Seed priming with selenium and zinc nanoparticles modifies germination, growth and yield of direct-seeded rice (*Oryza sativa* L.)

Saju Adhikary

Bidhan Chandra Krishi Viswavidyalaya

Benukar Biswas (✉ kripahi@yahoo.com)

Bidhan Chandra Krishi Viswavidyalaya

Debashis Chakraborty

Indian Agricultural Research Institute

Jagadish Timsina

Global Evergreening Alliance

Srikumar Pal

Bidhan Chandra Krishi Viswavidyalaya

Saon Banerjee

Bidhan Chandra Krishi Viswavidyalaya

Akbar Hossain

Bangladesh Wheat and Maize Research Institute

Sovan Roy

Department of Science & Technology and Biotechnology Government of West Bengal

Jagadish Chandra Tarafdar

Ex-Scientist, Central Research Institute for Dryland Agriculture, 17E/361A, C. H. B., Jodhpur 342008, India

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**Seed priming with selenium and zinc nanoparticles modifies germination,
growth, and yield of direct-seeded rice (*Oryza sativa* L.)**

Saju Adhikary^a, Benurkar Biswas^{a,*}, Debashis Chakraborty^{b*}, Jagadish Timsina^c, Srikumar Pal^d, Jagadish Chandra Tarafdar^e, Saon Banerjee^f, Akbar Hossain^g, Sovan Roy^h

^aDepartment of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India 741 252.

^bDivision of Agricultural Physics, Indian Agricultural Research Institute, New Delhi 110 012.

^cGlobal Evergreening Alliance, 1 Vision Drive, East Burwood, VIC 3151, Melbourne, Australia, and Institute for Study and Development Worldwide, Sydney, NSW, Australia.

^dDepartment of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India 741 252.

^e Ex-Scientist, Central Research Institute for Dryland Agriculture, 17E/361A, C. H. B., Jodhpur 342008, India and 32/E/2/1, Banamalipur Barasat, Kolkata, West Bengal, India 700124.

^fDepartment of Agricultural Physics and Meteorology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India 741 252.

^gBangladesh Wheat and Maize Research Institute, Dinajpur 5200, Bangladesh.

^hDepartment of Science & Technology and Biotechnology, Vigyan Chetana Bhavan, Kolkata, West Bengal, India -700064.

*Correspondence authors: kripahi@yahoo.com (BB) and debashisiari@gmail.com (DC)

Abstract

Direct-seeded rice (DSR) seeds are often exposed to multiple environmental stresses in the field, leading to poor emergence, growth and productivity. Appropriate seed priming agents may help to overcome the challenges by ensuring uniform seed germination, and better seedling stand establishment. To examine the effectiveness of sodium selenite, sodium selenate, and zinc oxide nanoparticles (ZnO-NPs) as priming agents for DSR seeds, a controlled pot experiment followed by a field experiment over two consecutive years was conducted on a sandy clay loam soil (*Inceptisol*) in West Bengal, India. Priming with combinations of all priming agents had advantages over the hydro-priming treatment (control). All the combinations resulted in the early emergence of seedlings with improved vigour. In the field experiment, the combinations of all priming agents increased the plant chlorophyll, phenol and protein contents, leaf area index and duration, crop growth rate, uptake of nutrients (N, P, K, B, Zn and Si) and yield of DSR over the control. Our findings suggest that seed priming with the combination of ZnO-NPs, Na-selenite, and selenate could be a viable option for the sustainable intensification of DSR.

Introduction

Direct-seeded rice (DSR), could potentially be a viable alternative to traditional puddled-transplanted rice (PTR) due to less labour requirement, increased resource (water, nutrient, energy, etc) use efficiency and higher returns (Chakraborty et al., 2017; Wang et al., 2017) in rice-growing regions of the world. Direct seeding can be achieved by sowing on the dry soil either mechanically or manually (Dry DSR), or wet soil through broadcasting (Wet DSR), or

in standing water through broadcasting (Water DSR) ^{1,3}. Dry DSR could be more advantageous over the other two methods ^{4,5} due to less time required for sowing, which economizes time and labour, and the water is saved, which otherwise is essential for wet-seeding. Though PTR is the main rice transplanted system in Asia, DSR has also been gaining popularity (Timsina and Connor, 2001; Mahajan et al., 2012). For example, in China, DSR covers 28% of the total rice area ⁶, while in Sri Lanka and Malaysia, areas under DSR were >93% (1.03 M ha) and 95% (0.67 M ha) respectively of their respective total rice area ⁷. In the Indian Punjab alone, DSR was adopted in 0.601 M ha during 2021 ⁸. Poor germination and seedling stand establishment were identified as major factors for the low productivity of DSR in South Asia ^{6,9-11}. Often in DSR when seeds are sown directly in the field, plants are exposed to multiple environmental stresses particularly during emergence and early development ¹²⁻¹⁴. These stresses depress the kinetics of many physiological and metabolic processes ¹⁵, generate a large number of reactive oxygen species (ROS) in plant's cells that trigger lipid peroxidation in membranes ¹⁶⁻¹⁸. These are followed by damage to biomolecules including proteins, carbohydrates and DNA ^{17,19}, and reduce respiration rate and energy supply to growing plant tissue ²⁰. All these processes affect sequentially the germination and seedling establishment, plant stand, growth and resource utilisation, and ultimately the yield of DSR.

