduced germination by 10 % below control. Micro-biotic nature of the *Dhawai* seeds with very thin seed coat made it prone to longer duration of hot air ovenation (Baskin and Baskin 2004). Exposing these seeds with longer duration of hot air ovenation might have damaged the seed coat injuring or killing embryos including endosperm resulting in lower germination (McDonnell et al 2012). Among the thermo-priming treatments, 24 hours refrigeration was found the best for germination of *Dhawai* followed by 12 hours refrigeration. Low temperature was reported to enhance germination and various parameters due to influence membrane permeability regulating the movement of gibberellins toward their activity places while, also increasing the soluble protein content in the germinating seed (Salisbury and Ross 1992, Lovegrove and Hooley 2000).

Botanical priming: Priming of *Dhawai* seeds with different concentrations of botanical or plant extracts significantly influenced germination and various germination parameters (Table 6). All the plant extracts used significantly increased germination over control (20 %) by 19-25 % for moringa extract, 9-21 % for neem extract, 2-66 % for papaya extract, 22-51 % for pongamia extract and 37-61 % for *Dhawai* extract. Increasing the concentration of all these extracts from one to five per cent significantly decreased the germination of *Dhawai* except moringa and neem extracts with which mixed response of germination was observed. Lowest concentration of one per cent used for papaya and *Dhawai* extract gave 86 % and 81 % germination, respectively with significant difference between the two treatments. Further increase in concentration to three and five per cent reduced germination more in papaya as compared to *Dhawai* though the reductions were significant in both the cases. Increasing concentration from one to three per cent reduced germination by 59 % with papaya extracts and 11 % with *Dhawai* extract while, from one to five per cent the reductions were 64 % and 24 %, respectively though the reductions were lesser from three to five per cent with 5 % and 13 %, respectively as compared to increasing concentration from one to three per cent. Next to one per cent papaya and *Dhawai* extract priming, another better priming treatment found was one per cent pongamia extracts with 71 % germination. The influence of all these botanical extracts priming on the germination parameters followed the same trend as was observed with germination (Table 6).

Among the botanical-priming treatments, one per cent papaya extracts priming gave best results. The extracts were prepared from young leaves which were reported to contain alkaloids which might have increased germination due to enhanced enzymatic and other metabolic activities of the germinating seeds (Devkota et al 2013, Ghadge 2018). Priming the seeds with plant extracts prior to germination was reported to enhance seed metabolism helping faster germination and increasing seedling vigour (Devkota et al 2013). These plant origin natural priming agents are cheap, safe, eco-friendly over synthetic priming chemicals with stimulatory effect on germination and seedling growth (Devkota et al 2013, Ghadge 2018).

Bio-priming: Bio-priming the seeds with different concentrations of *Azotobacter* (AZB) and Phosphobacteria (PSB) significantly influenced germination and various

Table 5. Effect of thermo-priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	MDG	PV	GV	SVI	SVIm
Control		7.5^{a}	19.0^{a}	22.0^{e}	0.73^{e}	0.33^{de}	0.24^{de}	27.72^{e}	2.97^{e}
	6 hours	5.0^{b}	16.0^{b}	74.0^{b}	2.28^{b}	0.71^{c}	1.62^{c}	97.72^{b}	12.23^{b}
Refrigeration	12 hours	5.0^{b}	16.0^{b}	85.0^{a}	2.66^{a}	0.88^{b}	2.33^{b}	111.76^{a}	14.24^{a}
	24 hours	5.0^{b}	17.0^{ab}	89.0^{a}	2.62^{a}	1.07^{a}	2.79^{a}	118.38^{a}	13.80^{ab}
Hot air ovenated at 40ºC	1 hour	5.0^{b}	17.0^{ab}	68.0^{c}	2.00^{c}	0.86^{b}	1.71^{c}	87.06^{c}	9.87^{c}
	3 hours	5.0^{b}	16.5^{ab}	37.0^{d}	1.13^{d}	0.38^{d}	0.43^{d}	47.74^{d}	5.65^{d}
	6 hours	7.5^{a}	17.0^{ab}	12.0^{f}	0.35^{f}	0.25^{e}	0.09^{e}	15.24^{f}	1.68^{e}
Mean		5.36	16.36	55.29	1.68	0.64	1.31	72.23	8.63

