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Germination and Growth Performance of Water-Saving and Drought-Resistant Rice Enhanced by Seed Treatment with Wood Vinegar and Biochar under Dry Direct-Seeded System

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Abstract: Dry direct-seeded rice (dry-DSR) is an efficient, resource-saving and environmentally friendly cropping system. The employment of water-saving and drought-resistant rice (WDR) for dry direct-seeding can better meet the needs of dry-direct seeding systems. However, the decline in seedling emergence rate and poor seedling growth are the main bottlenecks under current direct-seeded rice production. Seed treatment is a sustainable and effective technique to overcome these issues. Therefore, growth chamber and field experiments were conducted to assess the impact of poplar wood vinegar (WV) priming and rice straw biochar (BC) coating on emergence, establishment, growth, physio-biochemical events, and ultimate yield. We treated the seeds of WDR viz., Hanyou 73 with WV, BC, and co-treatment WV + BC. The results showed that seed priming with 1:50 WV concentration and coating with 20% BC content was the optimal ratio for promoting germination and seedling growth. The field evaluation indicated that individual WV and BC markedly promoted the final emergence by 58% and 31%, respectively, while co-treatment WV + BC increased by 67%. Likewise, WV and BC significantly enhanced total seedling biomass by 26% and 10%, respectively, and the respective enhancement of WV + BC was 31%. For ultimate yield, WV and BC produced 12% and 19% higher grain yield, respectively, whereas WV + BC yielded 20%. The above results revealed that WV and WV + BC were the most effective treatment. Our findings may provide new avenues for advancing pre-sowing seed treatments facilitating the stand establishment and grain yield of dry direct-seeded rice.

Keywords: wood vinegar; biochar; dry direct-seeded system; grain yield; emergence; stand establishment



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1. Introduction

Rice is the dominant cereal foodstuff and staple food for nearly half of the world's population [1]. In China, conventional transplanted rice (TPR) accounts for nearly 77% of the total rice grown planting area [2]. Nevertheless, the sustainability and productivity of this production system are threatened by higher labor and water demand, lower net benefits [3], and emissions of large amounts of greenhouse gas [4]. Dry direct-seeded rice technology (dry-DSR) is proposed as a socio-economically viable and environmentally promising alternative to TPR. In dry-DSR, rice seeds are directly sown with a machine or manually broadcast in the non-puddled soil, rather than nursery raising and transplanting seedlings in a puddled field [5].

However, poor emergence and non-uniform stand establishment, as well as higher weeds incidence, are some of the constraints to the large-scale adoption of DSR [6,7]. There

are many factors for uniform crop emergence and the establishment of dry-DSR, including land preparation, soil moisture, seed depth, and weed control [6,8]. Moreover, soil moisture is crucial to the seedling establishment of dry-DSR, particularly during the emergence phase [9]. Soil moisture depletion, that is, severe water deficit, may sometimes yield the complete inhibition of seedling emergence [10]. Furthermore, water deficit hampered the stand establishment during the seed imbibition stage [11], thereby inhibiting early seedling growth [12]. Therefore, effective and practical techniques need to be adopted to ameliorate crop emergence and enhance the early seedling growth of dry-DSR.

In recent years, various strategies were employed to improve the emergence attributes and seedling establishment, particularly under unfavorable environmental conditions. Pre-sowing seed treatment, i.e., seed priming and seed coating, is a sustainable and effective approach to enhance the rapid and uniform emergence and seedling quality. Seed priming refers to a seed treatment technology that controls the seed hydration to a point where the pre-germination metabolisms are triggered, but radicle emergence does not occur [13]. Recently, various researchers reported that several priming agents increased the speed of seed germination and triggered seedling growth in different crops, including rapeseed [14], common bean [15], and wheat [16]. Wood vinegar (WV), also known as pyroligneous acid, is a liquid during biochar production that originated from the slow pyrolysis of woody biomass by water vapor condensation with limited oxygen [17]. Recent reports indicated that wood vinegar with seed priming promoted the early seedling development of various plants, such as wheat [18], as well as pepper and tomato [19]. However, the impacts of wood vinegar priming on the emergence and seedling growth of rice under the dry-DSR system have not been explored yet.

Seed coating applies various materials, such as fertilizers, moisture attractive or repulsive agents, growth stimulants, microbes, chemicals, and pesticides to the seed surface by adhesive agents, which improves seed viability and vigor [20]. For example, seed coating with super absorbent polymer improved the energy and rate of seed germination, along with promoting seedling growth [21]. A previous study also reported that the hydro-absorber coating increased seedling performance under water-limited conditions, particularly by enhancing root growth [22]. However, the existing materials with seed coating are chemical, and the majority of them have little effect on seed vigor, germination characteristics, and seedling growth. Furthermore, certain coating agents, such as binders and powders, may pose a threat to the seed vigor, delaying emergence time and reducing germination rate. Biochar, as a fine and carbon-rich organic material, is produced by pyrolysis of plant biomass or residues under the same conditions as wood vinegar [23,24]. Biochar possesses a porous structure and high surface area; therefore, biochar has the potential to slowly absorb and release moisture and elements in the soil, thereby improving water and nutrient utilization efficiency [25,26]. Currently, it has been confirmed that seed coating with biochar could not only decrease nitrogen leaching losses but also provide more nutrients to the rice plants [27]. However, little information is available about the effects of seed coating based on biochar on plant emergence and growth.

Water-saving and drought-resistance rice (WDR) is a novel type of cultivated rice, which has a similarly high yield potential and good quality compared to wild-type varieties [28]. Additionally, WDR could exhibit higher drought tolerance and rice production [29]. Hanyou 73, one of the elite WDR, was developed and released to farmers for commercial rice production and obtained a high and sustainable yield [29]. However, the influence of seed treatments on the emergence, seedling growth, and yield of the WDR variety under the dry-DSR system has not been investigated yet. Therefore, research experiments from both the growth chamber and field were conducted with the objective of evaluating the effectiveness of the individual and co-application of wood vinegar and biochar on germination, stand establishment, physiology, crop growth, and yield of WDR under the dry-DSR system, and provide crucial technologies for the efficient cultivation of WDR.

2. Materials and Methods

2.1. Experimental Materials

Seeds used in this study were widely grown in China; Hanyou73 (HY73), which is an elite indica three-line hybrid WDR variety, originated from Shanghai Agrobiological Gene Center (SAGC), Shanghai, China. The cultivar had a germination percentage of $\geq 90\%$ at $25\text{ }^{\circ}\text{C}$, which was used to sow materials in the growth chamber experiments and field experiments.

Wood vinegar (WV) as the priming agent was obtained from poplar charcoal smoke, provided by Hubei Chutian Biomass Energy Technology Development Co., Ltd., Wuhan City, China. Biochar (BC), as one of the coating agents, was derived by pyrolysis of rice straw at a high temperature of $600\text{ }^{\circ}\text{C}$ produced by Hubei Jinzhi Eco-Energy Co., Ltd., Xiaogan City, China. Biochar with a pH of 9.42 containing 0.74% nitrogen, 47.14% carbon, 1.62% hydrogen, 11.85% oxygen, 0.32% phosphorus, 18.90% potassium, and 19.43% ash was used in the experiment. Other coating agents, including talc, attapulgitite, and a seed coater (Model RH-325), were produced by Qingdao Ruihua Agricultural Technology Co., Ltd., Qiaodao City, China.

