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EFFICACY OF SEED PRECONDITIONING WITH SALICYLIC AND ASCORBIC ACID IN INCREASING VIGOR OF RICE (*ORYZA SATIVA* L.) SEEDLING

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Abstract

Poor germination is one of the main problems in adopting direct seeded rice culture. The objective of the work was to enhance the efficiency of direct seeded rice in terms of germination and seedlings vigor. In this study, the role of hormonal priming (salicylic acid and ascorbic acid) and hydropriming for 20 and 40 h in increasing seed and seedling vigor both in *Petri* dishes was assessed. Seed conditioning with salicylic acid caused an increase in seed vigor (10%) and protease activity (18%) in rice at 4^{th} day of sowing, while significant enhancement (6%) was recorded with ascorbic acid in seedlings protease activity. Nitrate reductase activity (NRA) was increased in the 8 days old rice seedlings with all the pre-soaking treatment, the maximum increase was recorded in seedlings raised from seeds treated with salicylic acid (20mg L^{-1}) for 20 h. Based on overall performance, seed priming with salicylic acid increased seed and seedling vigor in fine rice, but the response depended on concentration and seed soaking time.

Introduction

The traditional crop cultivation practices are not sufficient enough to produce rice grains according to the needs of ever increasing world population. It has been projected that the world's annual paddy production must have to increase up to 781 million tons by 2020, and over a billion tons by the next century (Sass and Ciecerone, 2002). In the traditional transplanting system, the rice growers face looming water crises and increasing labour cost challenges. Direct seeding could be an attractive alternative to the traditional transplanting culture (Balasubramanian & Hill, 2002), but poor germination, uneven crop stand and high weed infestation are the main constraints to the adoption of direct seeded rice cultivation (Du & Tuong, 2002). Of different techniques being used to overcome the problem of poor seed germination in crop plants, pre-sowing seed treatment with different chemicals has gained much importance recently because of its effectiveness and cheapness (Harris *et al*., 1999; Iqbal & Ashraf, 2005, 2007, 2010a; Basra *et al*., 2006). Among different priming techniques (hydropriming, osmoconditioning, matripriming, etc.), seed acceleration (priming with plant growth regulators) has been shown to be much effective under both normal and stressed environments (Cayuela *et al*., 1996; Iqbal & Ashraf, 2005, 2007; Iqbal & Ashraf, 2010a; Mahmood *et al*., 2010; Ertekin, 2010; Rafique *et al*., 2011).

Salicylic acid (SA), an endogenous growth regulator of phenolic nature influences many physiological processeses such as, seed germination (Cutt & Klessing, 1992), ion permeability (Barkosky & Einhelling, 1993), photosynthesis and plant growth rate (Khan & Abdullah, 2003; Mahmood *et al*., 2010; Rafique *et al*., 2011) and endogenous hormone levels (Iqbal *et al*., 2006) in crop plants. Salicylic acid also prevents the damaging action of various stress factors in many plant species (Sakhabutdinova *et al*., 2003; Afzal *et al*., 2005; Iqbal & Ashraf, 2010b). Ascorbic acid (AsA), another important organic molecule serves as detoxification of reactive oxygen species (Conklin & Barth, 2004; Khan *et al.*, 2006) and a co-factor for many enzymes (Arrigoni & De Tullio, 2000). It is also involved in biosynthesis of many other plant hormones, including ethylene, gibberellic acid, and abscisic acid (Barth *et al.*, 2006).

The beneficial effects of seed priming are associated with different physiological and biochemical changes like, enzyme activation and endosperm weakening (Habib *et al.*, 2010), mobilization of storage proteins, changes in hormonal balance (Iqbal & Ashraf, 2005, 2007, 2010a, b) and synthesis of proteins that play an important role during germination in several plant species (Gallardo *et al*., 2001). High seed proteins contents have been shown to increase seedling vigor in cereals. However, the enhanced protease activity results in concomitant decline in storage and enzymatic proteins, decreasing seedling vigor of different plants. Moreover, nitrate reductase activity (NRA) of rice seedling reaches maximum at 7 to 10 days after seed imbibition and could be used as a measure of seedling vigor in rice plants (Yang & Sung, 1980).