See Table 1 for germination parameter details

germination parameters of *Dhawai* (Table 7). Both AZB and PSB priming treatments significantly increased germination over control (20 %) by 30-59 % for AZB and 19-44 % for PSB. Increasing the concentration of these bio-primers from 10 to 25 % significantly increased the germination by 20-29 %. The germination achieved with 20 % AZB priming (79 %) was comparable to germination achieved with 24 hours hydro-priming (Table 1); 100, 250 & 500 ppm GA_3 priming (Table 3); 4 mM Glutamine & 0.25 % Proline priming (Table 4); 12- & 24-hours refrigeration (Table 5) and 1.0 % papaya and *Dhawai* extracts priming (Table 6). The influence of both AZB and PSB bio-priming treatments on the germination parameters followed the same trend as was observed with germination (Table 7). Both the AZB and PSB significantly improved germination and its parameters. The possible mechanism of these plant growth promoting bacteria on the germination process is that these useful bacteria can excrete phytohormones such as auxins and gibberellins along with vitamins thereby improving seed germination and early development (Gowthamy et al 2017, Vasava et al 2018). Besides, during metabolism the bacteria excrete organic acids like citric acid and malic acid as well; thus, helping nutrient uptake at a later stage of growth (Gowthamy et al 2017, Vasava et al 2018).

Table 6. Effect of botanical priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	20.0^{i}	1.26^{c}	0.13^{c}	2.62^{j}
Moringa	1 %	5.0^{bc}	16.0^{bc}	42.0^{ef}	1.33^{bc}	0.16^{ab}	6.61^{efg}
	3 %	5.5^{b}	16.5^{ab}	39.0^{fg}	1.28^{bc}	0.15^{bc}	5.65^{g}
	5 %	5.0^{bc}	16.5^{ab}	45.0^{e}	1.34^{ab}	0.17^{ab}	7.32^{ef}
Neem	1 %	4.5^{cd}	15.5^{bc}	36.0^{g}	1.31^{bc}	0.16^{ab}	5.73^{g}
	3 %	5.0^{bc}	15.0^{c}	41.0^{f}	1.29^{bc}	0.16^{ab}	6.22^{fg}
	5 %	6.0^{a}	16.0^{abc}	29.0^{h}	1.27^{c}	0.13^{c}	3.76^{hi}
Papaya	1 %	4.0^{d}	16.0^{abc}	86.0^{a}	1.39^{a}	0.18^{a}	14.79^{a}
	3 %	5.0^{bc}	17.0^{a}	27.0^{h}	1.31^{bc}	0.16^{ab}	4.12^{h}
	5 %	5.5^{ab}	16.5^{ab}	22.0^{i}	1.31^{bc}	0.16^{ab}	3.46^{hi}
Pongamia	1 %	5.5^{ab}	16.0^{abc}	71.0^{c}	1.29^{bc}	0.15^{abc}	10.36^{cd}
	3 %	5.5^{ab}	17.0^{a}	54.0^{d}	1.29^{bc}	0.15^{bc}	7.83^{e}
	5 %	5.0^{bc}	17.0^{a}	42.0^{ef}	1.30^{bc}	0.15^{abc}	6.30^{fg}
Dhawai	1 %	4.0^{d}	16.0^{abc}	81.0^{b}	1.32^{bc}	0.17^{ab}	13.37^{b}
	3 %	4.0^{d}	16.0^{abc}	70.0^{c}	1.33^{bc}	0.16^{ab}	11.03^{c}
	5 %	5.0^{bc}	15.5^{bc}	57.0^{d}	1.35^{ab}	0.17^{ab}	9.70^{d}
Mean		5.03	16.22	47.63	1.31	0.16	7.43
Sig.		0.00^{*}	0.03^{*}	0.00^{*}	0.03^{*}	0.01^{*}	0.00^{*}

See Table 1 for germination parameter details

Table 7. Effect of bio-priming on germination and its parameters of *Dhawai*

Treatments		IG	CG	GP	TSL	TSW	SVIm
Control		7.5^{a}	19.0^{a}	20.0^{f}	1.27^{c}	0.13^{b}	2.53^{d}
AZB	10 %	6.0^{ab}	13.5^{c}	50.0^{cd}	1.33^{a}	0.16^{a}	8.13^{b}
	15 %	5.0^{c}	15.0^{b}	53.0^{c}	1.28^{bc}	0.14^{ab}	7.64^{b}
	20 %	4.0^{d}	15.0^{b}	79.0^{a}	1.34^{a}	0.16^{a}	12.78^{a}
PSB	10 %	5.5^{b}	14.0^{bc}	44.0^{de}	1.31^{ab}	0.16^{a}	6.89^{bc}
	15 %	5.0^{c}	15.0^{b}	39.0^{e}	1.26^{c}	0.15^{ab}	5.75^{c}
	20 %	4.0^{d}	15.0^{b}	64.0^{b}	1.27^{c}	0.13^{b}	8.32^{b}
Mean		5.14	14.64	49.71	1.29	0.15	7.43
Sig.		0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.02^{*}	0.00^{*}