2.2. Experimental Setup

2.2.1. Growth Chamber Experiment

The experiments were conducted in growth chambers (HP250GS-C, Ningbo Southeast Instrument Co., Ltd., Ningbo, China), adjusting the day/night temperature at $30/25\text{ }^{\circ}\text{C}$ with 12 h light (8000 lx) as well as 12 h dark. Rice seeds were sowed in a $12.0\text{ cm} \times 12.0\text{ cm} \times 6.0\text{ cm}$ germination box.

Screening the Effective Concentration of Wood Vinegar Priming

Fifty sterilized seeds were soaked in sterilized water supplemented with 0 (hydro-priming, HP), 1:10, 1:25, 1:50, 1:100, 1:200, and 1:400 different volumes of WV (primary WV:ddH₂O (*v:v*)), respectively, for 24 h. The seed priming treatment was performed as described in Hussain et al. [30]. Then, the soaking seeds were sowed in the sterile germination box with three layers of filter paper saturated with 10 mL of sterilized water. Seeds were dampened with 5 mL of water every day for one week. The number of seeds germinated was counted at 3 days after sowing (DAS), 7 DAS, respectively. Seeds were considered to be germinated until the radical length reached up to 2 mm.

Screening the Efficient Ratio of Biochar Coating

Biochar content in the seed coating agents were set as 20% (BC20), 30% (BC30), 40% (BC40), and 50% (BC50, *w/w*) in a coating formula with talc:attapulgitite = 5:2 (*w/w*). The ratio of seed weight to the coating agent's weight was set as 1:2 (recommended by the producer). Prior to coating, the dried biochar was crushed to a particle size of 0.60 mm. The rice seeds were coated with a seed coater using the following procedures. Firstly, biochar, talc, and attapulgitite were placed in a round-bottomed container, where the coating agents were stirred and evenly mixed. Then, untreated, dry seeds were put into the cylindrical drum at the rotating speed of 200 rpm; after, water was injected by opening the water valve for about 8 s to ensure that the seed surface was moist but not sticky. Thirdly, half of the total coating agents were placed into the feeding port so that the coating agents slowly fell into the cylindrical drum; meanwhile, the water was injected for about 1 min while rolling. This step was repeated. Finally, the cylindrical drum was continuously rotated for 2 min after adding the coating agents. The coated seeds were air-dried at $25\text{ }^{\circ}\text{C}$. Germination boxes were filled with 500 g of air-dried soil. After filling, the soil was kept at field capacity. Thirty-five seeds for each treatment were equally sown on the soil surface at a depth of 0.5 cm. Seedlings were evaluated at 8 DAS.

Optimum Seed Treatments and Treatment Combination

Based on the better performance of germination and seedlings development seed treatment with wood vinegar and biochar, two optimum treatments, namely WV and BC,

were selected. To unravel the stand establishment and biochemical changes induced by WV, BC, and the co-treatment of WV + BC under dry direct-seeded conditions, 35 seeds were equally sown in the germination boxes filled with 500 g of dried soil. The soil's relative water content was maintained at 30% of field capacity. The experiments were laid out in a randomized block design with six replications. Three replications of boxes were used to record seedling attributes. The seeds and seedlings in the other three replications were sampled at 0, 2, 5, and 8 DAS to determine biochemical parameters, and all samples were rapidly frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ before determination. Seedlings were evaluated at 14 DAS.

2.2.2. Field Experiment

The field experiments were performed at the experimental field of Huazhong Agricultural University. The plots with 10 m^2 ($2\text{ m} \times 5\text{ m}$) were arranged in a randomized block design with three replicates. Before sowing, the soil was dry plowed and harrowed without puddling. Dry seeds and treated seeds were evenly sown in 25 cm wide rows using a seed rate of 22.5 kg ha^{-1} by hand drill in June 2021, and then seeds were covered with soil immediately. The soil was kept moist to facilitate crop establishment during emergence. After which, the rainfed mode was maintained throughout the whole growing season, except in extremely dry conditions, and then water replenishment was maintained wet without a water layer. Fertilizer was applied at 180 kg ha^{-1} N, 90 kg ha^{-1} P, and 90 kg ha^{-1} K in the form of urea (46%) and compound fertilizer (45%). All the P, K, and half of the N (compound fertilizer) were applied as basal fertilizer at the final soil preparation. The remaining half of the N (Urea) was applied at the 4-leaf stage. Weeds, insects, and diseases were intensively controlled during the course of the experiment to avoid yield loss.

2.3. Data Collection

2.3.1. Germination and Seedling Attributes

Germination of seeds was recorded daily according to AOSA until it became constant [31]. Seedlings' emergence in the field was observed from two adjacent rows of 1.5 m length from the third row, expressed as seedlings m^{-2} . Ten randomly selected seedlings were sampled to record their shoot length and root length, then were oven-dried at $75\text{ }^{\circ}\text{C}$ to constant weight for measuring shoot dry weight (shoot DW) and root dry weight (root DW), respectively. Seedlings attributes, such as germination energy (GE), final germination/emergence (FG/FE), emergence index (EI) [31], mean emergence time (MET) [32], seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II) [33], were calculated by the formulae below, respectively:

$\text{GE} = 100 \times N/n$, where N is the number of germinated seeds at 3 DAS and n is the total number of tested seeds.

$\text{FG/FE} = 100 \times N/n$, where N is the number of normal emerged seedlings and n is the total number of tested seeds.

$\text{EI} = \sum n/D$, where n is the number of germinated seeds at a given day and D is the corresponding day number.

$\text{MET} = \sum(D \times n)/\sum n$, where n is the number of germinated seeds on day D and D is the number of days recorded from the beginning of emergence.

$\text{SVI-I} = \text{FG/FE} \times (\text{shoot length} + \text{root length})$ in cm per seedling.

$\text{SVI-II} = \text{FG/FE} \times (\text{shoot DW} + \text{root DW})$ in mg per seedling.

2.3.2. Measurement of Seedling Root Morphology

The roots of the seedlings from each treatment were scanned after these harvested seedlings were dissected into roots and shoots. The fresh root was scanned using an Epson V800 scanner (Epson Seiko Epson Corporation, Nagano Prefecture, Suwa, Japan) at 300 dpi. The scanned images were then analyzed by WinRHIZO 2017a software (Regent Instruments, Quebec City, QC, Canada) to measure root morphological traits, including total root length, surface area, average diameter, root volume, and the number of tips.

2.3.3. Determination of α -Amylase Activity, Soluble Sugar, and Soluble Protein

The α -Amylase activity (α -AMS), soluble sugar, and soluble protein contents in the rice seeds or seedlings were measured according to the manufacturer's protocol of the kit from the Nanjing Jiancheng Bioengineering Institute, Nanjing, China. In total, 0.2 g of dry seeds (0 DAS) and seedling samples (2, 5, 8 DAS) were weighed and mixed with 1.8 mL of distilled water. The mixture was ground thoroughly into a homogenous liquid at a low temperature for 5 min, following which it was centrifuged at 10,000 rpm for 10 min. The supernatant was collected to determine the α -AMS and soluble protein according to the introduction of the " α -AMS detection kit" and "Soluble protein detection kit", respectively. Similarly, for soluble sugar determination, the homogenous liquid was bathed at 100 °C for 10 min, which was centrifuged at 10,000 rpm for 10 min after cooling. The supernatant was collected to determine the soluble sugar according to the introduction of the "Soluble sugar detection kit".

