sustainability

MDPI

Article

Seed Coating in Direct Seeded Rice: An Innovative and Sustainable Approach to Enhance Grain Yield and Weed Management under Submerged Conditions

Talha Javed [1,2], Irfan Afzal [1,*] and Rosario Paolo Mauro [3,*]

[1] Seed Physiology Lab, Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan; talhajaved54321@gmail.com
[2] College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[3] Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Università degli Studi di Catania, Via Valdisavoia, 5-95123 Catania, Italy
* Correspondence: iafzal@uaf.edu.pk (I.A.); rosario.mauro@unict.it (R.P.M.)

Abstract: Dry direct-seeded rice is an alternative cropping technique that should require less water and labor than the classical method of transplanted-flooded rice. Weed competition is the major biological constraint in this resource-conserving production technique reducing the crop yield by 30–80%. This study evaluated the effects of different seed coating treatments on the performance of dry direct seeded rice under field conditions. The seed coating treatments used were preliminarily optimized under lab conditions. The rice seeds were coated with sodium lauryl sulphate (20:1), calcium peroxide (CaO_2) (20:6), alginate (20:6), and plant growth promoting bacteria *Bacillus* sp. KS-54 (20:6 g:mL) on a dry weight basis. Among treatments, seed coating with CaO_2 resulted in higher field emergence (85%) and suppressed the fresh and dry biomass of weeds at 15 and 35 days after sowing which subsequently improved the seedling growth of direct seeded rice followed by other treatments and the control. Rice seeds coated with CaO_2 and *Bacillus* sp. KS-54 were effective at enhancing morphological, yield and yield related attributes as compared to other treatments and the control under field conditions. The better morphological attributes and yield of rice plants raised from seeds coated with CaO_2 and *Bacillus* sp. KS-54 were associated with higher concentrations of reducing sugars and enhanced antioxidant enzymes activities.

Keywords: direct seeded rice; seed coating; weed dynamics; sustainable production

check for updates

Citation: Javed, T.; Afzal, I.; Mauro, R.P. Seed Coating in Direct Seeded Rice: An Innovative and Sustainable Approach to Enhance Grain Yield and Weed Management under Submerged Conditions. *Sustainability* **2021**, *13*, 2190. https://doi.org/10.3390/su13042190

Academic Editor: Carlos M. Correia

Received: 31 December 2020
Accepted: 9 February 2021
Published: 18 February 2021

1. Introduction

Rice (*Oryza sativa* L.) is a dominant food crop for more than half of the global population [1], while it is a principal source of livelihood for the majority of the population in Asia and Africa [2]. Increasing water scarcity, rising production costs and labor shortage are threatening food production in conventional transplanted rice system resulting in drastic shift to direct seeding of rice [3]. Dry direct seeding of rice (DSR) involves dry-seeding onto well prepared seedbeds at a field capacity level [4]. However, direct seeded crop is detrimental due to severe weed infestations, especially during the seedling establishment period which subsequently reduces crop growth and yields in this system [5].

Due to planting without the use of flooding, direct seeded crops exhibit slow growth compared to weeds, therefore, improving early seedling growth can reduce the crop–weed competition [6]. Early vigor can be achieved through growing cultivars which have better competitive ability or through agronomic ways. Among the different approaches, seed enhancements including certain pre-sowing treatments have been successfully employed in order to improve seed quality and overcome seed related issues (biotic and abiotic stressors) both during and after emergence [7,8]. These seed enhancements techniques produce healthy crop stand, additionally improve the growth and physio-morphological attributes of the plant [9]. Among these techniques, seed coating is one of the most promising methods aimed at improving seed quality in terms of vigor and field performance through the use of appropriate coating agents applied at suitable concentrations [10]. Seed coating reduces the time required for the accomplishment of germination phases and promotes the better survival of seedlings under unfavorable environmental conditions [11]. In addition, early flooding conditions especially during the critical hours of weed–crop competition can be effective in managing weeds with a lower/eliminated risk of decreased productivity. However this requires seedling survival with rapid crop development [3].

Calcium peroxide contains peroxide molecules, which when as a seed coating agent have been observed to be useful for improving the emergence of seedlings due to its higher water holding capacity and ability to promote early accomplishment of the imbibition phase during seed germination [12]. Healthy, uniform and vigorous crop establishment has been reported with seeds coated with calcium peroxide in rice under flooded conditions attributed to improved biochemical attributes [13]. Beneficial microbes are reported to play a significant role in improving the performance of agricultural crops [10], therefore, coating seeds with rhizobia improves the ability of plants to obtain essential nutrients both directly through symbiotic association and indirectly from the soil [14]. In addition, sodium lauryl sulphate [15] and alginate [16] have been reported to promote crop stand establishment and seeding growth especially under water deficit conditions, due to their higher water holding capacity. Furthermore, using these compounds and microbes could be helpful in improving the competitive ability of crops against weeds, under submerged conditions during the early stages of crop growth in direct seeded rice [17].

Indeed, previously very little information was available to evaluate the potential of seed coating using different chemicals and plant growth promoting bacteria, in conjunction with the early submerged condition in direct seeded rice. Therefore, it was hypothesized that seed coating will restrict weed growth and improve direct seeded rice performance by using low cost coating agents under submerged conditions.

2. Materials and Methods

2.1. Seed Source

Seeds of widely grown Indica rice (*Oryza sativa* L.) cultivar Basmati-515 were obtained from Engro Corporation Farm, Saikhum, Sheikhupura, Punjab, Pakistan (30.37° N, 69.34° E). The initial germination of the cultivar was >80% and the initial seed moisture content was 11% on a wet weight basis. All the seeds used in experiment were selected from the same seed lot.

2.2. Seed Coating Treatments

Rice seeds were coated with four different widely used commercial coating agents (calcium peroxide, *Bacillus* sp. KS-54, alginate, and sodium lauryl sulphate). For lab screening, the ratios of seed to each coating agent were 20:1, 20:3, 20:6, and 20:9 on dry weight basis (g), except *Bacillus* sp. KS-54 (g:mL). For field evaluation, based on preliminary emergence results under laboratory conditions, the ratio of seed to sodium lauryl sulphate (20:1), calcium peroxide (CaO_2) (20:6), alginate (20:6), and *Bacillus* sp. KS-54 was kept at 20:6 (g:mL) on a dry weight (g) basis for seed coating. Moreover, *Bacillus* spp. (KS-54) was applied in liquid form to seeds during the process of seed coating. Noncoated rice seeds were used as a control. For seed coating, a semiauto Rotary Seed Coater (Model R310, Rhino Research Thailand) was used. For seed coating treatment, rice seeds were introduced from inlet in the coating pan having width, length and height 820 × 1170 × 1500 mm, respectively, followed by the application of coating agent. Arabic gum was used as a binder to attach the coating agent with seeds. After seed coating, the seeds were extracted from the seed coating machine through outlet and were transferred to oven (air drying) at 25 °C for two days to reduce seed moisture contents to 11%.