Considering the involvement of SA and AsA in various physiochemical processes of plants, we assessed their role as priming agents in making direct seeding more efficient in fine rice. In addition, we determined whether SA and AsA priming induced seed and seedling vigor could be related to the altered activities of protease and nitrate reductase in rice.

Materials and Methods

A series of pilot experiments (data not given) to determine the effective concentrations from 1 to 25mg L^{-1} of 2 priming agents, salicylic acid (SA) and ascorbic acid (AsA), and the suitable priming duration from 10 to 40 h on seed and seedling vigor of rice (*Oriza sativa* L. cv. Super Kernel Basmati) were conducted. Based on the results of pilot study, 2 levels of SA and AsA (10 and 20 mg L^{-1}) were selected for further experimentation. Rice grains (250 for each treatment) were surface sterilized with 0.1% mercuric chloride and then treated with different solutions of SA (10 and 20 mg L^{-1}) and AsA (10 and 20 mg L^{-1}) for 20 or 40 h. Seeds were soaked in 250 ml plastic cups containing 200 ml solution of each treatment of SA, AsA or water at room temperature. Untreated seeds were kept for 20 or 40 hours at room temperature. After respective time of soaking, 25 seeds of each treatment were placed in *Petri* dishes lined with double layer of Whatman filter paper No. 42 moistened with distilled water. The *Petri* dishes were placed in a growth room at 25 ± 2°C in a completely randomized design with four replications. The efficacy of the same treatments was also checked under field conditions and seed vigor was tested in terms of emergence rate and germination percentage. The field experiment was conducted under natural environmental conditions at the research area of department of Botany, GC University Faisalabad, Pakistan. Seed priming was carried out as described above and seeds were sown with row to row distance of 12 inches.

Seed germination percentage and seedling shoot and root fresh or dry mass were recorded after eight days of sowing to assess seed and seedling vigor (Iqbal & Ashraf, 2007). The data for the germination rate was calculated by using the modified Timson Index (Khan & Ungar, 1984). Nitrate reducetase activity was determination by Sym (1984). Seedlings were chopped in 0.01 *M* phosphate buffer (pH 7.0) containing 0.02 M KNO_3 and 0.01% Triton X-100. After one hour incubation period, medium was mixed with sulphanilamide (10 g L^{-1}) in HCl and N-1naphthyl ethylenediamine dihydrochloride (0.1 g L^{-1}) solutions. The dye solution was centrifuged for 3 min. at 10000 *g* using (Z 216 MK, Hermle Labortechnin GmbH). The absorbance was measured at 542nm on spectrophotometer (UV-4000, ORI, Germany).

Protease activity was determined up to four days after seeds germinated to assess seed and seedling vigour in rice. Germinating seeds were homogenized in pre-chilled mortar with cold 50m*M* phosphate buffer (pH 7.8). The homogenates were kept in the cold (4°C) for 30 min. and then centrifuged (Z 216 MK, Hermle Labortechnin GmbH) at 12000*g* for 15 min. at 4°C. The Protease activity was determined by the casein digestion assay as described by Drapeau (1974). The supernatant then reacted with casein solution (1%) for one hour and protease activity was estimated by determining the amount of casein left in the solution. Total soluble proteins in germinating seeds were determined up to four days after sowing according to the Bradford (1976) method. Seedlings were extracted in phosphate buffer (pH 7.0) and centrifuged at 2000g for 10 min. The supernatant was treated with Bradford reagent and incubated for 30 min. at room temperature. The absorbance was measured at 590nm using spectrophotometer (UV-4000, ORI, Germany) and total soluble proteins were recorded using BSA as standard.

The experiments were laid out in a completely randomized design with 4 replicates for each experimental unit. Data for all attributes were subjected to analysis using the GLM module of Costat (CoHort Software, Monterey, CA, USA). LSD was used to separate treatment means in case of a significant F-test at $p \leq 0.05$.

Results

Seed priming with 10mgL^{-1} SA for 40 hours was very effective in increasing germination rate (28 %) in rice under growth room conditions (Fig. 1). Seed treatment with SA did not increase plumule fresh weight. However, seed soaking with lower concentration of SA (10 mg L^{-1}) for 20 h increased plumule dry weight compared with unsoaked ones (Fig. 2). Seedlings raised from seed treated with SA (10 mg L^{-1}) for 20 h showed greater radicle fresh and dry weights when compared with untreated seeds. Radicle and plumule fresh weight of rice seedlings increased up to 45% and 32% respectively in plants raised from seeds soaked in lower concentration (10 mg L^{-1}) of AsA for 20 h than that of seedlings raised from unsoaked seeds (Fig. 2). However, reduction in growth in terms of fresh weight of both radicle and plumule (30% and 24%, respectively) were observed in seedlings raised from seeds treated with same concentration (10 mg L^{-1}) of AsA for longer periods (40 h).