While the conventional breeding approach is time-consuming, genetic engineering is highly controversial albeit with its high potential benefits ²¹. A large number of abiotic stress-tolerant rice genes have been characterized during the last three decades which is being further exploited for varietal development with higher productivity in rice ^{22,23}. Seed priming has proven utility in optimising seed vigour and plant physiological processes, which makes the plant ready to face multiple probable stresses in the field more efficiently ²⁴⁻²⁶. Several

successful priming agents have been reported in the literature ranging from salts, polyamines, hormones, compatible solutes, and aqueous plant extracts (Farooq et al., 2009).

Zinc (Zn), an essential micronutrient, is the only metal that forms a part of the six different classes of enzymes²⁸. It is closely involved in many biochemical and physiological processes²⁹. Seed priming with Zn has shown positive effects on seed vigour, germination, early seedling growth and biomass production, photosynthetic efficiency, and in the contents of sugar, total nitrogen, protein and micronutrients in many crops (Faizan et al., 2018; Moghaddasi et al., 2017; Zhang et al., 2018). In the recent past, biologically synthesised ZnO-NPs with their enhanced physical and biochemical characteristics and low environmental toxicity showed their efficacy as priming agents^{36,37}. Uniformity in seedling emergence and improved growth of rice plants using ZnO-NPs as priming agent was reported with higher production of antioxidant enzymes against ROS damage³⁸.

In plants, selenium (Se) as a constituent of selenoproteins, has been reported to enhance starch and ATP synthesis³⁹, regulate water status, prevent chlorophyll loss during drought^{40,41}, and delay senescence⁴². In addition, it regulates redox reactions^{43,44} and rROS concentration, and consequently lipid peroxidation⁴⁵. Priming with sodium selenite has been shown to trigger germination in rice⁴⁶. Subsequent studies reported the efficacy of rice seed priming with sodium selenate (Du et al., 2019) and selenite-selenate combination⁴⁸ with their contributions to the assimilation and storage pathway respectively. Oxyanions of Se can promote the adsorption of Zn and enhance their bioavailability within the plant system by synergy^{49,50}. To our knowledge, there is no literature on the combined application of zinc or nano-zinc with Se as seed priming agents on germination and seedling establishment and growth and yield of DSR.

In response to above gaps, We hypothesized that (1) the (synergistic) effect of ZnO-NPs and Se as seed priming agents in DSR cultivation influences germination, and the

emergence of healthy and robust seedlings capable of mitigating environmental stresses; and (2) the synergistic effect could result in higher DSR yield compared to that without the priming. To test these hypotheses, a controlled pot experiment and a field experiment was undertaken with different treatments of ZnO-NPs, sodium selenate, and sodium selenite single or in combination as priming agents in DSR.

Materials and methods

Experimental site. A pot experiment (examined for seed vigour) was conducted followed by a field experimentation (examined for plant growth traits and yield) in each year (2019 and 2020) on DSR at Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India (23°5.3' N, 83°5.3' E; subtropical; 9.75m amsl). The soil was sandy clay loam (sand 64.8%, silt 10.4% and clay 24.8%) with a pH of 7.3 and EC of 0.296 dSm⁻¹ ⁵¹. Initial soil properties of study site were: 11.2 g kg⁻¹ oxidizable organic carbon (Walkley and Black, 1934), 315 kg ha⁻¹ available N ⁵³, 41.6 kg ha⁻¹ available P ⁵⁴, and 156.4 kg ha⁻¹ available K ⁵⁵. The climate is tropical moist sub-humid, with hot summer, and moderately cold winter. Average maximum and minimum temperatures ranged as 25-36 °C in summer and 10-25 °C in winter (Supplementary Figure S1).