2.3. Lab Screening

To screen the best coating ratio, the lab experiment was conducted at Seed Physiology Lab, University of Agriculture, Faisalabad, Pakistan. The plastic pots were filled with 6 kg of dried soil collected from the field experiment site. After filling, the soil was irrigated till filed capacity. Ten rows of rice seeds (each row had 10 seeds) were sown in each pot. Completely randomized design having eight replications was used. Pots were placed in a growth chamber maintained at 25 °C temperature and 65% relative humidity during the course of the lab study. Emergence of seeds was recorded on a daily basis and was expressed as percentage at 10 days after sowing (DAS) [18].

2.4. Crop Husbandry

Field experiment was carried out at Engro Corporation Farm, Saikhum Village, Sheikhupura District, Punjab Province, Pakistan (30.37° N, 69.34° E) from June 2018 to October 2018. The textural class of the soil was clay loam having pH 7.7 and EC_e 2.19 dS m^{-1} at the depth of 0–15 cm. To create submerged conditions during 15–35 days of crop growth period, the field was flooded and puddled prior to sowing. Excess water was drained out to perform sowing at field capacity. The experiment was laid out in Randomized Complete Block Design with three replications. For each treatment, net plot size was 6 m^2 (3 × 2 m) with 12 rows in each experimental unit. Coated and uncoated seeds were drilled manually using hand seed drill at 25 cm row to row distance. Fertilizer NPK at 120, 90 and 60 kg ha^{-1} as Urea, Diammonium phosphate (DAP) and Sulphate of Potash (SOP) were mixed and side drilled along seeding rows. Half dose of nitrogen was applied at sowing time while the remaining nitrogen was top dressed in first irrigation while phosphorus and potassium were applied during sowing.

2.5. Stand Establishment

Daily emergence of rice seeds was recorded in the field according to the seedling evaluation Handbook of Association of Official Seed Analysis [19]. Mean emergence time was computed by the equation given by Ellis and Roberts [20].

$$\text{Mean Emergence Time (MET)} = \frac{\sum \text{Dn}}{\sum \text{n}}$$

where n is the number of seeds which emerged on day D, and D is the number of days counted from the beginning of emergence.

According to formula suggested by Association of Official Seed Analysis [19], emergence index was determined.

$$\text{Emergence Index (EI)} = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

2.6. Rice Seedling Growth and Weed Dynamics

Weeds were harvested from an area of 1ft^2 at 15 and 35 DAS. The fresh and dry weights (g) of weeds were determined by using weighing balance (Uni Block AUX220, Shimadzu Corporation, Kyoto, Japan). At 35 DAS, five uniform seedlings were randomly selected from each experimental unit to measure the fresh and dry weights. For measuring dry weight, the seedlings were placed in an oven at 80 °C for 24 h [21]. Fresh and dry weights were averaged to get mean values for a seedling.

2.7. Morphological and Yield Related Attributes

At physiological maturity, plant height and flag leaf length of five randomly selected plants from each experimental unit were determined and averaged. Stem diameter was measured by using a Vernier caliper (Vorel 15240, Karachi, Pakistan). Panicle length, number of spikelet's per panicle, number of grains per panicle, 1000-grain weight and grain yield from 3 m^2 were measured after harvesting the crop.

2.8. Biochemical Assays

The reducing sugars were measured by dinitro-salicylic acid (DNS) method from the powdered seed sample (0.1 mg) extracted in 5 mL sodium phosphate buffer (pH 6.9). The tubes containing extracted mixtures were placed at room temperature to allow the impurities to settle down. Then the supernatant was obtained with the help of a pipette, 1.5 mL DNS solution was added in that followed by boiling for 5 min at 100 °C. About 1 mL of 1% sodium potassium tartrate solution was added in the mixture and reading was measured at 510 nm wavelength by using a spectrophotometer (T60 U, Spectrophotometer, PG Instruments Ltd. Leicestershire, UK) [22].

For determination of antioxidant enzymes, about 1g sample of fresh leaves from each experimental unit was grinded in 16 mL of phosphate buffer. After grinding 0.4 g quartz sand was added to it and homogenized. Centrifugation of sample was performed for 20 min at 4 °C. After centrifugation, supernatant (enzyme extract) was obtained using a pipette. To measure superoxide dismutase (SOD) activity, the reaction solution (3 mL) contained 50 µL enzyme extract, 50 µM nitroblue tetrazolium (NBT), 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA, and 50 mM phosphate buffer (pH 7.8). The reaction solution was kept under illumination of fluorescent lamp for 15 min. By using a spectrophotometer (T60 U, Spectrophotometer, PG Instruments Ltd. UK) absorbance was measured at 560 nm wavelength [23]. The catalase (CAT) reaction solution (3 mL) contained 0.1 mL enzyme extract, 50 mM phosphate buffer (pH 7.0), and 15 mM H_2O_2. Whereas, peroxidase (POD) reaction solution (3 mL) contained 50 mM sodium acetate buffer (pH 5.0), enzyme extract (0.1 mL), and 40 mM H_2O_2. The absorbance for CAT and POD was measured at 240 and 470 nm, respectively, by using a spectrophotometer (T60 U, Spectrophotometer, PG Instruments Ltd., Leicestershire, UK) [24].

2.9. Statistical Analysis

Analysis of variance was performed by using a statistical tool 'Statistix-8.1' (https://www.statistix.com/ (accessed on 7 January 2019)). Least significant difference (LSD) test at 0.05 probability level was used to compare the treatment means. Data collected during the experimentation regarding different attributes were presented as the mean values ± standard errors (SE). The complete set of original data used for all tables and figures as well as the complete statistical results are available as Supplementary File.

2.10. Economic Analysis

The economic feasibility of seed coating treatments was evaluated following benefit–cost analysis. The amount of *Bacillus* sp. KS-54, sodium lauryl sulphate, CaO_2, and alginate used in the study was 3.6 L ha^{-1}, 600 g ha^{-1}, 3.6 kg ha^{-1}, and 3.6 kg ha^{-1}, respectively. The cost of KS-54, sodium lauryl sulphate, CaO_2, and alginate was Pak Rs. 300 per liter, Pak Rs. 1500 per kg, Pak Rs. 10 per kg, and Pak Rs. 1000 per kg, respectively. Moreover, the cost of polymer color and gum Arabic was Pak Rs. 1000 per liter and Pak Rs. 820 per kg, respectively. The production cost included all agronomic practices starting from land preparation up to harvesting and threshing.