Protease activity was affected due to hormonal seed treatments and soaking periods as well (Fig. 3). However, as compared to unsoaked seeds, minimum protease activity was recorded on 1st day of sowing when the seeds were treated with higher concentration of SA (20 mg L^{-1}) irrespective of soaking periods. Afterwards, protease activity decreased and it was the minimum on 4th day of sowing. The maximum increase in total soluble proteins was recorded in 20 mg L^{-1} SA treated rice seeds for 40 h on 4th day of sowing (Fig. 4). Maximum protease activity was recorded on 1st day and 3rd day of sowing in seedlings raised from seeds treated with 10 mg L^{-1} AsA for 20 h as compared to unsoaked seeds (Fig. 3). However, the maximum increase in total soluble proteins was recorded in 20 mg L^{-1} SA treated rice seeds on 4th day of sowing (Fig. 4).

Pre- sowing seed soaking treatment with a lower concentration (10 mg L^{-1}) of SA for 20h was the most effective in increasing shoot NRA in rice and resulted 83% increase in NRA compared with unsoaked seeds (Fig. 5). Lower concentration of AsA (10 mg L^{-1}) also showed significant increase up to 54% when seeds were conditioned for 20 h.

Results from field experiment indicated that germination percentage was maximum (100%) in seeds treated 20 mg L^{-1} of SA or 10 mg L^{-1} of AsA for 40 h and 20 mg L^{-1} of AsA for 20 h (Table 1). The emergence rate was also high in seedlings raised from seeds treated with above mentioned levels of SA or AsA. Rice seeds treated with 10mg L^{-1} of SA for 20 h showed minimum values for seed germination and seedlings emergence rates among all the pre-sowing seed treatments.

Discussion

Although seed priming techniques have been found to be effective to some extent in increasing germination and early seedling growth of rice (Ruan *et al*., 2002; Mathew & Mohanasarida, 2005), poor germination is one of the main problems in adopting direct seeded rice culture. Direct seeding could be an attractive to the traditional transplanting system, which requires great amount of water for transplantation. In the present study, priming with SA for 20h was proved much effective in increasing germination rate as reported in earlier priming techniques like osmohardening, vitamin priming and hydropriming (Basra *et al.,* 2006; Farooq *et al.,* 2007). Although treatments were not different significantly for germination percentage under growth room conditions, there were significant differences when sown under field conditions. Seed treatment in 10mg L^{-1} SA for 40h significantly increased germination percentage compared with control under field conditions (Table 1). When priming duration increased upto 40h, hydropriming was also effective in improving germination percentage. Hydropriming, however, was not as beneficial in improving seed germination rate as in germination percentage.