2.3.4. Gas Exchange Parameters and Physiological Indicators

Gas exchange parameters of the flag leaf, including net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_{st}), and water use efficiency (WUE), were measured with an LI-6800 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) at HD, which were performed between 10:00 a.m. and 3:00 p.m. in full sunshine under the below environmental conditions: PPFD 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 concentration 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, flow rate 500 $\mu\text{mol s}^{-1}$, air humidity 60–80% [34].

After measuring the photosynthetic parameters, the determined leaves were stored in a freezer to obtain soluble sugar and soluble protein content. The soluble sugar and soluble protein content were determined following the same method as in the pot experiment.

2.3.5. Measurement of Agronomic Traits

Two adjacent rows at 0.5 m from each experimental unit were sampled at mid-tillering (MT), panicle initiation (PI), heading stage (HD), and physiological maturity (PM). After measuring plant height and tiller number, samples were separated into leaves and stems. The leaf area was measured with a leaf area meter (Licor-3100; LICOR, Lincoln, NE, USA). Leaf area index (LAI) was calculated as leaf area divided by land area. Plant material was put in an oven at 75 °C for 48 h to estimate total dry weight.

2.3.6. Yield and Yield Components

The components of rice yield were determined from two adjacent rows with a length of 1 m from each plot at PM. The total number of panicles was counted to estimate the panicle number per square meter. Additionally, all spikelets on the branches were threshed by hand. The number of spikelets per panicle, grain filling rate, and 1000 grain weight were obtained by an engineering prototype of the Yield Traits Scorer (YTS), as stated by Yang et al. [35]. To determine grain yield, a sampling area of 5 m² was selected in each plot and then adjusted at 14% moisture. The harvest index was computed as the ratio of grain weight to biological yield (total dry weight).

2.4. Statistical Analysis

The data were statistically analyzed using Statistix 8.1 with a randomized block design from three biological replications. Untreated, dry seeds without any soaking or coating served as a control (CK). The mean among treatments was compared on the basis of the least significant difference test (LSD) at the 5% probability level. The graphical representation of the data was plotted using the ggplot2 package in RStudio [36].

3. Results

3.1. Seedling Attributes and Seedling Growth

3.1.1. Screening the Effective Concentration of Wood Vinegar Priming

WV50 was the optimal concentration for seed germination, and seedling establishment and high concentrations (WV10) had adverse effects (Table 1, Figure 1) compared to CK. Nevertheless, WV priming had better effects than HP only in WV50 and WV100. Compared with hydro-priming, GE, FG, shoot length, root length, total DW, SVI-I, and SVI-II in WV50 were improved by 89%, 5%, 20%, 11%, 18%, 23%, and 24%, respectively. Therefore, WV50 was used in the following experiments.

Table 1. Effect of different concentrations of WV on WDR seed germination and seedling growth under growth chamber experiment.

Treatment	GE (%)	FG (%)	Shoot Length (cm)	Root Length (cm)	Total DW (mg Seedling ⁻¹)	SVI-I	SVI-II
CK	19.3 ± 1.8 cd	94.0 ± 1.2 bc	5.2 ± 0.1 e	3.3 ± 0.1 c	6.0 ± 0.1 d	790.2 ± 13.8 e	560.5 ± 18.3 e
HP	29.3 ± 3.3 bc	94.7 ± 0.7 bc	5.4 ± 0.1 de	3.5 ± 0.1 bc	6.3 ± 0.2 cd	839.4 ± 9.6 de	594.0 ± 18.6 de
WV10	16.0 ± 1.2 d	86.7 ± 1.8 d	5.5 ± 0.1 de	3.5 ± 0.1 bc	6.4 ± 0.1 cd	780.1 ± 12.8 e	558.1 ± 8.7 e
WV25	39.3 ± 2.9 b	93.3 ± 0.7 c	6.2 ± 0.1 ab	3.7 ± 0.1 ab	7.0 ± 0.3 ab	917.8 ± 9.9 bc	651.1 ± 24.6 c
WV50	55.3 ± 5.8 a	99.3 ± 0.7 a	6.5 ± 0.2 a	3.9 ± 0.1 a	7.4 ± 0.1 a	1029.3 ± 5.7 a	735.6 ± 16.6 a
WV100	54.7 ± 5.7 a	98.0 ± 1.2 ab	6.2 ± 0.2 abc	3.8 ± 0.1 ab	7.2 ± 0.1 a	972.3 ± 5.7 ab	708.7 ± 8.9 ab
WV200	55.3 ± 4.4 a	95.3 ± 1.3 abc	5.8 ± 0.1 bcd	3.8 ± 0.1 ab	7.1 ± 0.2 ab	912.0 ± 32.3 bc	677.8 ± 22.7 bc
WV400	50.7 ± 1.3 a	94.0 ± 3.1 bc	5.7 ± 0.2 cd	3.6 ± 0.1 ab	6.7 ± 0.1 bc	879.9 ± 44.4 cd	627.0 ± 21.6 cd

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, HP: hydro-priming. Numbers after WV denote the WV concentration used for seed priming. GE: germination energy, FG: final germination, DW: dry weight, SVI-I: seedling vigor index-I, SVI-II: seedling vigor index-II.

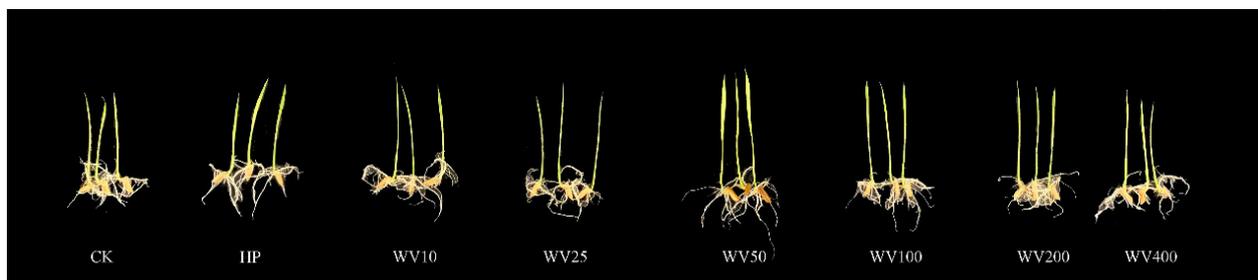


Figure 1. Pictorial illustration of different concentrations of WV treated rice seedlings at 7 DAS under growth chamber experiment. CK: untreated seeds, HP: hydro-priming. Numbers after WV denote the WV concentration used for seed priming.

3.1.2. Screening the Efficient Ratio of Biochar Coating

Seed coating with BC20 was the optimum proportion for WDR emergence and stand establishment. Probably due to the alkaline of biochar (pH 9.42), higher contents exerted negative effects as compared to CK (Table 2, Figure 2). BC20 and BC30 were considerably higher than other coating treatments and the control regarding seedling quality, and these two treatments were not statistically significant. When compared with control, BC20 promoted FE, EL, shoot length, root length, shoot DW, root DW, SVI-I, and SVI-II by 11%, 31%, 9%, 6%, 19%, 30%, 19%, and 34%, respectively. Furthermore, BC20 significantly lowered MET, and accordingly, BC20 was selected for the following experiments.