3. Results

3.1. Stand Establishment

3.1.1. Preliminary Controlled Screening

Rice seeds coated with the ratio of seed to CaO_2 20:6 expressed significantly higher final germination (%) and lower mean germination time (days) followed by seed to CaO_2 20:9, 20:3, 20:1 ratios and control, respectively. Similarly, rice seeds coated with *Bacillus* sp. KS-54 (20:6) expressed significantly higher final germination and lower mean germination time compared to the rice seeds coated with 20:3, 20:9, 20:1 ratios and control, respectively. Rice seeds coated with the ratios of seed to sodium lauryl sulphate (20:1) and alginate (20:6) also expressed higher final germination (%). Overall, rice seeds coated with calcium peroxide (20:6), *Bacillus* sp. KS-54 (20:6), sodium lauryl sulphate (20:1), and alginate (20:6) expressed significantly higher final germination and lower mean germination time compared to control and other seed to coat ratios, therefore, were used under field conditions (Figure 1).

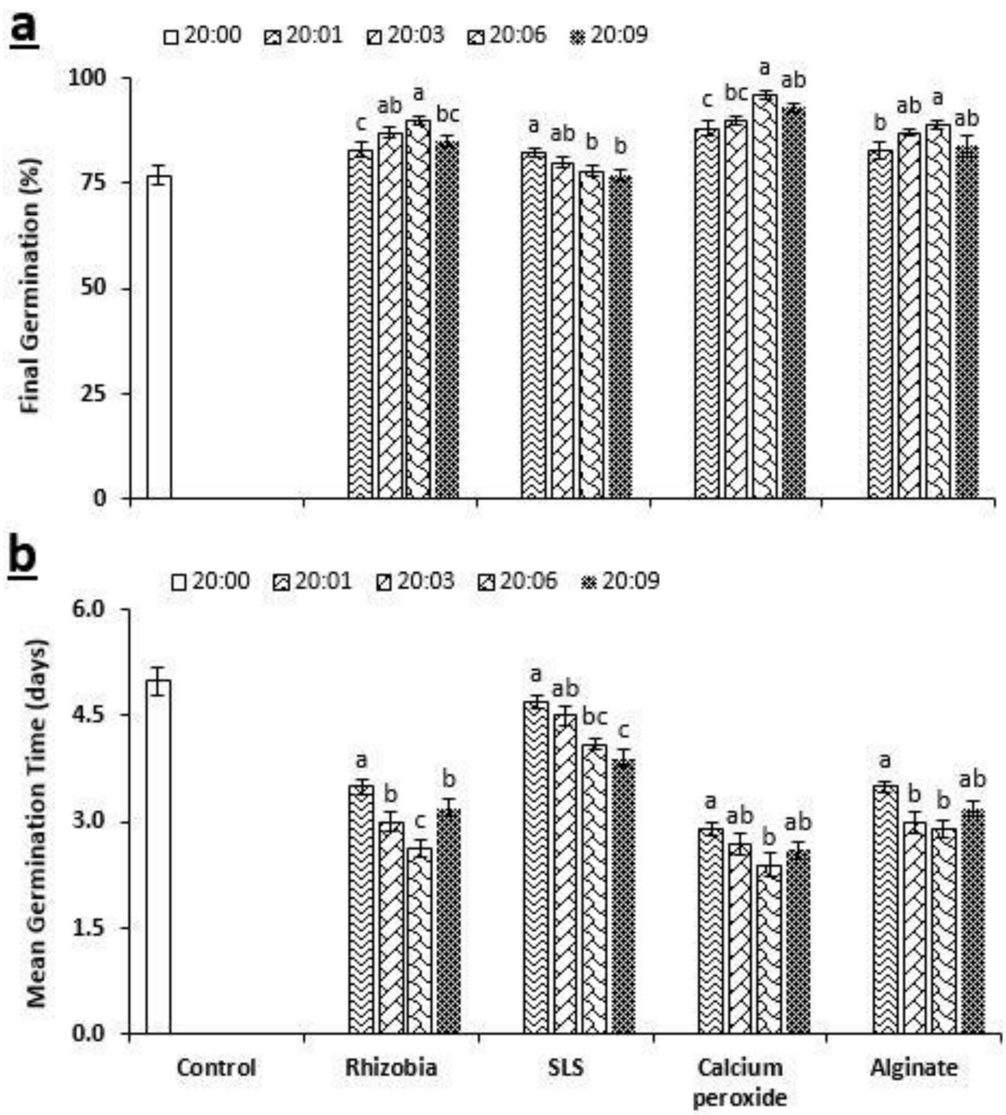

Figure 1. Germination potential of coated and non-coated rice seeds under lab conditions. (**a**): Final Germination (%); (**b**): Mean Germination Time. Values are means ± standard errors. Means followed by the same letters above vertical bars are not significantly different at 5% level of probability. SLS = sodium lauryl sulphate, Rhizobia: *Bacillus* sp. KS54.

3.1.2. Field Study

Coated seeds showed significant variation in stand establishment compared to non-coated rice seeds (Figure 2). Mean emergence time (MET) elaborates average time taken for seed emergence. Whereas, emergence index (EI) indicates the power of emergence. High value of EI represents fast and synchronized germination, while lower reveals the slow and erratic germination. Rice seeds coated with calcium peroxide and *Bacillus* sp. KS-54 showed significantly enhanced emergence and increased values of emergence index compared to noncoated seeds. Rice seeds coated with calcium peroxide also showed reduced mean emergence time. While minimum values for these traits (final emergence % and emergence index) were observed for control without seed coating in direct seeded rice.

