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RESEARCH ARTICLE

Effect of Various Seed Priming Agents on Germination, Seedling Vigour and Grain Yield of Dry Direct-Seeded Rice in the Northwestern Indo Gangetic Plains of India

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Abstract

Dry direct-seeded rice (DSR) grown in sub-tropical northwestern Indo-Gangetic Plains of India is often exposed to multiple environmental stresses during germination phase, leading to poor crop establishment and productivity. Seed priming may help overcome these stresses by ensuring better crop germination and establishment. The present study was carried out during 2022 and 2023 to assess the effectiveness of priming agents *viz*., hydropriming, nanopriming (25 ppm ZnO nanoparticles), hormonal priming (50 ppm Gibberellic acid, GA_3), halopriming (1% KNO_3) and ascorbic acid (100 ppm) priming in two rice cultivars under laboratory as well as dry direct seeded field conditions. All priming agents significantly improved seed germination and seedling growth, seedling length and seedling vigour under laboratory conditions and stand establishment of the crop in the field. Hormonal priming was found to be significantly superior to all the other treatments under laboratory conditions, but all the priming agents were equally effective under field conditions. The priming increased number of panicles/m^2 and ultimately grain yield of field crop significantly over the unprimed control, obviously due to improvement in crop stand (number of plants/m^2).

Keywords: DSR, Germination, Hormonal Priming, Nano Priming, Plant Stand

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Introduction

Rice (*Oryza sativa* L.) is the most important staple food of the world and not only feeds more than half the global population but also serves as a cultural and economic cornerstone in many regions (Asma *et al*., 2023). In India, it is a significant rainy season crop in the sub-tropical region of the northwestern Indo-Gangetic Plains (IGP), particularly in the states of Punjab and Haryana, and plays a vital role in the country's food security. The region is famous for growing fine-grain aromatic (basmati) rice in addition to high-yielding coarse-grain rice. Rice in this region is generally grown by the transplanting method of crop establishment, in which rice seedlings first grown in a nursery and then seedlings are transplanted (usually manually) into the puddled main field. But the transplanted rice (TPR) requires a large amount of water not only for field preparation (puddling) but also for maintenance of submergence for most part of the crop duration. Brar *etal*., (2015) reported that TPR requires a very high quantity of irrigation water (1357-1666 mm) to obtain a higher yield. The water requirement of the rice crop is largely met through the use of groundwater, as it is the principal source of irrigation in the region. This has resulted in the over-exploitation of groundwater resources in the region, resulting in a decline of the water table to the extent that pumping out of groundwater by tube wells is increasingly becoming a difficult and costly affair due to the high cost of installation and maintenance of large pump sets (deep tube wells) as well as high electricity consumption to operate them.

Therefore, it is the need of the hour to adopt water-saving rice establishment techniques as sustainable alternative to conventional water-intensive transplanting methods, aimed at maintaining rice production in the region while conserving valuable groundwater resources. Direct seeding of rice is an establishment technique in which rice seeds are sown directly in the main field rather than transplanting the seedlings from a nursery and is grown in aerobic conditions (irrigated at frequent intervals) as compared to TPR, which requires continuous flooding during most part of the growing period. The direct seeding offers a good alternative to transplanting as it saves the extra water required for puddling and maintenance of submerged conditions, throughout growing season, besides eliminating the need for nursery raising and manual labour required in conventional transplanting. The direct-seeded rice (DSR) has been reported to save up to 35% of irrigation water over the conventional TPR while saving up to 46% labour and 32% production cost (Saha *et al*., 2022). In recent years, direct-seeded (aerobic) rice has gained momentum in irrigated lowland areas of northwestern IGP, where rainfall is inadequate and pumping groundwater from deep wells is highly expensive (Sandhu *et al*., 2019).