Experimental treatments. Eight treatment combinations of ZnO-NPs and Se were selected: T₁: Hydropriming with distilled water (Control); T₂: Sodium selenite, Na₂SeO₃ @ 50 µmol (as Na₂SeO₃;Sigma-Aldrich USA); T₃: Sodium selenate, Na₂SeO₄ @50 µmol (as Na₂SeO₄;Sigma-Aldrich USA); T₄: Sodium selenite @ 50 µmol + Sodium selenate @ 50 µmol; T₅: ZnO-NPs @ 10 µmol (IFFCO; Tarafder et al., 2014); T₆: ZnO-NPs @ 10 µmol + Sodium selenite @50 µmol; T₇: ZnO-NPs @ 10 µmol + Sodium selenate @ 50 µmol; and T₈: ZnO-NPs @ 10 µmol + Sodium selenite @ 50 µmol + Sodium selenate @ 50 µmol]. Indica

inbred semi-dwarf type medium slender grain rice variety Ajit IET 22066 ⁵⁶ was used in both pot and field experiments.

Pot experiment. The pot experiment was conducted in a completely randomized design under factorial arrangements with three replications. A 25 g of seeds were placed in a 200 ml conical flask containing 125 ml initiator solution as per the treatment (ZnO-NPs, Na₂SeO₃, Na₂SeO₄), and aerated distilled water for the control and seeds were kept for imbibition or 24 h. The flasks were placed in an incubator [darkness, 27 ± 3 °C and 80% relative humidity] and were shaken once every 6 h. After priming, the seeds were filtered, placed in distilled water for 20 minutes and rinsed five times with ultrapure water. Autoclaved glass Petri dishes were lined with double layers of filter paper and were placed on a laboratory bench for air drying for a period of 24 h. Thereafter, 10 ml sterile water was aseptically pipetted into and 100 seeds were placed in each petri-dish. Each seed soaking treatment was performed in triplicate petri-dishes, which were kept inside an incubator at 26±0.5°C under aseptic condition.

Seeds were sown in plastic pots (15.6 cm height, 18.2 cm top diameter and 12.5 cm lower diameter), which were filled with air-dried and well-mixed field soil (2000 g) collected from the study site. Seeds were sown uniformly in each pot with soil moisture at field capacity (−0.03 MPa). Pots were placed in a screen house under the natural condition with a 14/10 h light/dark photoperiod and uniformly irrigated with distilled water as per the requirement to avoid water deficit. The seed emergence was counted daily according to the Association of Official Seed Analysts (AOSA) ⁵⁷ until a constant count was achieved. A seed was taken as emerged when the hypocotyl length was ≥2 mm. Time taken to 50% emergence of seedlings (E₅₀) was calculated according to the modified formula of Farooq et al. (2005):

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)} \quad (1)$$

where N is the final number of emerged seeds; n_i and n_j are the cumulative number of seeds emerged by adjacent counts at times t_i and t_j where $n_i < N/2 < n_j$. Mean emergence time (MET), an indicator of relative emergence of seedling in a day was calculated according to Ellis and Roberts (1981):

$$MET = \frac{\sum (D * n)}{\sum n} \quad (2)$$

where n is the number of new emerging seeds on day D (number of days from the beginning of emergence). The emergence index (EI), which is a measure for percentage and rate of germination, was calculated as described by AOSA (1990):

$$EI = \frac{\text{Number of emerged seeds}}{\text{Days of the first count}} + \dots + \frac{\text{Number of emerged seeds}}{\text{Days of the final count}} \quad (3)$$

Vigour index (VI) was calculated after Wang et al. (2018).

$$VI = S_d \times \sum \left(\frac{G_t}{t}\right) \quad (4)$$

where S_d is the seedling dry weight at the end of the test period (7 days), G_t is the number of germinated seeds on day t from the beginning of the test.

Root and shoot lengths were measured on 18 days after sowing (DAS) from each treatment. For these, five randomly selected seedlings were oven-dried at 70 °C for 48 h to get the dry biomass of root and shoot, and both the components were summed up to record the total seedling biomass.

Field experiment. The experiment was laid out in a factorial randomized complete block design with three replications. Fields were prepared by cultivating twice using a disc harrow (Unison Exports, Ludhiana, Punjab, India), followed by leveling with a wooden board. Pre-germinated primed seeds were sown manually by a single-row planter having an inclined plate mechanism @ 25 kg ha⁻¹ at 20 cm row-to-row spacing. The field was surface-irrigated immediately after sowing. Soil water potential was monitored with tensiometers installed at

20 cm depth, and the field was irrigated at -30 kPa potential. Fertilizers were applied as per soil test-based recommendations ⁶² with a basal application of N at 25 kg ha⁻¹, P₂O₅ at 50 kg ha⁻¹, K₂O at 50 kg ha⁻¹, and ZnSO₄ at 5 kg ha⁻¹ as urea, single super phosphate and muriate of potash, respectively. Additionally, 75 kg N ha⁻¹ in the form of urea and ammonium sulphate (AS) was applied in two splits: one half at the active tillering stage (urea and AS in equal proportion) and the other half at the panicle initiation stage (only through AS). Weeds were controlled by a pre-emergence herbicide [Pendimethalin @0.75 kg a.i. ha⁻¹] at 2 DAS, followed by a post-emergence herbicide [Bispyribac-sodium @ 25 g a.i. ha⁻¹] at 20 DAS. Weeds that escaped these treatments were removed manually at 42 DAS. Chloropyriphos @50 g a.i. ha⁻¹] and propiconazole @62.5 g a.i. ha⁻¹] were used to control insects and diseases, respectively. Irrigation was withdrawn 15 days before harvest. All agronomic management practices were the same in both years. Grains were harvested at 15–18% grain moisture content.