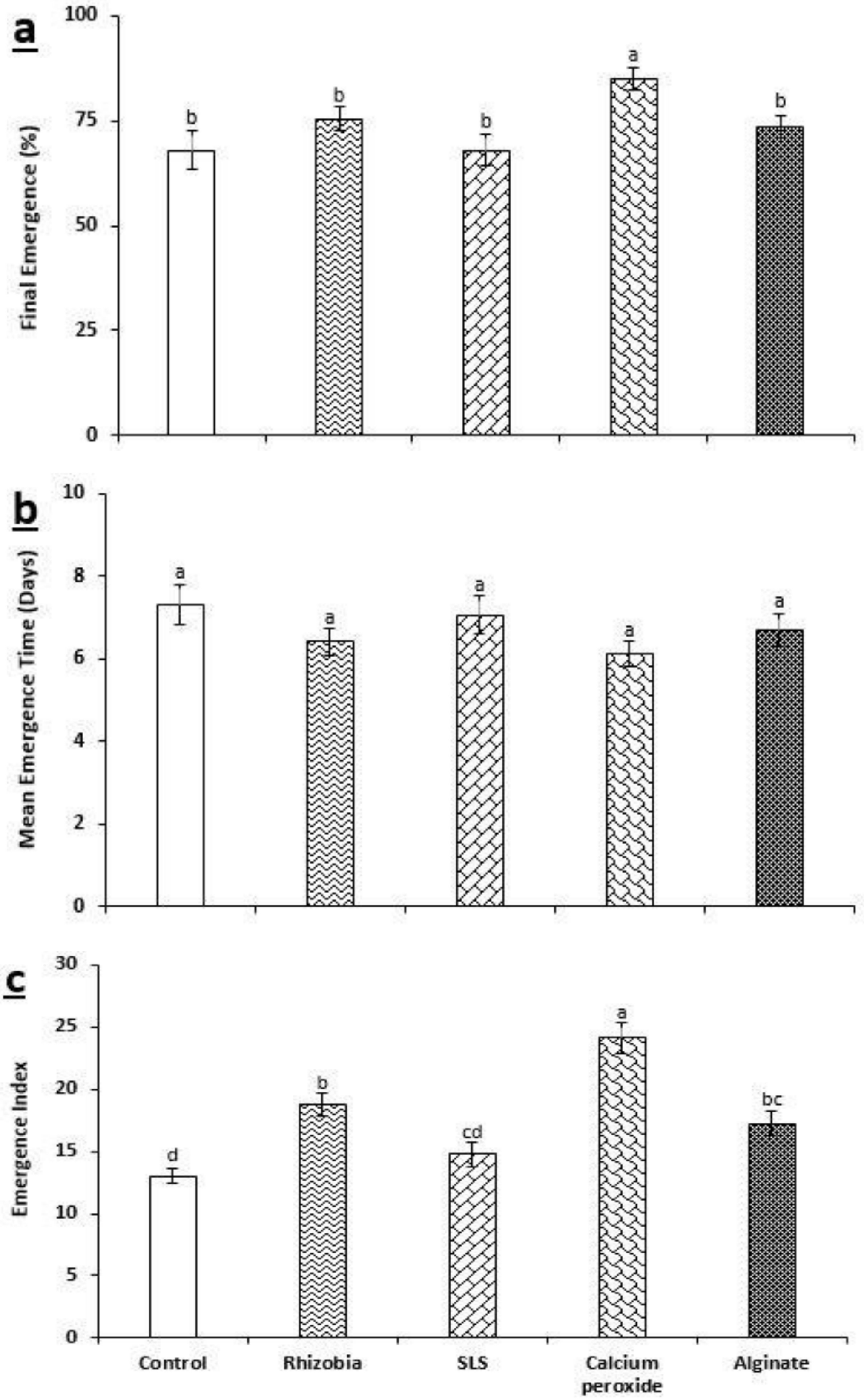

Figure 2. Emergence dynamics of coated and noncoated rice seeds during field evaluation. (**a**): Final Emergence (%); (**b**): Mean Emergence Time; (**c**): Emergence Index. Values are means ± standard errors. Means followed by the same letters above vertical bars are not significantly different at 5% level of probability. SLS = sodium lauryl sulphate, Rhizobia: *Bacillus* sp. KS54.

3.2. Weed Dynamics

Seed coating treatments effectively ($p \leq 0.05$) suppressed the weeds in direct seeded rice under submerged conditions during field evaluation (Figure 3). At 15 DAS, no difference was observed among weed fresh and dry weights for noncoated and coated seeds. At 35 DAS, seed coating with CaO_2 reduced weed fresh and dry weights in dry direct seeded condition.

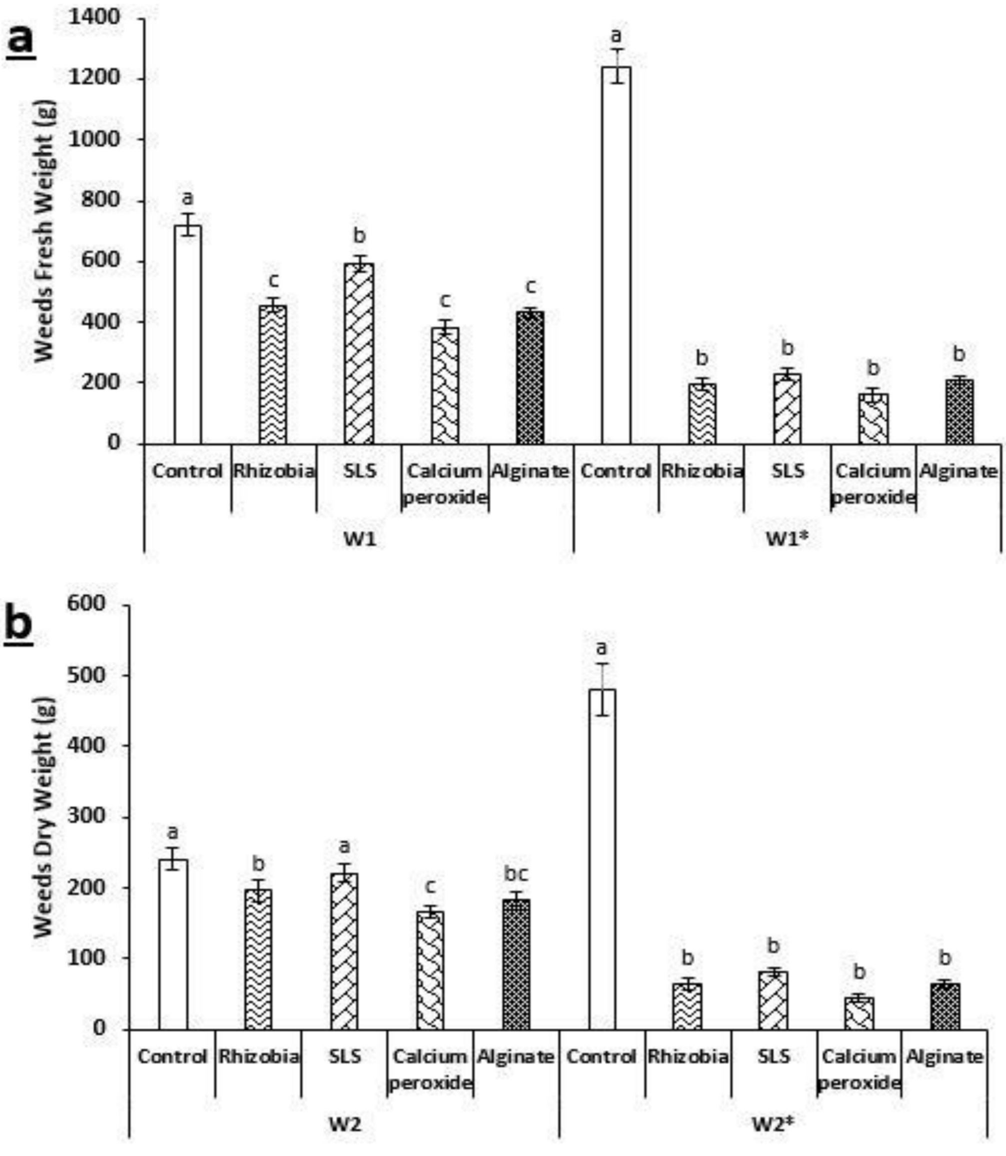

Figure 3. The effect of seed coating agents and submergence on weed fresh and dry weight in rice during field evaluation. (**a**): Weeds Fresh weight; (**b**): Weeds Dry Weight. SLS = sodium lauryl sulphate, Rhizobia: *Bacillus* sp. KS54, W1 and W2: weed fresh and dry weight before submergence at 15 DAS, W1* and W2*: weed fresh and dry weight after submergence at 35 DAS. Vertical bar represents mean of four independent determinants ± SE. Means followed by the same letters above vertical bars are not significantly different at 5% level of probability.