Direct seeding of rice can be done either by sowing in moist soil following pre-sowing irrigation (locally known as *vattar* DSR) or by sowing in dry soil followed by irrigation (dry DSR). However, dry DSR is more common (conventional) practice in the are as where DSR is growingin Haryana and Punjab (Dhillon *et al*., 2021). But there are several constraints to the widespread adoption of the direct seeding technique of rice cultivation. In addition to heavy weed infestation, poor germination and poor or non-uniform crop establishment are the major constraints to the adoption of DSR in northwestern IGP (Sandhu *et al*., 2021; Dhillon *et al*., 2021). Poor crop establishment of DSR can be caused by several factors that determine the soil moisture conditions during crop emergence (Alam *et al*., 2020). In conventional DSR, the field inundation caused by unexpected rains during the crop germination period (before seedling emergence) creates temporary excess water stress. This may adversely affect the seed germination and emergence of seedlings, resulting in poor crop establishment and ultimately poor crop yield (Dhillon *et al*., 2021). Moreover, in many cases, the topsoil dries up quickly after the rains or irrigation, resulting in the formation of a hard-thin layer (crust) which can hinder the emergence of the seedlings, leading to poor crop stand. Rapid and uniform germination as well as early seedling vigour, are crucial to improve the performance of rice under direct-seededsituations (Mahender *et al*., 2015). Therefore, sustainable and effective technologies are needed to improve germination, seedling emergence and early growth of DSR to obtain its higher yield.

Seed priming is a controlled hydration technique that involves soaking the seeds in a solution of priming agents to induce pre-germination metabolic processes, without actual germination. This technique improves germination and seedling vigour under adverse environments. The seeds are re-dried to a moisture content below the threshold required for triggering radicle emergence to complete the priming, and the primed seed is then transferred to a growing medium (such as soil) where it can reabsorb water until the radicle protrudes and grows. Priming reactivates metabolic processes crucial for germination, including respiration, protein synthesis and enzyme activation (Ali and Elozeiri, 2017). Seed priming has emerged as a practical and effective approach to enhance seed performance under various environmental stresses in crop plants, including rice (Dhillon *et al*., 2021; Paul *et al*., 2022). In addition to soaking of seeds in water (hydropriming), seed priming in rice can also be done by treating seeds with specific salts (halopriming), growth regulators (hormonal priming), nano-particles(nanopriming) or other chemicals (Khalid *et al*., 2019; Dhillon *et al*, 2021; Adhikary *et al*., 2022; Zhang *et al*., 2024), but the chemical and its concentration need to be identified for the dry DSR.

However, there is a dearth of data on the effects of various priming agents in rice, particularly in the dry DSR system of the northwestern IGP. Despite the proven benefits of seed priming, challenges such as reduced seed longevity post-priming and variability in crop or varietal-specific responses remain (Rhaman *et al*., 2020), necessitating tuning of variables such as priming agents for specific conditions (Pagano *et al*., 2023). Moreover, many earlier studies have been limited to laboratory environments, concentrating mostly on emergence, germination and early development, with a very few evaluating the crop productivity (Zheng *et al*. 2016; Dhillon *et al*., 2021). The present investigation was, therefore, conducted to assess the efficacy of various priming agents on the germination and growth of rice seedlings in the laboratory as well as on stand establishment and performance of the crop in the field under dry direct seeded aerobic conditions.

Materials and Methods

Laboratory and field experiments were conducted during rainy (*kharif*) seasons of 2022 and 2023 at CCS Haryana Agricultural University, Rice Research Station, Kaul (Latitude 20°51´ N and Longitude 76°39´ E), Hisar, Haryana to study the effects of different seed priming treatments on germination and seedling vigour of rice under laboratory conditions and yield of rice crop grown in the field under dry direct seeded (aerobic) conditions. The treatments consisted of two rice varieties *viz.,* HKR 128 (V_1), a coarse grain high yielding variety of rice, and Pusa Basmati 1121 (V_2), a fine grain variety of basmati (aromatic) rice, and six priming treatments *viz.,* no priming or control (T_1), hydropriming (T_2), nanopriming with ZnO nanoparticles @ 25 ppm (T_3), hormonal priming with gibberellin (GA_3) @ 50 ppm (T_4), halopriming with KNO_3 @ 1% (T_5) and priming with ascorbic acid @ 100 ppm (T_6), resulting in twelve treatment combinations. The treatments were laid out in a factorial randomized block design with three replications for each combination. For priming, dry seeds (12-13% moisture) of the two rice varieties (500 g each) were soaked in solutions of various priming agents for 16 hours in an equal volume of either solution (for T_3, T_4, T_5, and T_6) or water (for T_1). After priming, the seeds were redried (in shade) to their original moisture content. The primed seeds were put in polythene bags and kept in a refrigerator at 5±2°C until they were used.