Table 2. Effect of different content of BC coating on WDR seed germination and seedling growth under growth chamber experiment.

Treatment	FE (%)	EI	MET (d)	Shoot Length (cm)	Root Length (cm)	Shoot DW (mg Seedling ⁻¹)	Root DW (mg Seedling ⁻¹)	SVI-I	SVI-II
CK	89.5 ± 1.0 b	22.9 ± 0.8 c	6.3 ± 0 a	12.3 ± 0.1 b	9.3 ± 0.1 bc	7.4 ± 0.3 b	2.8 ± 0.1 b	1929.0 ± 16.1 b	917.7 ± 17.7 b
BC20	99.0 ± 1.0 a	30.0 ± 0.6 a	6.0 ± 0 b	13.4 ± 0.1 a	9.8 ± 0.1 a	8.9 ± 0.1 a	3.7 ± 0.3 a	2298.4 ± 26.6 a	1240.1 ± 32.3 a
BC30	97.1 ± 1.6 a	27.8 ± 1.5 ab	6.1 ± 0.1 b	12.9 ± 0.1 a	9.7 ± 0.2 ab	8.4 ± 0.3 a	3.2 ± 0.3 ab	2193.1 ± 60.8 a	1133.8 ± 22.9 a
BC40	94.3 ± 3.3 ab	24.8 ± 1.6 bc	6.2 ± 0.1 ab	11.5 ± 0.2 c	9.0 ± 0.2 c	6.8 ± 0.3 bc	3.2 ± 0.1 ab	1928.8 ± 78.7 b	946.3 ± 69.3 b
BC50	89.5 ± 2.5 b	23.4 ± 1.6 c	6.2 ± 0.1 ab	11.0 ± 0.3 c	8.8 ± 0.2 c	6.5 ± 0.3 c	3.0 ± 0.1 b	1773.0 ± 70.0 b	853.3 ± 64.5 b

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, numbers after BC denote different BC content used for seed coating. FE: final emergence, EI: emergence index, MET: mean emergence time, DW: dry weight, SVI-I: seedling vigor index-I, SVI-II: seedling vigor index-II.

Table 3. Emergence characteristics of different seed treatments of WDR under dry direct-seeded system.

Experiment	Treatment	FE	EI	MET (d)	SVI-I	SVI-II
Growth chamber	CK	83.8 ± 2.5 c	13.2 ± 1.4 c	8.4 ± 0.1 a	1631.8 ± 69.5 d	1055.9 ± 52.8 d
	WV	95.2 ± 1.0 ab	21.6 ± 1.6 ab	7.9 ± 0.1 bc	2483.8 ± 69.2 b	1456.0 ± 22.0 b
	BC	91.4 ± 1.6 b	19.2 ± 1.1 b	8.0 ± 0.1 b	2310.3 ± 36.4 c	1274.6 ± 11.2 c
	WV + BC	98.1 ± 1.9 a	26.0 ± 1.6 a	7.6 ± 0.1 c	2692.7 ± 12.7 a	1616.9 ± 51.7 a
Field	CK	48.0 ± 4.8 c	10.9 ± 1.4 b	9.0 ± 0 a	984.5 ± 106.3 b	552.6 ± 43.0 c
	WV	76.0 ± 2.8 ab	18.6 ± 1.9 a	8.9 ± 0.1 a	1854.4 ± 94.0 a	1104.9 ± 44.6 a
	BC	62.7 ± 6.9 b	14.5 ± 1.6 b	9.0 ± 0.1 a	1395.1 ± 142.3 b	795.5 ± 77.8 b
	WV + BC	79.6 ± 6.5 a	20.4 ± 1.5 a	8.8 ± 0 a	1950.3 ± 152.2 a	1200.3 ± 86.0 a

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating. FE: final emergence, EI: emergence index, MET: mean emergence time, SVI-I: seedling vigor index-I, SVI-II: seedling vigor index-II.



Figure 2. Pictorial illustration of different content of BC coating treated rice seedlings at 8 DAS under growth chamber experiment. CK: untreated seeds, numbers after BC denote different BC content used for seed coating.

3.1.3. Optimum Seed Treatments and Treatment Combination

All seed treatments notably improved the emergence of WDR both in the growth chamber experiment and field experiment (Figure 3). In the incubator chamber experiment, the emergence percentage of WV, BC, and WV + BC at 6 DAS was recorded as 65.7%, 54.3%, and 83.8%, respectively, while CK was only 19.1% (Figure 3a). Evaluation of FE depicted that emergence of growth chambers in WV, BC, and WV + BC were significantly increased by 14%, 9%, and 17%, respectively, while the respective increments in field conditions were 58%, 31%, and 67%, respectively in comparison with CK (Table 3). Both WV and WV + BC greatly enhanced EI under controlled experiments and natural experiments. All seed treatments under growth chamber conditions significantly reduced MET compared to CK.

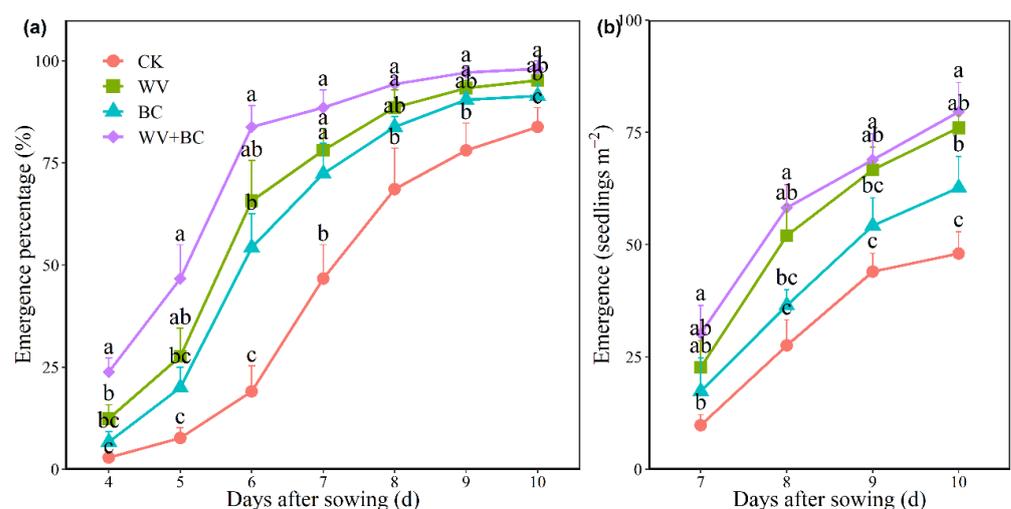


Figure 3. Emergence dynamics of different seed treatments of WDR under dry direct-seeded system. (a) growth chamber experiment. (b) field experiment. Different letters show a significant difference between treatments at $p = 0.05$ according to LSD test. Error bars indicate standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

Pre-sowing seed treatments also greatly influenced SVI-I and SVI-II. In the growth chamber experiment, WV and WV + BC averagely enhanced SVI by 86% and 103%, respectively, compared with CK, while the respective enhancements for the field experiment were 92% and 105%, respectively. Overall, the best emergence performance was WV + BC, followed by WV.