3.3. Seedling Growth Attributes

During field evaluation, a significant difference in seedling fresh weight was observed among seed coating treatments. Rice seeds coated with CaO_2 showed highest seedling fresh weight as compared to other coated rice seeds and the control. No significant difference was observed between *Bacillus* sp. KS-54 (1.47 g seedling^{-1}) and alginate (1.41 g seedling^{-1}) coated rice seeds for the seedling fresh weight. However, rice seeds coated with *Bacillus* sp.

KS-54 and alginate showed significantly higher seedling dry weight as compared to the control (Table 1). A similar trend was observed for seedling dry weight among coated and noncoated seeds. Rice seed coated with CaO_2 showed significantly higher plant height, stem diameter, and flag leaf length as compared to the control. No obvious difference in stem diameter and flag leaf length was found in seedlings grown from seeds coated with *Bacillus* sp. KS-54 and alginate (Table 1).

Table 1. Effect of seed coating agents on morphological attributes of direct-seeded rice during field evaluation.

Coating Treatments	Fresh Weight (g Seedling^{-1})	Dry Weight (g Seedling^{-1})	Plant Height (cm)	Stem Diameter (cm)	Flag Leaf Length (cm)
Control	1.19 ± 0.07 c	0.43 ± 0.05 d	104.9 ± 1.86 d	1.54 ± 0.03 c	38 ± 0.93 d
Bacillus sp. KS-54	1.47 ± 0.05 ab	0.66 ± 0.03 b	125.0 ± 1.72 b	1.73 ± 0.02 ab	48 ± 0.39 b
Sodium lauryl sulphate	1.32 ± 0.07 bc	0.53 ± 0.05 c	106.8 ± 1.98 d	1.57 ± 0.03 c	42 ± 0.83 c
Calcium peroxide	1.54 ± 0.03 a	0.76 ± 0.03 a	133.2 ± 1.66 a	1.83 ± 0.03 a	51 ± 0.66 a
Alginate	1.41 ± 0.06 ab	0.64 ± 0.04 b	121.5 ± 1.86 c	1.70 ± 0.02 b	46 ± 0.87 b
LSD at $p \leq 0.05$	0.0634	0.0501	0.0034	0.0098	0.0067

Means followed by the same letters are not significantly different at 5% level of probability.

3.4. Agronomic and Yield Traits and Economic Analysis

During field evaluation, seedlings grown from seeds coated with CaO_2 showed significant improvement in panicle length, panicle diameter, number of spikelets per panicle, number of grains per panicle, 1000-grain weight, and yield as compared to the control. No difference in panicle length, 1000-grain weight, and yield was observed among seedlings grown from seeds coated with sodium lauryl sulphate and noncoated control. Rice seeds coated with *Bacillus* sp. KS-54 (3.44 t/ha) and alginate (3.35 t/ha) were also effective for improving yield as compared to the control (2.89 t/ha) (Table 2). Seed coating with CaO_2 also showed higher benefit–cost ratio as compared to other coating agents including the control (Table 3).

Table 2. Effect of seed coating agents on agronomic and yield traits of direct-seeded rice during field evaluation.

Coating Treatments	Panicle Length (cm)	Panicle Diameter (cm)	Number of Spikelet's per Panicle	Number of Grains per Panicle	1000-Grain Weight (g)	Yield (t ha^{-1})
Control	25.33 ± 0.91 b	4.04 ± 0.08 d	8.48 ± 0.13 d	131.00 ± 1.32 c	19.33 ± 0.76 c	2.89 ± 0.05 c
Bacillus sp. KS-54	29.33 ± 0.78 ab	4.54 ± 0.07 b	12.44 ± 0.09 ab	170.33 ± 1.13 ab	26.33 ± 0.69 b	3.44 ± 0.04 b
Sodium lauryl sulphate	27.33 ± 0.88 ab	4.28 ± 0.08 c	10.00 ± 0.08 cd	145.67 ± 1.27 bc	22.00 ± 0.81 bc	3.17 ± 0.06 bc
Calcium peroxide	33.00 ± 0.63 a	5.11 ± 0.06 a	14.11 ± 0.07 a	193.00 ± 0.98 a	33.33 ± 0.59 a	4.16 ± 0.03 a
Alginate	28.33 ± 0.80 ab	4.54 ± 0.07 b	11.03 ± 0.09 bc	165.67 ± 1.31 ab	25.67 ± 0.77 bc	3.35 ± 0.04 bc
LSD at $p \leq 0.05$	0.2859	0.0012	0.0039	0.0173	0.0013	0.0051

Means followed by the same letters are not significantly different at 5% level of probability.

3.5. Biochemical Attributes

During field evaluation, highest values for reducing sugars were obtained in rice seeds coated with CaO_2 (4.16 mg/g) followed by *Bacillus* sp. KS-54 (3.97 mg/g) and alginate (3.62 mg/g). Significantly highest activities of CAT (28.33 unit mg^{-1} protein) and POD (190.66 unit mg^{-1} protein) were obtained in rice seedlings raised from CaO_2 coated seeds as compared to the control. No significant difference for SOD was observed in all experimental units, however, highest SOD activity was noted in rice seedlings raised from CaO_2 coated seeds. Rice seedlings raised from *Bacillus* sp. KS-54 and alginate coated seeds also proved to be effective for improving the activities of antioxidant enzymes. Lowest values for antioxidant enzymes were obtained in rice seedlings raised from noncoated seeds (Figure 4).

Table 3. Effect of coating treatments on net income and benefit–cost ratio of direct seeded rice.

Coating Treatments	Total Expenditure (* PKR ha^{-1})	Net Field Benefits (PKR ha^{-1})	Net Return (PKR ha^{-1})	Benefit–Cost Ratio
Control	79,375	86,427	37,772	1.48
Bacillus sp. KS-54	80,725	96,239	47,584	1.59
Sodium lauryl sulphate	85,225	91,739	43,084	1.51
Calcium peroxide	79,945	141,145	92,490	2.16
Alginate	87,205	101,329	52,674	1.60

* 1.00 USD = 160.00 PKR.