One set of the primed seeds (300 seeds for each treatment combination) was used for the laboratory experiment and was divided into three replications (100 seeds/replication). In the laboratory experiment, the seeds of each replication were placed on moist germination papers and kept in a seed germinator under dark conditions at a temperature of 25±2°C and relative humidity of 90±1%. These seeds were evaluated for laboratory observations on germination (%) and seedling vigour parameters *viz.,* seedling length, seedling dry weight and vigour indices *viz.,* vigour index-I and vigour index-II (ISTA, 2019) after 14 days. The number of germinated seeds in each replication was counted to determine germination percentage. For seedling length, the shoot length of ten randomly selected seedlings per replication was measured from the base of the seedling to the tip of the longest leaf and averaged. After measuring the seedling length, these ten seedlings were oven-dried for 24 h at 80±1°C and subsequently weighed to determine the average seedling dry weight. The vigour indices were determined using the formula suggested by Abdul-Baki and Anderson (1973),according to which the vigour index-I was determined by multiplying the seed germination (%) by the average length of the seedlings, whereas the vigour index-II was determined as the product of the germination (%) and average dry weight of the seedlings.

The second set of primed seeds was used for a field experiment on the crop to be grown under conventional direct-seeded (aerobic) conditions. In the field experiment, the primed seeds for each treatment combination were sown in the field (at 3-4 cm depth) on a well prepared flat dry seed bed in plots of size 4.2 m × 3.0 m (for each replication) in rows 20 cm apart with a plant to plant (intra-row) spacing of 15 cm, sowing one seed per hill (place) in each row. The field was irrigated immediately after sowing as a standard agronomic practice for a dry direct-seeded crop. The recommended agronomic and plant protection practices, such as land preparation, weed management, irrigation, fertilization, and disease or insect-pest management, were followed uniformly across all plots. Observations on seedling establishment or plant stand (number of plants/m^2), physiological indices, yield attributes (number of plants/m^2, number of grains/panicles, and 1000-grain weight), and grain yield of the DSR crop were recorded from the net plot (3.6 m × 2.6 m) of each treatment. The number of plants/m^2 was determined at 15 days after sowing (DAS) by placing a quadrate (0.5 m × 0.5 m) randomly at three places along the rows in each plot and counting the total number of plants within the quadrat area and then converting the number on a per square metre basis. Physiological indices, *viz.* chlorophyll content (in terms of SPAD values) and chlorophyll fluorescence (CF), were recorded at flowering initiation and grain filling stages of the crop. The SPAD values were recorded by using a portable handheld chlorophyll meter (SPAD-502 Plus, Konica Minolta Optics, Japan), whereas chlorophyll fluorescence was measured with the help of a portable hand-held chlorophyll fluorometer (OS-30p+ Opti-Sciences, Inc.). The yield attributes and grain yield (kg/ha) of the crop were recorded at maturity. The number of panicles/m^2 was obtained by counting all effective (panicle-bearing) tillers within a quadrat (0.5 m × 0.5 m) placed randomly at three different places along the rows in each plot and then converting the count on per square-meter basis. The number of grains/panicles was estimated by harvesting a representative set of panicles (10 panicles) from each plot, threshing them separately, and counting their grains. The 1000-grain weight was determined by randomly taking a sample of grains from the harvested/threshed produce (grain) of each net plot, counting 1000-grains from the sample and recording their weight (g). Grain yield was measured as the total weight of grains obtained from each plot (after drying to 22% moisture) converted to kg/ha.