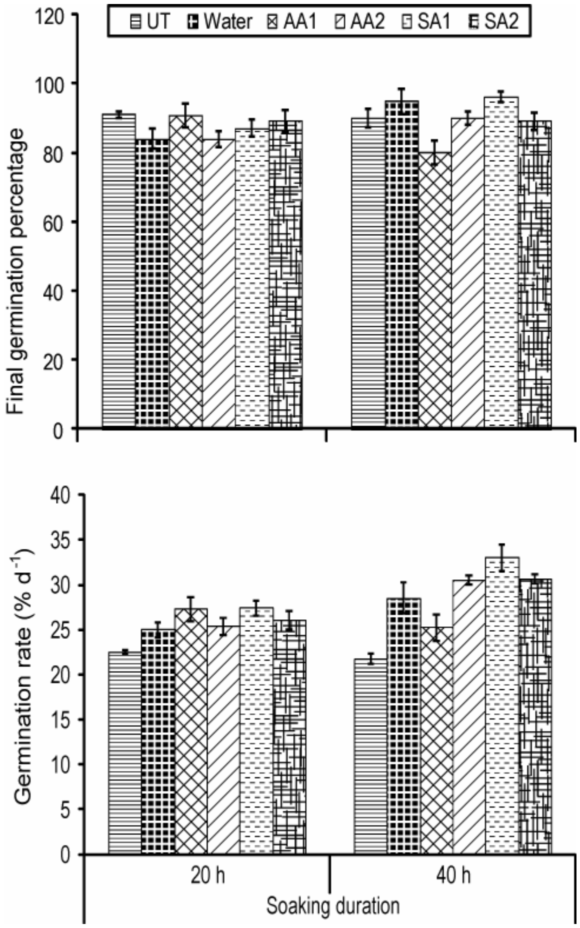

Fig. 1. Effect of ascorbic acid (AsA) and salicylic acid (SA) treatments for different soaking periods on germination percentage and germination rate of rice grown in growth room. UT, untreated; water, hydropriming; AA1 and AA2, 10 and 20 mg L^{-1} of ascorbic acid, respectively; SA1 and SA2, 10 and 20 mg L^{-1} of salicylic acid, respectively. ($n = 4$; bars showing means ± *SE*).

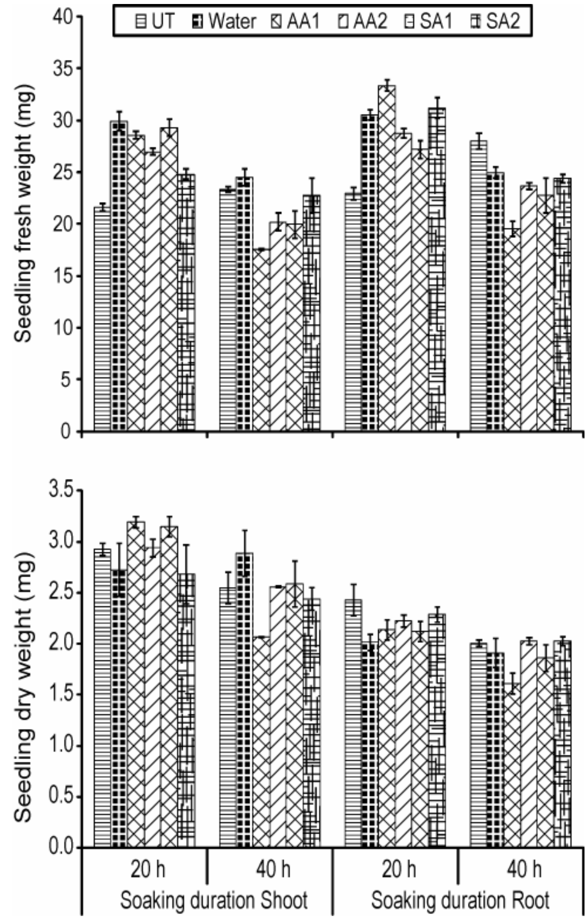

Fig. 2. Effect of ascorbic acid (AsA) and salicylic acid (SA) treatments for different soaking periods (20 h and 40 h) on shoot and root fresh and dry weights of 8 d old rice seedlings. UT, untreated; water, hydropriming; AA1 and AA2, 10 and 20 mg L^{-1} of ascorbic acid, respectively; SA1 and SA2, 10 and 20 mg L^{-1} of salicylic acid, respectively. ($n = 4$; bars showing means ± *SE*).