Similarly, seed treatments depicted a significant improvement in rice seedling growth attributes both under laboratory study and field study (Table 4 and Figure 4). Under laboratory conditions, the individual WV promoted the shoot length, root length, total length, shoot DW, root DW, and total DW by 33%, 35%, 34%, 26%, 13%, and 21%, respectively, as compared to CK, while the co-treatment WV + BC enhanced the respective seedling growth attributes by 43%, 37%, 41%, 35%, 22%, and 31% (Table 4). Although seed treatment in BC was the least effective treatment for these growth attributes, it recorded significantly higher seedling length and shoot dry weight. In addition, the performance of the field among various seed treatments was consistent with the results of the laboratory regarding seedling establishment. Generally, WV + BC and WV outperformed BC and the control.



Figure 4. Pictorial illustration of different seed treated rice seedlings at 14 DAS under growth chamber experiment. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

Significant variations in seedling root morphology were also found between different seed treatments and non-treated control under growth chamber experiment (Table 5 and Figure 5). Under incubator chamber conditions, 44%, 52%, and 55% increments of total root length were observed in WV, BC, and WV + BC, respectively. Compared to CK, the surface area was increased up to 25%, 24%, and 30% by WV, BC, and WV + BC, respectively. Furthermore, the number of root tips promoted 51%, 71%, and 73% by WV, BC, and WV + BC, respectively, in comparison with CK. However, in contrast to total root length and surface area, seed treatments notably decreased the average root diameter in WDR seedlings (Table 5). Such results might be due to an increase in the number of lateral roots. In terms of water absorption, thinner roots were more conducive to entering the small pores of the soil to obtain water.

Table 4. Seedling growth attributes of different seed treatments of WDR under dry direct-seeded system.

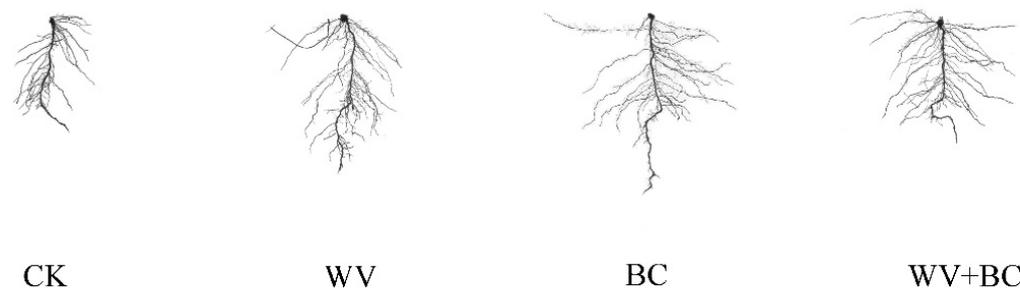
Experiment	Treatment	Shoot Length (cm)	Root Length (cm)	Total Length (cm)	Shoot DW (mg Seedling ⁻¹)	Root DW (mg Seedling ⁻¹)	Total DW (mg Seedling ⁻¹)
Growth chamber	CK	14.1 ± 0.3 d	5.3 ± 0.3 b	19.5 ± 0.6 c	8.4 ± 0.4 c	4.2 ± 0.2 b	12.6 ± 0.5 c
	WV	20.2 ± 0.1 b	7.3 ± 0.4 a	27.5 ± 0.5 ab	11.3 ± 0.1 ab	5.2 ± 0 ab	16.5 ± 0.1 a
	BC	16.9 ± 0.4 c	8.4 ± 0.4 a	25.3 ± 0.6 b	9.7 ± 0.2 b	4.3 ± 0 b	13.9 ± 0.2 b
	WV + BC	18.9 ± 0.2 a	7.2 ± 0.2 a	26.1 ± 0.4 a	10.5 ± 0.4 a	4.8 ± 0.3 a	15.3 ± 0.6 a
Field	CK	11.4 ± 0.1 c	9.1 ± 0.3 b	20.5 ± 0.2 c	7.3 ± 0.2 c	4.3 ± 0.3 b	11.6 ± 0.4 c
	WV	14.4 ± 0.4 a	10.0 ± 0.1 a	24.4 ± 0.4 a	9.0 ± 0 a	5.5 ± 0.2 a	14.5 ± 0.2 a
	BC	12.3 ± 0.1 b	10.0 ± 0.1 a	22.3 ± 0.2 b	8.1 ± 0 b	4.6 ± 0.3 b	12.7 ± 0.3 b
	WV + BC	14.4 ± 0.2 a	10.1 ± 0.2 a	24.5 ± 0.1 a	9.1 ± 0.4 a	6.0 ± 0.2 a	15.1 ± 0.3 a

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating. DW: dry weight.

Table 5. Seedling root morphology of different seed treatments of WDR under dry direct-seeded system under growth chamber experiment.

Treatment	Total Root Length (cm)	Surface Area (cm ²)	Average Diameter (mm)	Root Volume (cm ³)	Tips (Seedling ⁻¹)
CK	63.8 ± 1.4 b	5.9 ± 0.1 b	0.293 ± 0.010 a	0.042 ± 0.001 a	384.7 ± 18.5 b
WV	92.2 ± 7.3 a	7.3 ± 0.5 a	0.254 ± 0.004 b	0.047 ± 0.002 a	581.7 ± 39.6 a
BC	96.8 ± 5.0 a	7.3 ± 0.5 a	0.239 ± 0.005 b	0.043 ± 0.003 a	659.9 ± 100.1 a
WV + BC	99.0 ± 5.5 a	7.6 ± 0.4 a	0.246 ± 0.008 b	0.047 ± 0.002 a	667.0 ± 49.4 a

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

**Figure 5.** Representative root scanned images of different seed treated rice seedlings at 14 DAS under growth chamber experiment. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

3.2. α -Amylase Activity, Soluble Sugar, and Soluble Protein

Both WV and WV + BC significantly promoted α -AMS, soluble sugar, and soluble protein content during the emergence of WDR (Figure 6). When compared with CK, WV increased the α -AMS by 11–128%, presenting the maximum (128%) at 0 DAS, whereas WV + BC promoted the α -AMS by 14–153%, achieving the highest (153%) at 0 DAS. The content of soluble sugar in WV and WV + BC was enhanced by 36% and 51% at 0 DAS, 41% and 38% at 2 DAS, 35% and 50% at 5 DAS, and 14% and 17% at 8 DAS, respectively (Figure 6b). At 0 DAS, pretreated seeds displayed notably higher soluble protein than untreated seeds (Figure 6c). When compared to CK, the soluble protein content at 2, 5, and 8 DAS in WV was markedly improved by 46%, 49%, and 33%, respectively, while the enhancement of soluble protein for the respective time points in WV + BC was 35%, 51%, and 34%, respectively. WV and WV + BC were the most effective for promoting these biochemical contents.

Similarly, the experimental results of seed treatments were also shown in the field experiments. Seed treatments considerably enhanced soluble sugar and soluble protein,

except for BC treatment, which was comparable to CK (Figure 7). WV and WV + BC increased soluble sugar and soluble protein content by 19% and 11% and 80% and 63%, respectively, in comparison with the control.