See Table 1 for germination parameter details

Comparison of priming treatments in *Dhawai:* The different priming treatments in *Dhawai* were compared with the best two treatments in each category of primers with respect to germination achieved (Fig. 1). Among all the priming treatment of *Dhawai*, 500 ppm of GA_3 gave highest germination of 94.0 % with highest SVI and SVI- mass of 132.54 and 16.17, respectively (Fig. 1).

Nursery performance of seedlings of *Dhawai* : Priming the seeds of *Dhawai* with different priming treatments significantly influenced the seedling shoot length, collar diameter and number of leaves and but dry weight, root length and survival showed insignificant variation among the treatments (Fig. 2, Plate 3). Not all the priming treatments improved these parameters over control. Shoot length was longest when the seeds were primed with 500 ppm of GA_3 and shortest was recorded with control. Priming the seeds with 500 ppm GA_3 produced seedlings with longest roots and seedlings with shortest roots were recorded with control. Seeds primed with 500 ppm of GA_3 also produced seedlings with widest collar diameter of 0.23 mm while, 24 hours hydro-

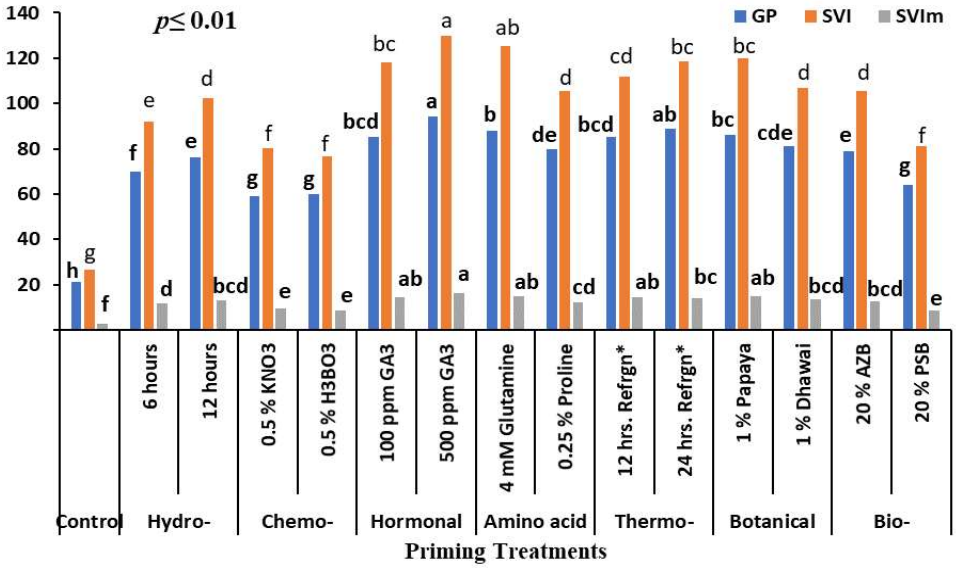

Fig. 1. Germination of *Dhawai* with best two priming treatments

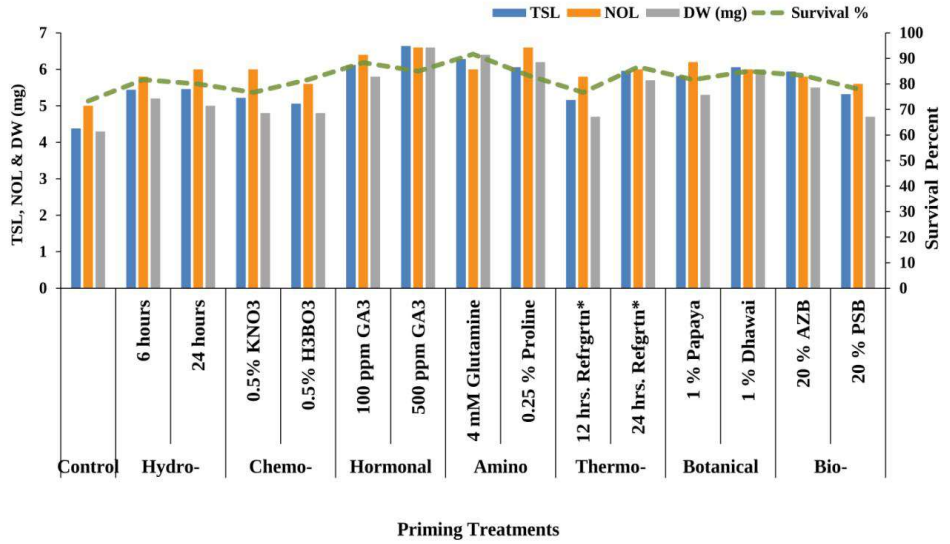

Fig. 2. Nursery performance of seedlings of *Dhawai*

priming, 0.5 % H_3BO_3 and control produced seedlings with narrowest collar diameter of 0.14 mm. Seeds primed with 500 ppm GA_3 and 0.25 % Proline produced seedlings with highest number of leaves (6.60) and lowest number of leaves (5.00) was recorded with control (Fig. 2). Dry weight of the seedlings was heaviest (6.60 mg) when the seeds were primed with 500 ppm of GA_3 and lightest (4.30 mg) was recorded with control (Fig. 2). Survival of the seedlings with these priming treatments was 73.33-91.67 % with a mean of 82.22 %. Priming treatments 4mM Glutamine resulted into highest survival of the seedlings and lowest survival was observed with control (Fig. 2). Among various priming treatments, 500 ppm of GA_3 was found the best priming treatment for improving growth parameters under nursery establishment study. GA_3 primed seeds recorded significantly higher seedling growth parameters as compared to control. This may be attributed to the key role of gibberellins in germination as growth regulator which are involved both in the break of dormancy and in the control of reserve hydrolysis on which the growing embryo depends (Lovegrove and Hooley 2000). The faster emergence of seeds primed with GA_3 might be due to its stimulation effect in the formation of enzymes during early phases of germination which helped for a fast radicle protrusion and hypocotyl elongation to penetrate the soil up (Salisbury and Ross 1992). These were further promoted due to exogenous application of GA as