Data on the laboratory and field observations were statistically analysed by the analysis of variance (ANOVA) technique with the help of an online statistical tool (OPSTAT) accessible at the website of CCS HAU, Hisar (www.hau.ac.in). The means of each treatment were compared with the help of the critical difference (CD) determined at 5% level of significance.

Results and Discussion

Seed germination and seedling vigour

Under laboratory conditions, rice variety Pusa Basmati 1121 exhibited significantly higher germination percentage (82.9-87.4%) and seedling length (27.9-35.0 cm) as compared to HKR 128 (75.4-81.0% and 24.3-30.2 cm) during the two years of laboratory investigation. Pusa basmati 1121 also showed significantly higher seedling vigour Index-I (2298-3088) and vigour index-II (8.37-8.57) than HKR 128 (1842-2426 and 7.77-7.78). Rice varieties may differ in terms of activities of amylase and other enzymes, as well as the contents of sugars and soluble proteins (Monajjem *et al*., 2023), which might be responsible for the differences in their germination potential.

The data on laboratory studies presented in Table 1 showed that priming of rice seeds with all the agents significantly increased the germination and seedling growth in terms of seedling length, seedling vigour-I, and seedling vigour-II over the unprimed control during both years. Among the priming treatments, the highest mean (averaged over the varieties) germination (84.0-90.3%), seedling length (29.2-37.2 cm), seedling vigour-I (2460-3359) and seedling vigour-II (8.79-9.03) was achieved when seeds were primed with GA3 @ 50 ppm (T_4) and this treatment was found significantly superior as compared to other priming treatments, including hydro-priming (T_2). Priming with GA_3 @ 50 ppm improved the germination, seedling length, vigour index-I and vigour index-II by 15.1-16.1, 22.0-26.0, 41.6-45.3 and 20.4-22.2 per cent, respectively, over the unprimed control during the two years of study. Seed priming with gibberellin (GA_3) improved the germination and seedling emergence, possibly due to breakage of seed dormancy and an increase in the activity of starch-metabolizing enzymes (hydrolases) such as α-amylase, which convert the stored starch in the endosperm into soluble sugars to provide energy for germination or growth of the embryo (Waqas *et al*., 2019; Wang *et al*., 2021). The better germination and seedling growth or vigour obtained with GA_3 priming

Table 1: Germination and growth of rice seedlings as affected by varieties and seed priming treatments under laboratory conditions

Treatment	Germination (%)		Seedling length (cm)		Seedling dry weight (g/seedling)		Vigour index-I		Vigour index-II	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Varieties										
V_1	81.0	75.4	30.2	24.3	0.096	0.103	2426	1842	7.78	7.77
V_2	87.4	82.9	35.0	27.9	0.098	0.101	3088	2298	8.57	8.37
CD 5%	5.5	3.4	2.1	1.9	NS	NS	255	120	0.92	0.53
Priming treatments										
T_1	77.8	73.0	29.2	23.2	0.095	0.100	2271	1693	7.39,11.0	7.30,13.0
T_2	83.8	80.0	32.3	26.3	0.097	0.103	2707	2117	8.13	8.23
T_3	86.3	80.2	32.4	25.8	0.095	0.103	2796	2059	8.20	8.25
T_4	90.3	84.0	37.2	29.2	0.100	0.105	3359	2460	9.03	8.79
T_5	83.5	79.0	32.2	25.8	0.098	0.100	2689	2017	8.18	7.90
T_6	83.5	78.7	32.4	26.3	0.097	0.101	2706	2074	8.10	7.95
CD 5%	3.2	1.9	1.3	1.2	NS	NS	147	69	0.53	0.41

V_1: HKR 128; V_2: Pusa Basmati 1121; T_1: No priming (control); T_2: hydropriming; T_3: priming with ZnO nanoparticles @ 25 ppm;T_4: priming with GA_3 @ 50 ppm;T_5: priming with KNO_3 @ 1%;T_6: priming with ascorbic acid @ 100 ppm; NS: non-significant.