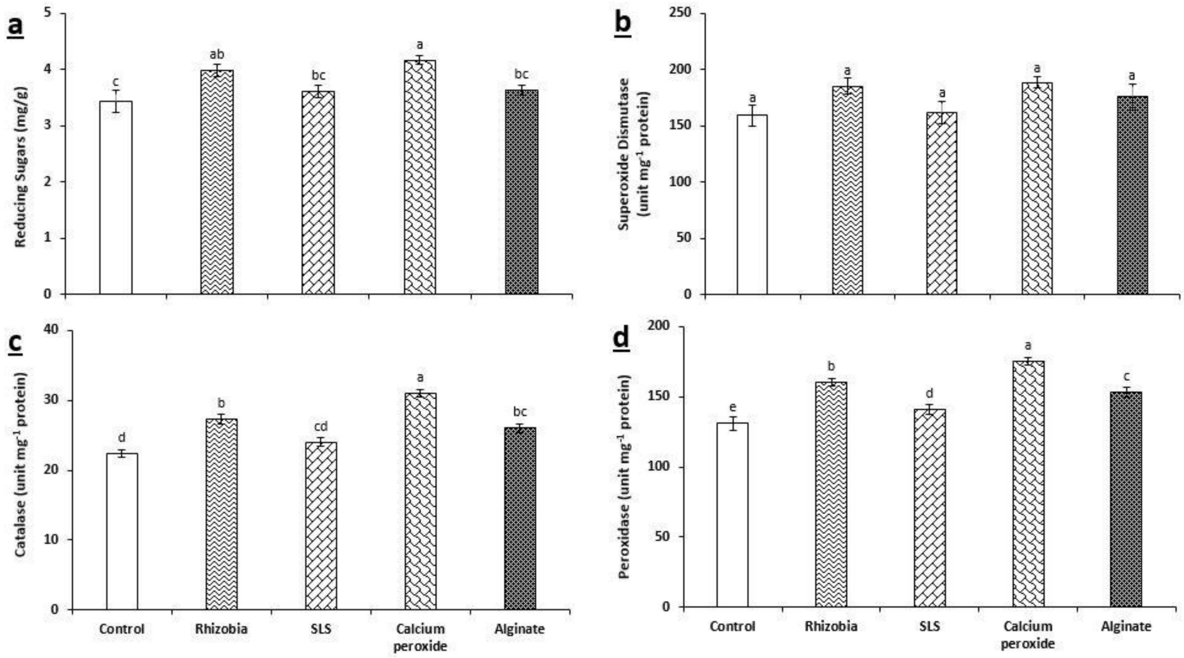

Figure 4. The effect of seed coating agents on biochemical attributes of direct seeded rice during field evaluation. (**a**): Reducing Sugars; (**b**): Superoxide Dismutase; (**c**): Catalase; (**d**): Peroxidase. Values are means ± standard errors. Means followed by the same letters above vertical bars are not significantly different at 5% level of probability. SLS = sodium lauryl sulphate, Rhizobia: *Bacillus* sp. KS54.

4. Discussion

Pre-sowing seed enhancements have potential to improve emergence potential of seeds, stimulate early growth and development, and finally enhance final economic output of crop [8]. Due to its low cost, ease of application and being environmentally friendly, coating technology can be implemented to enhance crop establishment in rice [25]. In the present study, seed coating with CaO_2 resulted in higher final emergence (%) and reduced mean emergence time of dry direct seeded rice followed by seed coating with *Bacillus* sp. KS-54 and alginate (Figure 2). The possible reason behind this mechanism might be the increased available oxygen for seed respiration during germination [13,26]. This resulted in earlier emergence and higher final emergence under submerged dry direct seeded conditions. Upon moisture availability, seeds completed pre-germination events such as imbibition and active metabolism earlier, therefore, resulted in earlier and higher emergence [27]. Our results are also consistent with those of Baker and Hatton [28], who reported that the available oxygen increased as the content of CaO_2 increased. Increased oxygen availability to germinating seed might be the reason for early completion of metabolic activities followed by early radicle protrusion and emergence of the seedlings [13]. The findings of Rafique et al. [29] also highlighted the enhanced emergence of *Bacillus* sp. MN54 treated seeds. The improved seedling establishment by seed coating with rhizobia (*Bacillus* sp. MN-54) could be attributed to enhanced production of siderophores, auxins, and ACC deaminase [30]. The reduction in emergence of rice seeds in the control was attributed directly due to the unavailability of seed coating agent or indirectly due to imbibition and metabolic imbalance [31]. Taking together, seed coating with calcium oxide and rhizobia separately or in combination could be effective in improving stand establishment of rice by using direct seeded rice technology.

Earlier and higher field emergence by seed coating reduced weed growth in direct seeded rice before and after submergence (Figure 3). It is reported that seed coating with calcium peroxide could improve seedlings vigor under waterlogged conditions [26]. Suppression of weeds might result from early and improved establishment of seedlings due to unavailability of oxygen from submerged conditions during critical period of weed emergence [32,33]. Anaerobic conditions created by standing water in rice resulted in poor germination and growth of weeds [34]. Moreover, submerged conditions have a suppressive impact on weed establishment while having no influence on survival and establishment of direct seeds rice due to presence of aerenchyma cells [35]. Submerged conditions were effective during critical weeds–crop competition and exhibited restricted weed growth [36]. Thus, weed suppression in a direct seeded rice system could be achieved by combined implication of seed coating agents and submerged conditions created during critical hours of weeds–crop competition.

The seed coating agents particularly calcium peroxide and *Bacillus* sp. KS54 were effective in enhancing morphological, yield, and yield-related attributes (Table 1; Table 2). Under submerged conditions, provision of extra oxygen contents by seed treatment with calcium peroxide results in higher respiratory and enzymatic activities. In previous literature, improved biochemical attributes (α-amylases, reducing sugars) stimulated the elongation of coleoptile as well as rapid leaf growth [13,37]. The present study is also supported by the result that rice seeds pelleted with ideal CaO_2 content expressed faster extension of coleoptile followed by rapid seedling growth as compared to nonpelleted seeds [13]. Similar results of improved plant height and seedlings vigor were also reported in mung-bean seeds treated with rhizobia [38]. The improved yield could be linked with improved stand establishment [39]. In previous literature, improved seedling emergence was exhibited due to the higher availability of oxygen contents [40]. Availability of optimal oxygen for respiratory activity can promote seedling length and survival under submerged environment [41]. Under anaerobic conditions, growth and development of rice plants could be promoted by seed pelleting with calcium peroxide [42]. It was reported that alfalfa seeds treated with rhizobia exhibited higher seedling growth as compared to control [43]. Thus, CaO_2 coating not only improved emergence, stand establishment, crop growth and yield, but also showed higher benefit–cost ratio (Table 3). Low cost and availability of CaO_2 in the local market would be an advantage for the farmers and seed companies to adopt in rice growing areas of the world. Calcium peroxide is an environmentally friendly oxidant for the control of pathogens in organic fertilizers and ensures phosphorus bioavailability to the growing crops [44]. Additionally, seed coating with *Bacillus* sp. KS-54 can be utilized for improving crop performance in organic production.