Growth traits analysis. Plants were collected from 1-m length within a row in each plot at initiation of tillering (14 DAS), panicle initiation (42 DAS), 50% flowering stage (70 DAS) and physiological maturity (108 DAS) to determine aboveground biomass and green leaf area index (LAI). Green leaves were separated and area was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska, USA). The LAI was expressed as leaf area per unit of area sampled for each plot. Dry weights of each plant part were determined after oven-drying at 80° C to calculate crop growth rate (CGR), net biomass assimilation rate (NAR), and leaf area duration (LAD) ^{63,64} for vegetative (up to 41 DAS), reproductive (42-69 DAS) and grain filling (70-108 DAS) stage using following equations:

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \quad (5)$$

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1} \quad (6)$$

$$LAD = \frac{L_2 + L_1}{2} \times (t_2 - t_1) \quad (7)$$

where W_1 and W_2 were dry weights of aerial plant parts per unit land area at time t_1 and t_2 , respectively; L_1 and L_2 were total leaf area of plants per unit land area at time t_1 and t_2 respectively.

Biochemical analysis of seedlings. Four random rice seedlings from each plot were collected on 18 DAS to determine total soluble phenolic compounds ⁶⁵, photosynthetic pigments (chlorophyll “a” and “b”) ⁶⁶, and soluble proteins ⁶⁷.

Nutrient uptake. Plant samples were collected at harvest, oven-dried, and ground. The N content on dry weight basis was estimated by Micro-Kjeldahl method ⁶⁸, P content by colorimeter method using vanadomolybdate yellow ⁶⁸, and K content by flame photometer ⁶⁹. Zinc was analyzed through diacid extract using Perkin-Elmer Atomic Absorption Spectrophotometer. Boron was estimated by dry ashing and azomethine-H methods ⁷⁰, and silica was estimated by blue silicomolybdic acid method ⁷¹. The nutrient uptake by grain and straw was expressed on per hectare basis ⁷².

Yield and yield components. Grain and straw yields were determined at physiological maturity. Harvest index was calculated as the ratio of dry grain yield to total dry biomass yield (both oven-dried at 70°C). Tiller and panicle densities were determined with a quadrat (0.4 m x 0.5 m) placed randomly in each plot at two locations. At the same time, five plants were selected randomly from each plot to measure the number of filled grains panicle⁻¹ and 1000-grain weight ⁷³.

Statistical analyses. All data from pot and field experiments were analysed using a mixed ANOVA model in SAS, considering year and treatment, and their two-way interactions as factors. Treatment adjusted means were separated by using HSD Tukey multiple range test at 5% level of significance (Wang et al., 2017). The coefficient of determination (R^2) and correlogram were calculated to assess the degree of association between two variables using the JMP Pro 16.

Results

Pot Experiment

Seed germination emergence and vigour, and enzymatic and biochemical activities. Seed priming improved gemination and vigour attributes, although year-to-year differences were evident (Supplementary Table S1). Priming with either Se or ZnO-NPs alone or in combination reduced the time to start of emergence (TSE), time to reach 50% germination (E_{50}) and mean emergence time (MET) compared to the control, while EI and VI parameters improved over the control (Table 1). Percent reduction in TSE (43.3), MET (25.5) and E_{50} (30.9), and percent increase in EI (66.4) and VI (112.3) were greater with the combined application of ZnO-NPs, selenate and selenite. Even selenite and ZnO-NPs combination favoured the germination, as evidenced in germination parameters except for the TSE.

A minimum leachate conductivity of seeds was recorded in the combination of all three priming agents (Supplementary Fig. S2). Selenate was more effective in increasing antioxidant enzyme activities compared to selenate, and the priming through the combination of selenite, selenate ZnO-NPs resulted in improved biochemical activities in DSR seeds (Supplementary Table S2).

Field experiment

Chlorophyll, phenol and soluble protein content in rice seedling. Total chlorophyll, phenol and soluble protein contents in rice seedlings increased through priming of seeds over the control (Table 2). The combination of selenite, selenate and ZnO-NPs recorded the highest total chlorophyll (5.39 mg g⁻¹), phenol (0.58 mg g⁻¹) and soluble protein (0.37 mg g⁻¹) contents, although comparable with ZnO-NPs+selenate treatment (chlorophyll, phenol and soluble protein were 5.27, 0.57 and 0.35 mg g⁻¹).