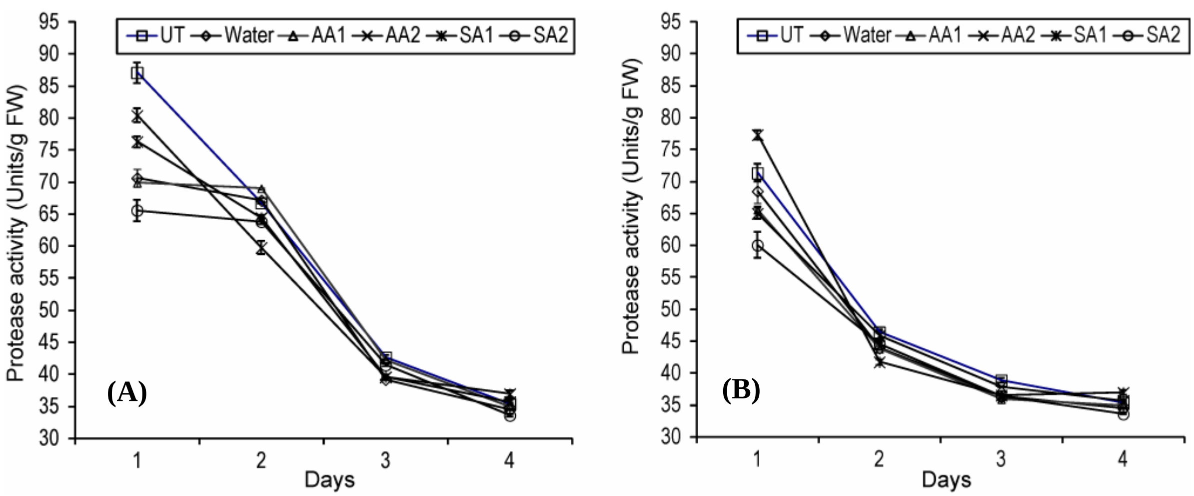

Fig. 3. Protease activity of rice seeds for four days after seed sowing when the seeds were treated with different concentrations of ascorbic acid (AsA) and salicylic acid (SA) for different soaking periods (A) 20 h and (B) 40 h. UT, untreated; Water, hydropriming; AA1 and AA2, 10 and 20 mg L^{-1} of ascorbic acid, respectively; FW, fresh weight; SA1 and SA2, 10 and 20 mg L^{-1} of salicylic acid, respectively. ($n = 4$; bars showing means ± *SE*).

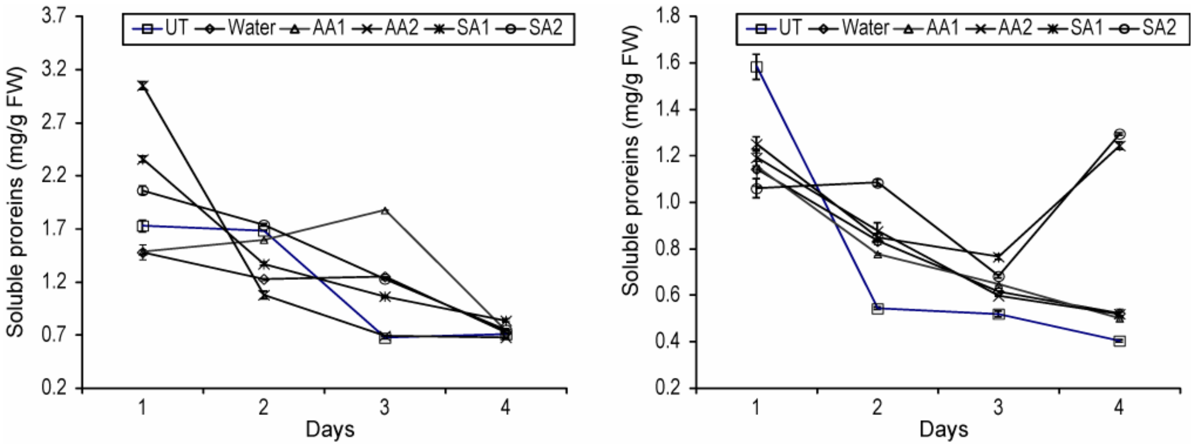

Fig. 4. Total soluble proteins of germinating rice seeds for four days after seed sowing when the seeds were treated with different concentrations of ascorbic acid (AsA) and salicylic acid (SA) for different soaking periods (A) 20 h and (B) 40 h. UT, untreated; Water, hydropriming; AA1 and AA2, 10 and 20 mg L^{-1} of ascorbic acid, respectively; FW, fresh weight; SA1 and SA2, 10 and 20 mg L^{-1} of salicylic acid, respectively. (*n* = 4; bars showing means ± *SE*).

Table 1. Effect of different pre-sowing seed treatments for different soaking periods on the emergence rate and germination percentage of direct seeded rice under field conditions. (*n* = 4).