might be due to the additional supply of growth-promoting substance (gibberellin).The remaining priming treatments (T_2, T_3, T_5, and T_6) were, however, at par with each other, improving the germination by 7.3-10.3% besides improving the seedling length (10.3-13.4%), seedling vigour-I (18.4-25.0%) and seedling vigour-II (8.2-13.0%) over the control during the two years. It has already been reported that hydropriming increases germination rate and seedling growth in rice by increasing the activity of α-amylase,which in turn results in better mobilization of carbohydrate reserves in the endosperm (Kata *et al*., 2014), thus shortening the metabolic lag time for seed germination (Khalid *et al*., 2019). In nanopriming, nanoparticles can penetrate the seed coat to enhance seed germination by increasing the activity of enzymes (such as amylase), proteins, and lipids in seeds to produce more soluble sugars, besides regulating the balance between Abscisic acid (ABA)and GA (Jisha *et al*., 2013; Sharma *et al*., 2021). Seed priming with KNO_3 might have improved the germination and seedling growth through improvement in water absorption and activation of reserve enzymes such as amylases, dehydrogenases and xylanase (Alietal., 2020).Priming with ascorbic acid is reported to induce a wide range of coordinated responses, including regulation of hormone synthesis and epigenetic control of gene expression, allowing primed seeds to germinate and grow under adverse environment (Terzjaghi and De Tullio, 2023).

Dry weight of the seedlings, however, was not affected significantly due to priming with any agent. Interaction between varieties and priming treatments was not found to be significant in respect of germination or seedling growth, indicating that the effect of all the priming treatments on both the varieties was more or less similar magnitude. Increase in the germination and growth of seedlings due to priming with gibberellic acid (Zhang *et al*., 2024), hydropriming (Matsunami *et al*., 2021), priming with KNO_3 (Dhillon *et al*., 2021), priming with ZnO nano-particles (Li *et al*., 2021), and priming with ascorbic acid (Zhang *et al*., 2024) have also been reported earlier.

Physiological indices and yield of crop

The data on mean value (averaged over the priming treatments) of various physiological indices, yield attributes and grain yield of the DSR crop grown in field conditions (Table 2) revealed that Pusa Basmati 1121 showed significantly higher plant stand (25.9-27.6 plants/m²), number of panicles/m² (311-324) and 1000-grain weight (28.2-29.6 g) than HKR 128 (23.3-24.2, 289-304 and 23.6-24.6, respectively) but number of grains/panicle (95.6-106.8) and grain yield (5939-6373 kg/ha) of HKR 128 was significantly higher than that of Pusa Basmati 1121 (57.7-63.6 and 4545-4946 kg/ha, respectively) during the two years. The higher yield of HKR 128, despite its lesser panicles/m² and lower 1000-grain weight, is attributable to its much higher number of grains/panicles. The data on physiological indices, *viz.,* chlorophyll content (SPAD) and Chlorophyll fluorescence (CF), showed that variety Pusa Basmati 1121 exhibited significantly higher values of SPAD (both at flowering initiation and grain filling stages) and CF (only at flowering initiation) as compared to HKR 128.

Table 2: Plant stand, physiological indices, yield attributes and grain yield of DSR as affected by varieties and priming treatments under field conditions