3.3. Gas Exchange Parameters and Physiological Indicators

Pre-sowing seed treatments exerted a positive effect on gas exchange parameters, e.g., A , g_{sw} , E , and WUE (Figure 8). WV, BC, and WV + BC at HD significantly improved A , g_{sw} , and WUE. When compared to the control, A was increased up to 19%, 31%, and 20% by WV, BC, and WV + BC, respectively (Figure 8a). The respective increments for g_{sw} were 26%, 66%, and 31%, respectively (Figure 8b). WV, BC, and WV + BC markedly promoted WUE by 15%, 11%, and 14%, respectively, compared to CK (Figure 8d). Additionally, BC markedly boosted 20% regarding E , recording the maximum (Figure 8c).

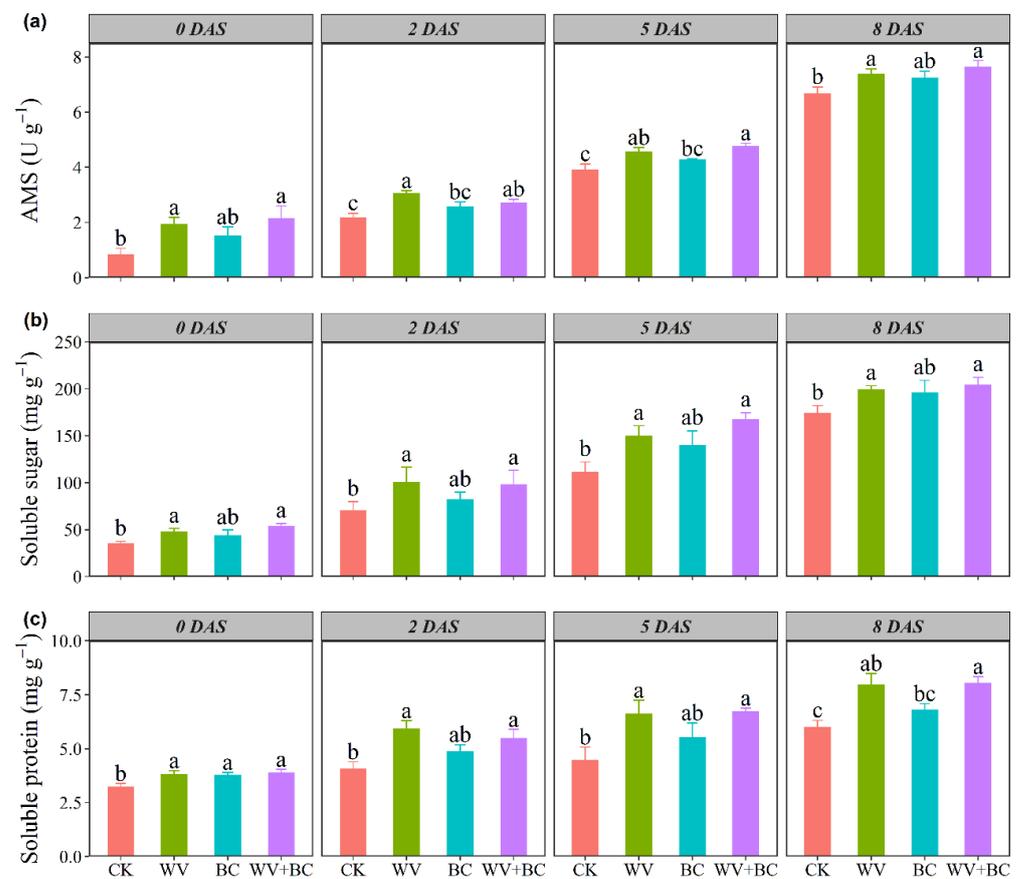


Figure 6. Dynamic changes in (a) AMS (b) soluble sugar (c) soluble protein of rice seeds and seedlings in different seed treatments at 0, 2, 5, 8 DAS under growth chamber experiment. Different letters show a significant difference between treatments at $p = 0.05$ according to LSD test. Error bars indicate standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

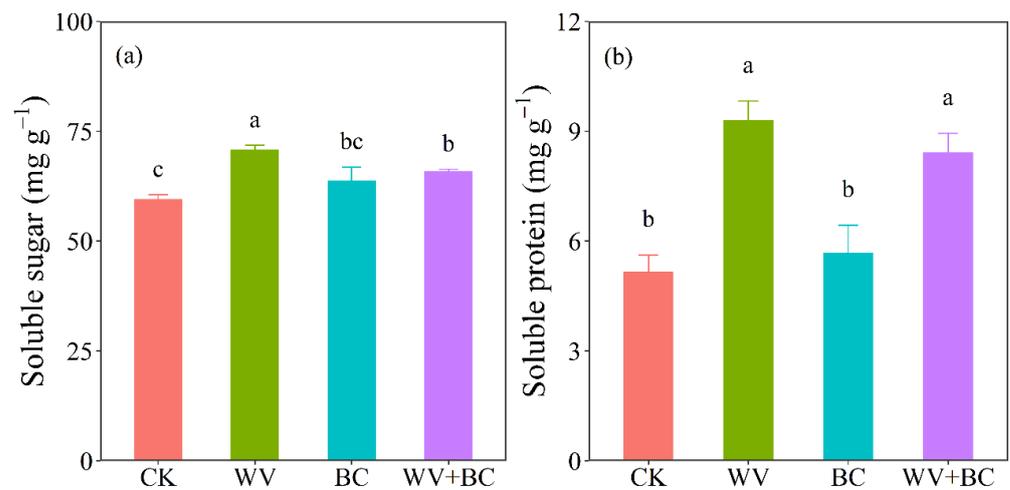


Figure 7. Effects of different seed treatments on (a) soluble sugar and (b) soluble protein of WDR at heading stage under field experiment. Different letters show a significant difference between treatments at $p = 0.05$ according to LSD test. Error bars indicate standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