Plant growth analysis. All priming treatments either singly or in combination, affected chlorophyll, phenolics and soluble protein contents of seedlings, growth behaviour, nutrient uptake of plants and crop yields at harvest (Supplementary Table S3). Year-to-year variation was also noticed in parameters with a few exceptions (soluble proteins, crop growth rate at vegetative stage and net assimilation rate at ripening stage). However, year-treatment interactions were mostly non-significant. The impact of priming of seeds is manifest in plant LAI (Fig. 1). The highest LAI was observed with ZnO-NPs-sodium selenite-sodium selenate combination in all stages - 1.66 (early tillering), 3.50 (panicle initiation), 5.21 (early grain filling) and 3.18 (physiological maturity); values were 81%, 93% 56%, and 55% higher, respectively over the control.

Seed priming impacted other physiological parameters like leaf area duration (LAD) (Fig. 2), crop growth rate (CGR) (Fig. 3) and net biomass assimilation rate (NAR) (Fig. 4) during early tillering to panicle initiation stage (14-42 DAS), panicle initiation to completion of pollination (42-70 DAS) and entire grain filling stage (72 DAS-108 DAS).

The impact of selenate on LAI and LAD was always greater than that of selenite, and the combined selenite-selenate was even more effective. A similar trend was recorded for CGR except during 42-70 DAS where selenate alone and with selenite had a comparable effect.

Seed priming by ZnO-NPs alone or with selenite did not bring a change in either LAI or LAD, although the effect was greater with selenate and was the best when combined with selenite and selenate. Likewise, a combination of ZnO-NPs with selenite and selenate together had the best priming effect on CGR. Overall, improved LAI and LAD with seed priming contributed 9-55% higher crop growth rate and 34-109% higher net assimilation rate in comparison to control. The NAR showed a mixed trend, with all treatments behaving similarly in 14-42 DAS although all three agents in combination showed less NAR compared to single ZnO-NPs. During 42-70 DAS, ZnO-NPs alone or in combination with both selenite and selenate recorded lower NAR, however, all treatments had a similar effect during 70-108, but higher than the control.

Nutrient uptake by plants. Nutrient uptake (153 kg N ha⁻¹, 48.6 kg P ha⁻¹, 78.7 kg K ha⁻¹, 24.6 kg B ha⁻¹, 321 g Zn ha⁻¹, and 188 kg Si ha⁻¹) was the highest with the combined use of selenite, selenate and ZnO-NPs as priming agents (Table 3). The efficacy of selenate was greater in harnessing soil nutrients compared to either selenite or ZnO-NPs.

Yield attributes, grain and straw yields. Seed priming improved the grain yield in DSR due to an increase in panicle density, grains per panicle and grain-filling (Table 4). Considering seed-priming effects, grain yield ranged from 3.91 t ha⁻¹ in the second year with hydropriming treatment to 6.15 t ha⁻¹ in the first year with combined application of ZnO-NPs, selenite and selenate. Yields were significantly lower in YII than in YI. Straw yields followed a similar pattern. Application of selenite and selenate with ZnO-NPs registered a 25.9% higher grain yield over the control, compared to 11-13% increase with application of ZnO-NPs with either selenite or selenate. Yield gains in treatment with the combination of all three priming agents could be ascribed to 12.0, 47.1 and 27.8 % increases in panicles, filled grains per panicle, and

grain filling percentage respectively over the control. Combined application of ZnO-NPS and selenite or ZnO-NPS and selenate as priming agents also resulted in 7.6-8.7, 32.8-32.9, 22.2-23.6% higher panicles, filled grains per panicle and grain filling efficiency respectively over control. However, sole use of selenite, selenate and ZnO-NPs also contributed 5.5, 6.2 and 5.0% higher grain yield over hydro priming.

Discussion

Seed priming by zinc oxide nanoparticles, Na-selenite and selenate either singly or in combinations, facilitated speedy germination and early vigour of seedlings in direct-seeded rice. Priming reduced time to start of emergence as well as time to reach 50% germination. Primed seeds have shown low MET, a measure of the rate and time-spread of germination⁷⁵, and enhanced seed emergence and vigour indices. There were increases in enzymatic antioxidant functions in seeds necessary to reduce the production of degenerative radicals and therefore, seeds would likely be protected from damage during environmental (growing condition) stress. Consequently, lengths of seedlings (root and shoot, on 18 DAS) largely increased (Supplementary Figure S3). All enzyme activities were positively correlated with seed vigour indices and the seedling growth following seed priming (Supplementary Figure S4). Sodium selenate had a clear advantage over sodium selenite when combined with zinc oxide nanoparticles. However, the combination of all three had the maximum impact.