Parameters		Treatments					
		Ascorbic acid		Salicylic acid		Hydro-priming	Untreated seed
		10 mg L^{-1}	20 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}		
Emergence rate (% d^{-1})	20 h	21.3^{g}	28.4^{f}	32.6^{cd}	37.8^{a}	31.8^{d}	30.6^{e}
	40 h	29.9^{e}	35.1^{b}	38.2^{a}	32.3^{cd}	33.0^{c}	29.4^{ef}
Germination percentage	20 h	54.7^{e}	84.0^{d}	85.3^{d}	100.0^{a}	84.0^{d}	90.7^{c}
	40 h	82.7^{d}	100.0^{a}	100.0^{a}	94.7^{bc}	96.0^{ab}	92.0^{bc}

Means with the same letter(s) do not differ significantly at $p<0.05$ within each attribute

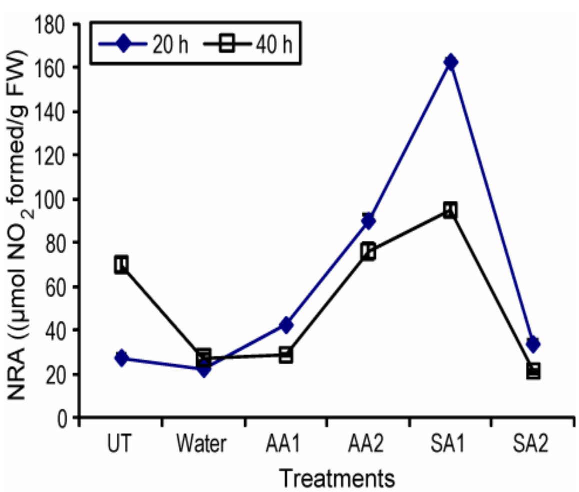

Fig. 5. Effect of ascorbic acid (AsA) and salicylic acid (SA) treatments for different soaking periods (20 h and 40 h) on nitrate reductase activity (NRA) of 8 day rice seedlings. UT, untreated; water, hydropriming; AA1 and AA2, 10 and 20 mg L^{-1} of ascorbic acid, respectively; SA1 and SA2, 10 and 20 mg L^{-1} of salicylic acid, respectively. (*n* = 4; bars showing means ± *SE*).

The increase in plumule dry matter after SA treatment as observed in present study might be a result of increased shoot lateral growth through increased cell division by maintaining the hormonal balance (IAA and cytokinin levels) in the plant tissues, which enhanced the cell division (Sakhabutdinova *et al.*, 2003). The increased root growth in terms of fresh and dry matter production due to SA treatments can be explained by the fact that SA enhances the cell replication in root tips, and thus increases root growth. Recently, Basra *et al*., (2006) reported that melon (*Cucumis melo* L.) seeds treated with 50 mg L^{-1} SA showed improved root and shoot length and number of roots. High seed protein content has been shown to increase seedling vigor in cereals. There are reports which show a positive relationship between NRA and seedling vigor in many crops (Yang & Sung, 1980; Below, 1995). Therefore, protease activity, total soluble proteins and NRA were determined so as to see the possible mechanism of priming treatments in increasing seed and seedling vigor in rice. The gradual decrease in protease activity and increase in soluble protein contents in SA treated seeds as observed in the present study suggested that SA-priming increased seedling vigor in rice.

Earlier works suggested that increasing levels of proteins help the plants to maintain their growth even under stressful environments (Agastian *et al*., 2000; Fidalgo *et al*., 2004; Sajid & Aftab, 2009; Ali *et al*., 2012). Thus, the AsA-mediated increase in seed protein contents at 4th day of sowing suggested that exogenous AsA increased seedlings rather than seed vigor in rice. Yamada *et al*., (2005) have reported 1.5 times increase in the protease activity during germination in brown rice under abiotic stresses. They suggested that this increase was due to increased proteolytic degradation.

The increase in fresh weight of radicles and pulmule of plants raised from AsA-treated-seeds might be due to increase in cell number within these tissues. Exogenously applied AsA has been suggested to be utilized in cell metabolism and to enhance the cell division efficacy of competent cells (Citterio *et al.,* 1994). Moreover, AsA has also been reported to be an important cofactor for a number of enzymes involved in hormone synthesis, e.g., gibberellins (Prescott & John, 1996). Recently, Afzal *et al*., (2005) reported significant increase in root and shoot length and fresh and dry weight of seedlings by exogenously application of AsA (50 mg L^{-1}) under both normal and saline conditions.

Based on overall performance of seed and seedlings, SA was the best priming agent to increase germination and subsequent seedling growth in fine rice. However, the seed and seedling vigor depended on the concentration of SA and the soaking time as well especially in our studies, seeds priming for 20 hours were more suitable for rice seed with low concentrations of both SA and AsA.

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