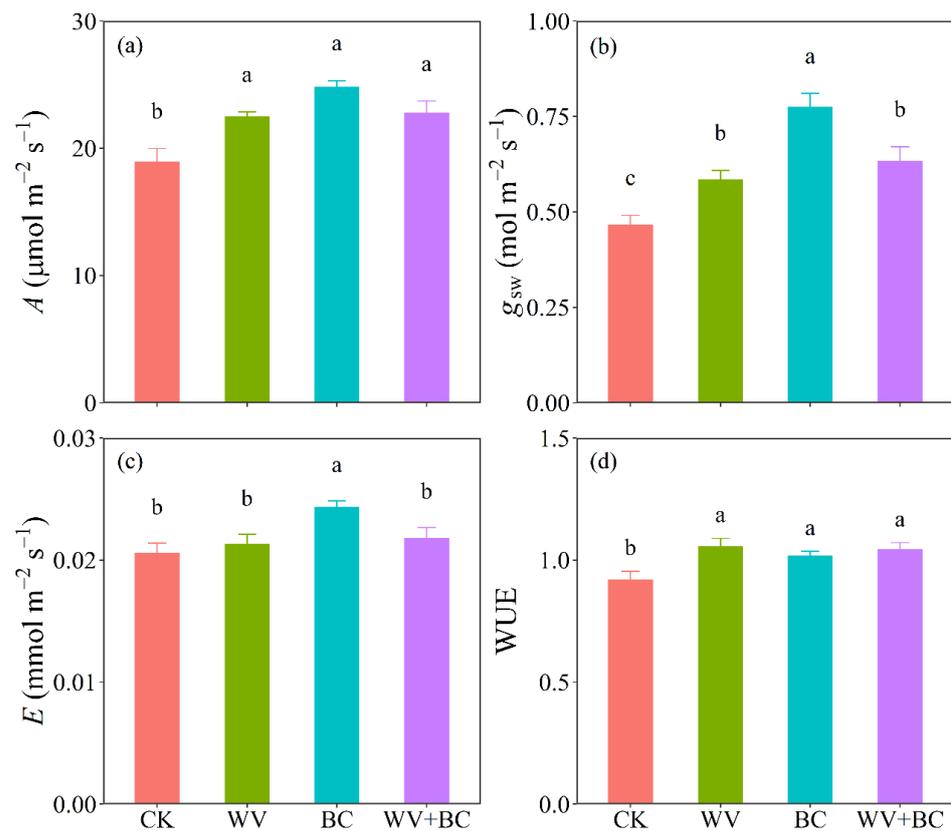


Figure 8. Effects of different seed treatments on (a) A , (b) g_{sw} , (c) E and (d) WUE of WDR at heading stage under field experiment. Different letters show a significant difference between treatments at $p = 0.05$ according to LSD test. Error bars indicate standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating. A : net photosynthetic rate, E : transpiration rate, g_{sw} : stomatal conductance to vapor diffusion, WUE: water use efficiency (A/E).

3.4. Plant Height, Tillers, LAI, and Total Dry Weight

Seed treatments significantly affected agronomic traits, i.e., tillers, LAI, and total dry weight (Table 6).

Table 6. Agronomic traits of different seed treatments of WDR at different growth stages under field experiment.

Trait	Treatment	MT	PI	HD	PM
Plant height (cm)	CK	88.8 ± 3.2 a	101.6 ± 3.8 bc	133.9 ± 2.1 a	136.6 ± 0.9 a
	WV	93.7 ± 3.9 a	108.1 ± 1.8 a	131.2 ± 2.8 a	134.1 ± 0.8 a
	BC	86.3 ± 3.6 a	97.9 ± 2.3 c	132.5 ± 1.8 a	133.5 ± 1.6 a
	WV + BC	91.9 ± 2.8 a	106.9 ± 4.4 ab	133.9 ± 0.1 a	134.3 ± 1.5 a
Tillers (m ⁻²)	CK	352 ± 12 c	311 ± 11 c	246 ± 4 b	211 ± 12 b
	WV	421 ± 16 ab	370 ± 9 ab	292 ± 6 a	260 ± 17 a
	BC	392 ± 23 bc	343 ± 20 bc	265 ± 8 b	231 ± 12 ab
	WV + BC	445 ± 12 a	398 ± 18 a	302 ± 8 a	261 ± 4 a
LAI	CK	2.33 ± 0.17 c	4.27 ± 0.27 b	4.54 ± 0.21 b	-
	WV	3.47 ± 0.44 ab	5.76 ± 0.36 a	6.37 ± 0.41 a	-
	BC	2.58 ± 0.36 bc	5.77 ± 0.12 a	6.21 ± 0.60 a	-
	WV + BC	3.85 ± 0.05 a	5.96 ± 0.53 a	6.47 ± 0.64 a	-
Total dry weight (t ha ⁻¹)	CK	2.61 ± 0.11 b	6.70 ± 0.29 b	11.30 ± 0.34 b	13.21 ± 0.70 b
	WV	3.72 ± 0.28 a	8.51 ± 0.31 a	13.12 ± 0.64 a	15.64 ± 0.69 a
	BC	2.78 ± 0.23 b	7.15 ± 0.18 b	11.98 ± 0.62 ab	14.00 ± 0.77 ab
	WV + BC	4.18 ± 0.10 a	8.70 ± 0.50 a	13.58 ± 0.45 a	15.96 ± 0.74 a

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating. MT: mid-tillering, PI: panicle initiation, HD: heading stage, PM: physiological maturity, LAI: leaf area index.

Pre-sowing seed treatments effectively influenced the tiller number. The tiller number of dry-DSR was gradually reduced with an increase in the growth stage. Furthermore, at the PM stage, the percent increase of tillers in the individual application of WV and BC was 23% and 9%, respectively, compared with CK. The co-application of WV + BC was the most effective, enhancing 24% higher tillers.

The greater LAI across all the seed treatments was detected during different growth periods, with LAI in all plots achieving its maximum at HD. Moreover, LAI induced by the application of WV and BC alone was increased by 40% and 37%, respectively, and the co-application of WV + BC was 43%, compared to CK (Table 6).

The effect of seed treatments on the total dry weight during growth periods was consistent with LAI. Likewise, total dry weight was gradually accumulated from MT to PM in all the plots, achieving its maximum at maturity. At the MT stage, WV treatment alone significantly increased the dry weight by 43%, whereas SC treatment alone was statistically similar to CK, increasing 6% biomass as compared to the control. The co-treatment WV + BC outperformed the untreated control and markedly increased the total dry weight by 60%.

3.5. Yield and Yield Components

Data regarding the rice yield and yield components at maturity are exhibited in Table 7. WV, BC, and WV + BC promoted grain yield, panicles, grain filling rate, and harvest index. Moreover, the improvement of grain yield was primarily attributed to the increment of panicle numbers and grain filling rate. The individual treatment of WV and BC produced 19% and 12% higher rice yield, respectively, compared to control, while the combination treatment of WV + BC resulted in a 20% higher yield. Similarly, WV and BC increased the panicles by 15% and 5%, respectively, and WV + BC increased the panicles by 15%. Compared to the control, the grain filling percentage in WV and BC was improved by 11% and 4%, respectively; however, the respective improvement in WV + BC was 8%. Additionally, WV and WV + BC enhanced the harvest index by 10% and 7%, respectively, compared with the control. Overall, WV and WV + BC had the best performance in grain yield.

Table 7. Grain yield, yield components, and harvest index of different seed treatments of WDR under field experiment.

Treatment	Grain Yield (t ha ⁻¹)	Panicles (m ⁻²)	Spikelets per Panicle	Grain Filling Rate (%)	1000 Grain Weight (g)	Harvest Index
CK	5.27 ± 0.30 b	182 ± 9 b	164 ± 4 a	64.0 ± 1.2 c	29.6 ± 0.3 a	0.450 ± 0.005 b
WV	6.25 ± 0.22 a	209 ± 6 a	162 ± 4 a	70.8 ± 0.8 a	28.7 ± 0.6 a	0.495 ± 0.003 a
BC	5.90 ± 0.27 ab	192 ± 8 ab	171 ± 10 a	66.3 ± 0.8 bc	28.9 ± 0.3 a	0.447 ± 0.005 b
WV + BC	6.32 ± 0.28 a	214 ± 1 a	159 ± 3 a	69.0 ± 0.7 ab	28.7 ± 0 a	0.483 ± 0.006 a

Different letters within the columns show a significant difference between treatments at $p = 0.05$ according to LSD test. \pm SE indicates standard error of three replicates. CK: untreated seeds, WV: wood vinegar seed priming, BC: biochar seed coating, WV + BC: co-treatment of wood vinegar priming and biochar coating.