Early vigour of seeds and seedlings is the most desirable trait in field crops to enhance water and nutrient uptake by plants and to provide competitiveness against biotic and abiotic stresses^{3,76}. Due to chemical priming of seeds, plants used soil nutrients (N, P, K, B, Zn, Si) more efficiently resulting in higher density of grains per panicle, and finally leading to higher yields in DSR compared to grain imbibition with pure water. Selenate improved rice seedling

germination and growth, and soluble carbohydrate and protein contents in plants compared to hydropriming ⁴⁷. The ZnO nano particles had similar positive effects on seed germination, seedling growth and dry biomass in rice ⁷⁷ and wheat ⁷⁸. The Zn content in primed rice seeds was several times higher compared to hydropriming ⁷⁷. The success with Zn nanoparticles may encourage future research on other micronutrients as priming agents ⁷⁷.

Chlorophyll is associated with photosynthetic activity, and hence it is an indicator of vegetative growth and vigour of a plant. Phenolics are secondary metabolites, which promote the adaptation capability of plants during stress. Soluble proteins have diverse roles in promoting growth including osmoregulation in plants under adverse growth environments ⁷⁹. A combination of ZnO nanoparticles, Na-selenite and Na-selenate priming had the best impact on chlorophyll, phenol and soluble protein contents in rice seedlings, resulting in higher leaf area index and duration, and crop growth rate. A combination of sodium selenite and selenate was more effective in promoting the growth of plants (higher leaf area index, leaf area duration and crop growth rate) compared to when these were used singly or even when seeds were primed by nano-zinc formulation only. However, the combination of both selenite and selenate with nano-zinc induced the largest changes in growth parameters. These led to higher yield-contributing factors such as grain-filling efficiency, test weight and harvest index. The highest DSR yield was, however, obtained with a combination of the three seed priming materials.

Our results established that combinations of Na-selenite, Na-selenate and zinc oxide nanoparticles were potential seed invigoration techniques, and the best results were obtained with all three chemicals in combination. Responses differed between years; however, positive impacts on the field crop were established. Seeds with high vigour are proxy of sustainable productivity, especially under adverse conditions, although suitable field studies are lacking. Uniform seed emergence, crop stand and establishment are major challenges for successful

DSR cultivation ¹⁰. Direct-seeded rice often fails to emerge uniformly due to uneven land preparation leading to imprecise water management, and therefore weed infestations take place heavily ⁸⁰. On the other side, low oxygen availability in rainfed lowlands owing again to poor water management, seeds fail to germinate ⁸¹. Priming of DSR seeds can give necessary vigour to the plants to sustain better under unfavourable growing conditions. The modified traits in seeds effectively translate into early seedling vigour and agility in growing plants, which is perfectly tuned with increased productivity in the field-grown rice. This small intervention (seed priming) will support the rice growers to embrace direct seeding of rice as a replacement of water- and labour-intensive puddled transplanted rice. Farmers will also get an opportunity to widen the window of sowing of wheat following rice in large tracts of rice-wheat rotation in Indo-Gangetic Plains of South Asia.

Conclusion

Both Zn and Se have found their effectiveness in seed-priming. It was observed that zinc oxide nanoparticles with both forms of Se were synergistic in their action, and provided further benefits compared to their single applications. The impact was evaluated in direct-seeded rice production often exposed to adverse growing conditions and therefore, not realizing full potential productivity. Priming of seeds with zinc oxide-sodium selenite selenate combination resulted in an early emergence due to more seed vigour, and extended to the improvement in plant growth and productivity in DSR in the field. This has implications in the vast rice-growing areas in South Asia in general, and to the rice-wheat system of Indo-Gangetic Plains of South Asia in particular.

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Table 1 Effect of seed priming on seedling emergence and seedling vigour of DSR (pooled over 2019 and 2020)

Treatment	TSE (days)	E ₅₀ (days)	MET (days)	EI	VI
	-----days-----				
T ₁	3.8a	5.8a	6.19a	13.5f	969f
T ₂	3.2b	5.1bc	5.26bc	17.5e	1290e
T ₃	3.3b	4.9bc	5.06bc	20.1c	1519d
T ₄	3.3b	4.9bcd	5.15bc	17.6e	1590cd
T ₅	3.2b	4.7cd	5.49b	18.1de	1221e
T ₆	2.7c	4.7de	4.96bc	19.4cd	1643c
T ₇	2.7c	4.4e	4.72c	20.4bc	1796b
T ₈	2.2d	4.0f	4.61c	22.4a	2057a