In the present study, seed coating agents significantly enhanced the biochemical attributes of rice compared with the control, nevertheless, the effect of sodium lauryl sulphate as a seed coating agent was limited (Figure 4). The rice seeds coated with CaO_2 significantly increased the reducing sugars (4.16 mg/g), CAT (28.33 unit mg^{-1} protein), and POD (190.66 unit mg^{-1} protein) compared with the control (Figure 4). Higher contents of reducing sugars and antioxidant activities such as SOD in rice seeds have been reported in seeds coated with rhizobia [21]. It has been reported that higher sugar contents in rice seeds coated with calcium peroxide reflected faster rate of starch breakdown, which presumably provides the base for early and improved stand establishment through increasing germination energy [13,45].

5. Conclusions

Seed quality is an important aspect of the agricultural sector that provides the basis for improved performance of crop. Seed coating with calcium peroxide and *Bacillus* sp. KS-54 were effective to significantly enhance the emergence, vigor, and yield of rice compared to noncoated control. Better performance of coated rice seeds was associated with enhanced antioxidants activity and reducing sugar contents as well as increased oxygen availability in direct seeded rice under submerged conditions. Increased yield by seed coating was associated with reduced weed growth resulting in early crop development and higher yield attributes.

Supplementary Materials: The following are available online at https://www.mdpi.com/2071-1050/13/4/2190/s1.

Author Contributions: Conceptualization, T.J. and I.A.; methodology, T.J.; software, T.J.; validation, I.A. and R.P.M.; formal analysis, T.J.; investigation, T.J.; resources, I.A.; data curation, I.A. and R.P.M.; writing—original draft preparation, T.J.; writing—review and editing, I.A. and R.P.M.; supervision, I.A.; project administration, I.A.; funding acquisition, I.A. and R.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: The experimental work was financially supported by Pakistan Science Foundation under a research project No. PSF/NSLP-489 entitled "Development of technology rich seeds for improving the performance of crops".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Muhammad Moaaz Ali for the valuable suggestions for data analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fukagawa, N.K.; Ziska, L.H. Rice: Importance for global nutrition. *J. Nut. Sci. Vitaminol.* **2019**, *65*, S2–S3. [CrossRef]
2. Food and Agriculture Organization of the United Nations—FAO. Rice and US. 2019. Available online: http://www.fao.org/rice2004/en/aboutrice.htm (accessed on 15 August 2020).
3. Dhakal, M.; Sah, S.K.; Kharel, G. Integrated weed management in direct-seeded rice: Dynamics and economics. *Int. J. Agric. Environ. Food. Sci.* **2019**, *3*, 83–92. [CrossRef]
4. Nawaz, A.; Awan, M.I.; Ijaz, M.; Hussain, M.; Ahmad, S.; Farooq, M. Direct seeding in rice: Problems and prospects. In *Agronomic Crops*; Springer: Singapore, 2019; pp. 199–222.
5. Rao, A.N.; Wani, S.P.; Ramesha, M.S.; Ladha, J.K. Rice production systems. In *Rice Production Worldwide*; Springer: Cham, Switzerland, 2017; pp. 185–205.
6. Farooq, M.; Usman, M.; Nadeem, F.; Rehman, H.; Wahid, A.; Basra, S.M.A.; Siddique, K.H. Seed priming in field crops: Potential benefits, adoption and challenges. *Crops Pasture Sci.* **2019**, *70*, 731–771. [CrossRef]
7. Afzal, I.; Javed, T.; Amirkhani, M.; Taylor, A.G. Modern Seed Technology: Seed Coating Delivery Systems for Enhancing Seed and Crop Performance. *Agriculture* **2020**, *10*, 526. [CrossRef]
8. Afzal, I.; Rehman, H.; Naveed, M.; Shahzad, M.A.B. Recent advances in seed enhancements. In *New Challenges in Seed Biology-Basic and Translational Research Driving Seed Technology*; InTech Publishing Ltd.: London, UK, 2016.
9. Anilkumar, L.; Malarkodi, K. Combined seed enhancement technique involving seed priming and coating for improvised anatomical potential and vigour of okra seeds (*Abelmoschus esculentus* L.). *J. Phytol.* **2019**, *11*, 25–30.
10. Rocha, I.D.S.; Ma, Y.; Souza-Alonso, P.; Vosátka, M.; Freitas, H.; Oliveira, R.S. Seed coating: A tool for delivering beneficial microbes to agricultural crops. *Front. Plant Sci.* **2019**, *10*, 1357. [CrossRef]
11. Qiu, Y.; Amirkhani, M.; Mayton, H.; Chen, Z.; Taylor, A.G. Biostimulant seed coating treatments to improve cover crop germination and seedling growth. *Agronomy* **2020**, *10*, 154. [CrossRef]
12. Asagi, N.; Ueno, H.; Sekiya, H.; Ogiwara, H. Establishment of rice seedlings by direct sowing of multiple seed pellets on paddy soil covered with legume living mulch. *Plant Prod. Sci.* **2008**, *11*, 361–365. [CrossRef]