4. Discussion

4.1. Effect of WV Seed Priming on Germination and Seedling Growth

The effect of WV priming on germination and seedling growth relied on the application of an optimal concentration due to the inhibition effects of high concentrations (Table 1). Previous studies widely reported the repression influences with high content wood vinegar [19]. The negative effects on germination might be attributed to the phenolic compounds [37] or the high acidity generated by WV [19]. Indeed, the seeds in our experiments primed with 1:10 WV displayed a brown color, which revealed that this high concentration caused damage to the rice seeds to some extent. The current study indicated that seed priming with 1:50 WV performed optimum promotion for WDR seedlings (Table 1). This promoting effectiveness can probably be explained by butanolide, acetic acid, and catechol, etc., which are present in wood vinegar [38]. Likewise, more attention should be paid to various alcohols, which are reported to have stimulatory effectiveness on germination and plant growth [39].

The results from the laboratory study and natural study showed that WV depicted a significant improvement in seedling length and dry weight (Table 4 and Figure 4). Our results on stand establishment are consistent with the finding reported by Simma et al. [40], who primed rice seeds with 1:300 wood vinegar and observed the stimulating effects on the germination percentage and seedling establishment. Furthermore, a few reports have documented that wood vinegar could produce positive influences on plant development [18,19]. This promotion effect could be ascribed to multiple compounds in wood vinegar, such as karrikinolide or karrikins-plant hormones, which were proved to improve early seedling growth of different plant species including maize [41] and tomato [42]. Starch metabolism is defined as the capability of plants to degrade starch into soluble sugars, which plays a crucial role in reflecting the seedling vigor during emergence and early seedling establishment. In rice, amylase activity is highly induced during germination [43]. Soluble proteins could provide the food supply and specific proteins, i.e., cell membrane transport protein, to young seedlings during the degradation of seed storage proteins [44]. The present results revealed that the relatively high soluble sugar and protein accumulation is induced by WV (Figure 6). Therefore, the higher starch metabolism and protein content may furnish the substrates necessary for generating the energy and consequently result in a significant increment in crop growth.

4.2. Effect of BC Seed Coating on Germination and Seedling Growth

The effect of biochar seed coating on seedling emergence and growth varied with the rates of biochar application, and the highest content (BC50) might be detrimental (Table 2). The inhibitory effects of the higher biochar can be responsible for the high alkalinity of biochar. Most biochars were generally alkaline, and high alkalinity could inhibit crop emergence at high doses [45]. Our explanation was also confirmed by the research of Williams et al. [46], which reported that biochar coating had adverse impacts on seed germination when employing excessive biochar (100% biochar content) to coated seeds.

In this regard, biochar coating with a 20% ratio presented a pronounced promotion in seedling establishment under dry direct-seeded conditions regardless of laboratory study and field study (Tables 2 and 4 and Figure 2). It could be speculated that the primary contributor to increasing emergence and growth coating with biochar is the enhancement of water availability around the seed, thereby producing favorable environments for seedling establishment. Additionally, due to the rich macro- and microelements and developed porous structure, biochar could absorb and provide nutrients and thus enable them to become slowly released [47]. Considerable literature reported that biochar could provide a habitat for bacterial proliferation and life due to their high porosity and elevated concentrations of organic carbon and nutrients [47,48]. As a result, biochar may stimulate the proliferation of beneficial microorganisms around the seedling, which positively affects seedling growth. Our speculations were also confirmed by the improvement of seedling root development (Table 5 and Figure 5). A is a determinant of plant growth and assimilation accumulation [49]. Furthermore, other parameters, such as g_{sw} , E , and chlorophyll fluorescence, govern plant health [50]. Our experimental results from the field revealed that BC treated plants maintained much higher A , g_{sw} , and E in comparison to untreated rice plants (Figure 8). The possible explanation would be that biochar coating provided an optimal environment for carbon assimilation, thereby promoting the photosynthetic capacity at heading stages due to the ability of biochar to retain moisture and nutrients [51].

4.3. Effect of Co-Treatment WV + BC on Germination and Seedling Growth

Combining wood vinegar priming and biochar coating as an approach to promote crop germination and growth is a new practice. In this study, the combined application of wood vinegar and biochar (i.e., WV + BC) can produce a certain positive interaction, which can effectively promote crop emergence characteristics and seedling growth than the application of wood vinegar or biochar alone (Tables 1 and 3–5).

Our findings suggest that WV has a distinct advantage in stimulating the potential growth capacity of WDR. WV priming treatment not only significantly improved seed vigor and emergence rate and promoted tillering during the early growth of the plant but also had an effect on the growth characteristics of the crop at the late growth stage, resulting in higher effective panicle numbers and improvement of grain yield (Table 7). Seed coating with biochar has considerable virtues, including enlarging seed size, improving the fluidity of precise sowing, providing a better supply of water and nutrient for seeds, and protecting seeds from pests and birds. Present results indicated that BC effectively influenced plant emergence and seedling establishment (Tables 2–5). However, BC has no stimulating effect on seeds and plants, so it has little effect on the crop growth during the middle and late stages but can ultimately increase grain yield by 12% (Table 7). Therefore, we believe that the co-application of wood vinegar and biochar into the seed can be considered an effective and sustainable seed treatment technology.

4.4. Sustainability and Circular Economy in Agriculture

It is reported that vast amounts of agricultural wastes, such as fruit orchards, crop straw, and forest woods, are annually produced, which lead to a series of environmental problems if they are casually discarded [52]. The generation of wood vinegar and biochar via the pyrolysis process could be a sustainable strategy for the adequate disposal of biomass residues; meanwhile, it may play an essential role in advancing a circular economy in the agriculture sector [53,54]. The diverse value-added and beneficial chemicals of wood vinegar could not only promote the production and quality of crops [55] simultaneously but also improve the disease resistance of plants, which is conducive to reducing the application of chemical pesticides [56]. Furthermore, the acquired biochar can be integrated into conventional fertilizer based on the seed coating technology to generate biochar-based slow-release fertilizer, which contributes to minimizing the negative impact of chemical fertilizer on the environment. Therefore, we consider that the application of wood vinegar

and biochar into the agricultural practice could promote sustainability and circular economy in agriculture.

5. Conclusions

The current studies showed WV priming with 1:50, and BC coating with 20% was the optimal ratio for promoting germination and stand establishment. The individual treatment of WV or BC facilitated emergence attributes, such as improving the emergence percentage and vigor index and reducing mean emergence time. The application of WV or BC alone also stimulated rice seedling growth, including biomass production and root development. Moreover, the combined application of WV and BC exerted long-lasting and persistent effects, which had a significant impact on tiller number, leaf area expansion, biomass accumulation, and panicle number, ultimately resulting in higher grain yield by 20%. The present study revealed that WV and WV + BC were the most effective treatment in both laboratory and field studies. However, further studies to quantify the positive effect of seed treatments, particularly in farmer fields, would contribute to scaling these seed invigoration technologies under the DSR system.

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