it increased the proportion of GA in the seed system promoting seedling growth and development. Similar influence of GA_3 on seedling growth of some field crops were also reported in earlier studies (Salisbury and Ross 1992, Lovegrove and Hooley 2000). In contrast, maximum survival was recorded with 4 mM Glutamine followed by 500 ppm GA_3 and 1 % *Dhawai*. Providing Glutamine exogenously might have supplemented the Glutamine synthetase activity, aiding nitrogen metabolism and acted as substrate for protein synthesis. Glutamate also leads to ammonium release in an oxidative deamination reaction catalyzed by glutamate dehydrogenase (Mifflin and Habash 2002).

Earlier studies with field crops exhibited excellent results with glutamine priming both under stress and normal conditions which was attributed to their influence on production of protein, seed sugar, oil, fiber content, moisture content, and ash (Ali et al 2013). Increased survival especially during stress was found with glutamine priming due to increased cell division, cell wall expansion, and other developmental processes. The micro-biotic nature with very fine seed coat might normally render the seeds to stress under normal germinating conditions restricting the seeds to germinating and seedling growth. Applying glutamine might have played a role of stress busters for the *Dhawai* seed aiding its germination, seedling growth and survival. Nevertheless, priming with 1 % *Dhawai* improved seedling survival; this might be due to synergetic effect of plant on its own species to germination, growth and survival. Similarly, improved germination and survival with *Dhawai* botanical extract seed treatment was also reported earlier (Devkota et al 2013).

CONCLUSION

The seed priming agents had positive effect in enhancement of germination and its parameters. Among all the priming treatments of *Dhawai*, it was found that priming with 500 ppm of GA_3 was the best followed by 4mM Glutamine and 24 hours refrigeration. Even though there is a high germination, seedling growth and establishment were recorded in both GA_3 and Glutamine seed priming treatments, 24 hours refrigeration treatment can be recommended for easy, effective and eco-friendly mode of quality planting material production in Terai region of West Bengal. In contrast, priming treatments such as the longer duration of hot air ovenation, higher concentration of acids, chemicals, hormones and other botanical priming agents deteriorated germination, seedling vigour and nursery establishment compared to non-primed seeds. However, further studies on seed biology, types of seed dormancy, genetic behaviour and pharmacological investigations can be undertaken along with studies on effect of priming on stresses (biotic and abiotic), seed storage, pests, diseases and yield parameter.

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