Treatment	Number of plants/m²		SPAD at flowering initiation		SPAD at grain filling		CF at flowering initiation (%)		CF at grain filling (%)		Number of panicles/m²		Number of grains/ panicle		1000-grain weight (g)		Grain yield (kg/ha)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Varieties																		
V_1	24.2	23.3	34.6	34.9	31.3	33.1	0.57	0.59	0.56	0.54	289	304	95.6	106.8	23.6	24.7	5939	6373
V_2	27.6	25.9	36.1	37.5	33.8	35.3	0.61	0.63	0.57	0.56	311	324	57.7	63.6	28.2	29.6	4545	4946
CD 5%	1.1	1.6	1.3	1.4	1.1	1.3	0.03	0.02	NS	NS	10	12	3.1	5.7	2.03	1.4	307	222
Priming treatments																		
T_1	22.8	21.6	34.7	34.4	32.1	33.3	0.58	0.58	0.55	0.53	278	289	76.0	84.6	23.6	26.8	4883	5214
T_2	26.6	24.4	36.5	35.7	34.5	33.6	0.60	0.61	0.56	0.54	303	318	77.8	85.3	25.8	27.2	5304	5728
T_3	26.8	25.6	36.4	37.3	32.1	34.6	0.61	0.62	0.53	0.54	303	317	77.0	85.1	25.4	27.2	5308	5715
T_4	27.1	26.0	34.2	36.6	32.2	35.0	0.57	0.59	0.57	0.55	304	320	76.4	85.5	27.1	27.2	5322	5770
T_5	25.7	25.4	34.1	36.9	31.4	34.2	0.60	0.62	0.60	0.59	307	319	76.1	85.4	27.2	27.0	5312	5753
T_6	26.4	24.6	36.4	36.3	33.1	34.4	0.61	0.63	0.57	0.56	307	320	76.7	85.2	26.4	27.2	5323	5777
CD 5%	2.5	2.0	NS	NS	NS	NS	NS	NS	NS	NS	16	20	NS	NS	NS	NS	378	385

SPAD: Chlorophyll content; CF: Chlorophyll fluorescence.

These varietal differences in physiological indices and yield attributes might be due to differences in the genetic constitution of the varieties.

All the priming treatments significantly increased the mean (averaged over varieties) number of established seedlings or plant stand (number of plants/m^2), number of panicles/m^2 and grain yield of the field crop over the unprimed control (T_1) under dry direct seeded (aerobic) conditions (Table 2). Where as, the number of grains and 1000-grain weight of the crop were not influenced significantly due to priming. A similar trend was recorded during both years. The increase in the number of plants/m^2 (stand establishment) over the unprimed control was to the tune of 12.8-15.8% during 2022 and 11.7-12.7% during 2023, whereas the increase in the number of panicles/m^2 was 11.3-12.8% during 2022 and 10.0-11.8% during 2023. The priming, irrespective of the priming agents, also increased grain yield of the DSR crop significantly (10.5-12.2% in 2022 and 9.8-11.1% in 2023) as compared to unprimed control (T_1). However, all the priming treatments, including the hormonal priming, were at par with each other in respect of their favourable effect on the stand establishment, panicle number/$m^{2,}$ and grain yield. This indicated that the additional benefit of hormonal priming over the other priming agents was absent under the field conditions, probably due to prohibitive soil conditions. The results clearly indicate that the priming increased the grain yield of DSR primarily by improving its stand establishment (number of plants/m^2) in the field, which resulted in more effective tillers or panicles/m^2 and ultimately higher yield. Halopriming with 2% potassium nitrate (KNO_3) and hormonal priming with 50 ppm GA_3have also shown good potential in boosting crop establishment and yield of direct-seeded rice in soils of western IGP (Dhillon *et al*., 2021). The increase in yield of DSR due to enhancement of germination, stand establishment and number of panicles/m^2 by hydropriming (Mahajan *et al.,* 2011), nanopriming with Zn nanoparticles (Adhikary *et al*., 2022), and priming with ascorbic acid (Zhang *et al*., 2024) has also been reported earlier. Physiological indices, *viz.,* SPAD and CF both at flowering and maturity did not vary significantly due to the priming treatments. Mahajan *et al.,* (2011) also did not find any variation in SPAD values due to priming. There was no significant interaction between the varieties and priming treatments with respect to their effect on yield attributes and the yield of DSR crop in the field, indicating that the effect of priming in both varieties was of a similar nature.

Conclusion

Priming with all the agents significantly improved the germination, seedling growth, and seedling vigour under laboratory conditions and also improved the plant stand, number of panicles/$m^{2,}$ and ultimately grain yield of dry DSR crop under field conditions over the unprimed control. Among the priming agents, priming with GA_3 @ 50 ppm showed the highest germination and seedling length under laboratory conditions. However, under field conditions all the priming agents were at par in respect of their favourable effect on plant stand, yield attributes, and yield of DSR crop.

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