13. Mei, J.; Wang, W.; Peng, S.; Nie, L. Seed pelleting with calcium peroxide improves crop establishment of direct seeded rice under waterlogged conditions. *Sci. Rep.* **2017**, *7*, 1–12. [CrossRef] [PubMed]
14. Ma, Y. Seed coating with beneficial microorganisms for precision agriculture. *Biotechnol. Adv.* **2019**, *37*, 107423. [CrossRef]
15. Ninfa, A.; Ballou, D.; Benore, M. *Fundamental Laboratory Approaches for Biochemistry and Biotechnology*; Wiley, John and Sons: Hoboken, NJ, USA, 2019; Available online: http://agproducts.basf.us/products/stamina-f3-cereals-fungicide-seed-treatment.html (accessed on 15 August 2020).
16. Liu, H.; Zhang, Y.H.; Yin, H.; Wang, W.X.; Zhao, X.M.; Du, Y.G. Alginate oligosaccharides enhanced *Triticum aestivum* L. tolerance to drought stress. *Plant Physiol. Biochem.* **2013**, *62*, 33–40. [CrossRef]
17. Kumar, R.; Kumawat, N.; Kumar, S.; Kumar, R.; Kumar, M.; Sah, R.P.; Kumar, U.; Kumar, A. Direct seeded rice: Research strategies and opportunities for water and weed management. *Oryza* **2016**, *53*, 354–365.
18. He, J.W.; Chen, A.Q.; Tan, D.S. Studies on seed-coating dosages of Hanyubaomu in raising rice seedlings in wet nursery. *Hybrid Rice* **2011**, *26*, 48–50.
19. Association of Official Seed Analysis. Rules for testing seeds. *J. Seed Technol.* **1990**, *12*, 101–112.
20. Ellis, R.A.; Roberts, E.H. The quantification of aging and survival in orthodox seeds. *Seed Sci. Technol.* **1981**, *9*, 373–409.
21. Thobunluepop, P.; Chitbanchong, W.; Pawelzik, E.; Vearasilp, S. Physiological and biochemical evaluation of rice seed storability with different seed coating techniques. *Int. J. Agric. Res.* **2009**, *4*, 169–184. [CrossRef]
22. Bernfeld, P. *Methods in Enzymology*; Academic Press: New York, NY, USA, 1955.
23. Giannopolitis, C.N.; Ries, S.K. Superoxide dismutase occurrence in higher plants. *Plant Physiol.* **1977**, *59*, 309–314. [CrossRef] [PubMed]
24. Chance, M.; Maehly, A.C. Assay of catalases and peroxidases. In *Methods in Enzymology*; Academic Press: New York, NY, USA, 1955.
25. Wang, W.; Chen, Q.; Hussain, S.; Mei, J.; Dong, H.; Peng, S.; Huang, J.; Cui, K.; Nie, L. Pre-sowing seed treatments in direct seeded early rice: Consequences for emergence, seedling growth and associated metabolic events under chilling stress. *Sci. Rep.* **2016**, *6*, 1–10. [CrossRef] [PubMed]
26. Biswas, J.K.; Ando, H.; Kakuda, K.; Purwanto, B.H. Effect of calcium peroxide coating, soil source and genotype on rice (*Oryza sativa* L.) seedling establishment under hypoxic conditions. *Soil Sci. Plant Nutr.* **2001**, *47*, 477–488. [CrossRef]
27. Afzal, I.; Hussain, B.; Basra, S.M.A.; Rehman, H. Priming with MLE reduces imbibitional chilling injury in spring maize. *Seed Sci. Technol.* **2012**, *40*, 271–276. [CrossRef]
28. Baker, A.M.; Hatton, W. Calcium peroxide as a seed coating material for padi rice. *Plant Soil.* **1987**, *99*, 379–386. [CrossRef]
29. Rafique, M.; Naveed, M.; Mustafa, A.; Akhtar, S.; Munawar, M.; Kaukab, S.; Ali, H.M.; Siddiqui, M.H.; Salem, M.Z. The combined effects of gibberellic acid and rhizobium on growth, yield and nutritional status in chickpea (*Cicer arietinum* L.). *Agronomy* **2021**, *11*, 105. [CrossRef]
30. Samreen, T.; Zahir, Z.A.; Naveed, M.; Asghar, M. Boron tolerant phosphorus solubilizing *Bacillus* sp. strain MN-54 improved canola growth in alkaline calcareous soils. *Int. J. Agric. Biol.* **2019**, *21*, 538–546.
31. Ferreira, J.S.; Baldani, J.I.; Baldani, V.L.D. Selection of peats inoculants with diazotrophic bacteria in to rice varieties. *Acta Scientiarum. Agron.* **2010**, *32*, 179–185.
32. Rao, A.N.; Johnson, D.E.; Sivaprasad, B.; Ladha, J.K.; Mortimer, A.M. Weed management in direct-seeded rice. *Adv. Agron.* **2007**, *93*, 153–255.
33. Mahajan, G.; Chauhan, B.S. Effects of planting pattern and cultivar on weed and crop growth in aerobic rice system. *Weed Technol.* **2017**, *25*, 521–525. [CrossRef]
34. Raj, S.K.; Syriac, E.K. Weed management in direct seeded rice—A review. *Agric. Rev.* **2017**, *38*, 41–50. [CrossRef]
35. Poonpaiboonpipat, T.; Poolkum, S. Utilization of *Bidens pilosa* var. *radiata* (Sch. Bip.) Sherff integrated with water irrigation for paddy weed control and rice yield production. *Weed Biol. Manag.* **2019**, *19*, 31–38.
36. Samoy-Pascual, K.; Martin, E.C.; Ariola, C.P. Effects of water and weed management on the weed density, grain yield and water productivity of wet-seeded rice. *Philipp. J. Sci.* **2020**, *149*, 139–149.
37. Magneschi, L.; Kudahettige, R.L.; Alpi, A.; Perata, P. Comparative analysis of anoxic coleoptile elongation in rice varieties: Relationship between coleoptile length and carbohydrate levels, fermentative metabolism and anaerobic gene expression. *Plant Biol.* **2009**, *11*, 561–573. [CrossRef] [PubMed]
38. Mehboob, I.; Zahir, Z.A.; Arshad, M.; Tanveer, A.; Khalid, M. Comparative effectiveness of different Rhizobium sp. for improving growth and yield of maize (*Zea mays* L.). *Soil Environ.* **2012**, *31*, 37–46.
39. Afzal, I.; Basra, S.M.A.; Shahid, M.; Farooq, M.; Saleem, M. Priming enhances germination of spring maize (*Zea mays* L.) under cool conditions. *Seed Sci. Technol.* **2008**, *36*, 497–503. [CrossRef]
40. Macewan, R.J.; Crawford, D.M.; Newton, P.J.; Clune, T.S. High clay contents, dense soils, and spatial variability are the principal subsoil constraints to cropping the higher rainfall land in south-eastern Australia. *Soil Res.* **2010**, *48*, 150–166. [CrossRef]
41. Siegel, S.M.; Gerschman, R. A study of the toxic effects of elevated oxygen tension on plants. *Physiol. Plant.* **2006**, *12*, 314–323. [CrossRef]
42. Sato, T.; Maruyama, S. Seedling emergence and establishment under drained conditions in rice direct sewn into puddled and levelled soil: Effect of calcium peroxide seed coating and sowing depth. *Plant Prod. Sci.* **2002**, *5*, 71–76. [CrossRef]

43. Zhou, J.; Deng, B.; Zhang, Y.; Cobb, A.B.; Zhang, Z. Molybdate in rhizobial seed coat formulations improves the production and nodulation of alfalfa. *PLoS ONE* **2017**, *12*, e0170179. [CrossRef] [PubMed]
44. Wieckol-Ryk, A.; Bialecka, B.; Thomas, M. Application of calcium peroxide as an environmentally friendly oxidant to reduce pathogens in organic fertilizers and its impact on phosphorus bioavailability. *Arch. Environ. Prot.* **2020**, *46*, 42–53.
45. Hwang, Y.S.; Thomas, B.R.; Rodriguez, R.L. Differential expression of rice alpha-amylase genes during seedling development under anoxia. *Plant Mol. Biol.* **1999**, *40*, 911–920. [CrossRef]