TSE - time to start emergence, E₅₀ - time taken to reach to 50% emergence, MET - mean emergence time, EI - emergence index, VI - Vigour index

T₁ - hydro priming or control, T₂ - sodium selenite at 50 µmol, T₃ - sodium selenate at 50 µmol, T₄ - sodium selenite at 50 µmol + sodium selenate at 50 µmol, T₅ - ZnO-NPs at 10 µmol, T₆ - ZnO-NPs at 10 µmol + sodium selenite at 50 µM, T₇ - ZnO-NPs at 10 µM + sodium selenate at 50 µM, T₈ - ZnO-NPs 10 µM + sodium selenite at 50 µM + sodium selenate at 50µMI⁻¹

Means not sharing a letter in common differ significantly at 5% probability level by HSD Tukey's test.

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Table 2 Effect of seed priming on total chlorophyll, phenolics and soluble protein contents (mg g⁻¹ fresh weight) in direct-seeded rice seedlings

Treatment	Total chlorophyll	Total phenolics	Soluble protein
Year			
YI	4.56n	0.55g	0.28k
YII	4.49o	0.54g	0.28k
Treatment			
T1	5.40h	0.50f	0.17j
T2	5.28h	0.52ef	0.25i
T3	4.68i	0.54def	0.30gh
T4	4.48j	0.56de	0.31gh
T5	4.26k	0.55de	0.25i
T6	4.17k	0.54def	0.26hi
T7	4.09l	0.56de	0.35fg
T8	3.82m	0.58d	0.36f
Year × Treatment			
YIT1	3.84g	0.50c	0.17e
YIT2	4.19ef	0.53abc	0.26bcd
YIT3	4.46cd	0.54abc	0.30abc
YIT4	4.75b	0.56abc	0.30abc
YIT5	4.19ef	0.58ab	0.25cde
YIT6	4.28de	0.55abc	0.26bcd
YIT7	5.32a	0.56abc	0.36a
YIT8	5.42a	0.59a	0.36a
YIIT1	3.81g	0.50c	0.18de
YIIT2	3.98fg	0.51bc	0.24cde
YIIT3	4.50cd	0.54abc	0.31abc
YIIT4	4.61bc	0.55abc	0.31abc
YIIT5	4.14ef	0.52abc	0.25cde
YIIT6	4.25e	0.53abc	0.27bc
YIIT7	5.24a	0.56abc	0.34ab
YIIT8	5.38a	0.57abc	0.36a

Means followed by different letters (Tukey ranking) differ significantly at 5% level of significance. Treatments details are given in Table 1.

Table 3 Effect of seed priming on total plant uptake (kg ha⁻¹) of primary nutrients and micronutrients by direct-seeded rice

Treatment	N	P	K	B	Zn	Si
Year						
YI	131o	38.4k	68.8l	21.1m	276k	162m
YII	127p	37.4l	66.7m	20.4n	266l	157n
Treatment						
T1	109n	28.9j	53.8k	15.8k	206j	122l
T2	119m	36.4h	64.2j	19.9i	260h	153j
T3	128l	38.4h	68.5ij	21i	275h	162j
T4	125lm	36.5h	66.9ij	10i	261h	154j
T5	118m	31.7i	67.3ij	17.4j	227i	134k
T6	137k	42.5g	69i	23.2h	304g	178i
T7	141k	43.8fg	73.6h	24.0g	313g	184hi
T8	153j	44.9f	78.7g	24.6g	321g	188h
Year × Treatment						
YIT1	121fg	29.2e	56.6ef	16.0f	208f	124fg
YIT2	97i	36.8c	51.0f	20.2d	264d	155d
YIT3	127efg	38.9bc	69.2cd	21.3cd	279cd	120g
YIT4	129def	37.3c	70.0cd	20.5d	268d	165bcd
YIT5	130def	32.1de	78.7a	17.7ef	231ef	158d
YIT6	132def	43.1a	63.4de	23.6ab	310ab	137ef
YIT7	136cde	44.5a	70.1bcd	24.5ab	318ab	180ab
YIT8	156a	45.6a	78.5a	25.1a	327a	187a
YIIT1	116gh	28.6e	64.2d	15.6f	204f	191a
YIIT2	122fg	36.0cd	64.2d	19.6de	256de	151de
YIIT3	129def	38.0bc	67.8cd	20.7cd	271cd	159cd
YIIT4	121fg	35.7cd	63.8d	19.5de	254de	150de
YIIT5	106hi	31.3e	55.9f	17.1f	223f	131fg
YIIT6	142bcd	41.9ab	74.7abc	22.8bc	298bc	176abc
YIIT7	146abc	43.2a	77.1ab	23.5ab	308ab	181ab
YIIT8	150ab	44.2a	78.9a	24.1ab	315ab	185a

Means followed by different letters (Tukey ranking) differ significantly at 5% level of significance. Treatments detail in Table 1.

Table 4 Effect of seed priming on yield and yield components of direct-seeded rice in a field experiment during 2019 and 2020

Treatment	Panicles m ⁻²	Filled grain panicle ⁻¹	Grain filling percentage	Test weight (g)	Grain yield (t ha ⁻¹)	Straw yield (t ha ⁻¹)	Harvest index (%)
Year							
YI	174j	114k	85g	20.24	4.53k	5.81j	43.8i
YII	165i	102j	86g	20.22	4.22j	5.57i	43.1i
Treatment							
T1	184h	85i	72f	20.10	4.01i	5.70g	41.3h
T2	188gh	105h	84e	20.40	4.23h	5.68g	42.6gh
T3	192g	110fg	88e	20.10	4.26h	5.66g	42.9fgh
T4	192g	110fg	87e	20.20	4.32h	5.68g	43.2fgh
T5	188gh	103g	83e	20.50	4.21h	5.62gh	42.8gh
T6	200ef	113f	88e	20.10	4.53g	5.80fg	43.9efg
T7	198f	113f	89e	20.17	4.45g	5.46h	44.9ef
T8	206e	125e	92d	20.28	5.05f	5.96f	45.9e
Year × Treatment							
YIT1	189d	88d	68c	20.13	4.10e	5.70e	40.8d
YIT2	191c	112b	85b	20.45	4.37cd	5.81c	42.4bcd
YIT3	196c	118b	90a	20.11	4.41c	5.75c	42.4bcd
YIT4	198bc	109bc	85b	20.17	4.49c	5.91b	43.2abcd
YIT5	194c	115b	84b	20.49	4.36c	5.66d	42.1bcd
YIT6	205ab	119b	91a	20.04	4.70b	5.92b	43.5abcd
YIT7	202ab	117b	88b	20.07	4.62b	5.59b	44.5abc
YIT8	209a	130a	91a	20.45	5.22a	6.15a	45.8a
YIIT1	179d	82d	76c	20.07	3.91e	5.68c	41.8cd
YIIT2	185c	98c	84b	20.35	4.08cd	5.55b	42.9bcd
YIIT3	188c	101bc	86b	20.09	4.10c	5.57b	43.4abcd
YIIT4	186c	112b	88b	20.23	4.14c	5.44b	43.2abcd
YIIT5	182c	90c	82b	20.51	4.05d	5.57b	43.5abcd
YIIT6	195b	106b	85b	20.16	4.36b	5.67b	44.3abc
YIIT7	194b	109b	90a	20.27	4.27b	5.32b	45.3ab
YIIT8	203a	121a	93a	20.11	4.87a	5.77a	45.9a

Within a column, means followed by the same letter are not different at the 0.05 level of probability. Treatments detail in Table 1.

Figure 1 Leaf area index of direct-seeded rice plants during vegetative (A, D), reproductive (B, E), and ripening (C, F) stages as affected by seed priming treatments in 2019 (A, B, C) and 2020 (D, E, F). Treatments with different letters (top of bars) represent significant differences ($P<0.05$) between treatments. Vertical lines with caps are $\pm$ standard error of the mean. Treatments details are given in Table 1

Figure 2 Leaf area duration of direct-seeded rice plants during vegetative (A,D), reproductive (B, E), and ripening (C, F) stages as affected by seed priming in 2019 (A, B, C) and 2020 (D, E, F) Treatment bars with different letters represent significant differences ($P < 0.05$) between means. Vertical lines with caps are $\pm$ standard error of the mean of treatments. Treatments details are given in Table 1

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Figure 3 Crop growth rate of direct-seeded rice during vegetative (A, D), reproductive (B, E), and ripening (C, F) stages as affected by seed priming in 2019 (A, B, C) and 2020 (D, E, F). Treatment bars with different letters represent significant differences ($P < 0.05$) between means. Vertical lines with caps are $\pm$ standard error of the mean of treatments. Treatments details are given in Table 1.

Figure 4 Net biomass assimilation rate by direct-seeded rice plants during vegetative (A, D), reproductive (B, E), and ripening (C, F) stages as affected by seed priming in 2019 (A, B, C) and 2020 (D, E, F). Treatment bars with different letters represent significant differences ($P < 0.05$) between means. Vertical lines with caps are $\pm$ standard error of the mean of treatments. Treatments details are given in Table 1.

706 **Declaration of interests**

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708 The authors declare that they have no known competing financial interests or personal

709 relationships that could have appeared to influence the work reported in this paper.

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711 The authors declare the following financial interests/personal relationships which may be

712 considered as potential competing interests.

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714 Authors have necessary permissions or licenses to collect plant/seed used in this study.

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