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Enhancing Seedling Performance Direct Seeded Rice under Anaerobic Germination Condition by Seed Invigoration

(Meningkatkan Prestasi Anak Benih Padi Pembenihan Terus di bawah Keadaan Percambahan Anaerobik dengan Pencergasan Benih)

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ABSTRACT

In an effort to address labor scarcity and enhance the cropping index in tidal swamp areas, farmers utilize direct seeding techniques. However, flooded conditions due to tides and ebb can hinder seed germination. This study aimed to obtain the best invigoration treatment for improving seedling establishment and growth in South Sumatra's tidal swamp hotspots. In the first part of the study, 19 different seed treatments were tested on the swamp rice variety, Inpara 9, in a greenhouse. These treatments included hardening, priming, thermal treatment, and controls. Soluble sugar content and α-amylase activities of treated seeds were analyzed in the lab. The results showed that the invigorated seeds outperformed the control group in terms of seedling growth and development when they were planted directly in the ground under both anaerobic germination (AG) and anaerobic germination with extra $FeSO_4$ added to the soil (AG+Fe) conditions. All treated seeds also exhibited higher soluble sugar and α-amylase activities. A follow-up experiment in a hotspot area assessed the effectiveness of the top nine seed treatments from the first experiment under AG conditions. The flooded level was managed at around 10 cm during the experiment and Inpara-9 was the variety planted. All invigorated seeds exhibited superior seedling establishment and growth compared to the control on *in-situ* swampy land. Hardening using *Trichoderma* yielded the highest seedling establishment and performance, indicated by greater root and shoot dry weight than other treatments, followed by hardening with *Methylobacterium* td-J7 and *Methylobacterium* td-tpb3.

Keywords: Anaerobic germination; hardening, *Methylobacterium*; rice; seedling establishment

ABSTRAK

Dalam usaha menangani kekurangan buruh dan meningkatkan indeks tanaman di kawasan paya pasang surut, petani menggunakan teknik pembenihan terus. Bagaimanapun, keadaan banjir akibat air pasang dan surut boleh menghalang percambahan benih. Penyelidikan ini bertujuan untuk mendapatkan rawatan pencergasan terbaik untuk meningkatkan penumbuhan dan pertumbuhan anak benih di kawasan paya pasang surut Sumatera Selatan. Kajian bermula dengan uji kaji rumah hijau dengan 19 rawatan benih terpilih, termasuk *hardening*, penyebuan, rawatan haba dan kawalan telah diuji pada varieti padi paya, Inpara 9. Kandungan gula larut dan aktiviti α-amilase benih dirawat dianalisis dalam makmal. Keputusan menunjukkan bahawa benih yang dicergaskan mengatasi prestasi kawalan dalam perkembangan dan pertumbuhan anak benih apabila ditanam secara langsung *ex-situ* di bawah kedua-dua percambahan anaerobik (AG) dan percambahan anaerobik dengan 3000-ppm FeSO4 ditambah kepada tanah (AG+Fe). Semua benih yang dirawat juga menunjukkan aktiviti gula larut dan α-amilase yang lebih tinggi. Percubaan susulan di kawasan titik panas menilai keberkesanan rawatan sembilan benih teratas daripada percubaan pertama di bawah keadaan AG. Paras banjir telah diuruskan pada kira-kira 10 cm semasa uji kaji dan varieti yang ditanam adalah Inpara-9. Kesemua benih yang dicergaskan menunjukkan penubuhan dan pertumbuhan anak benih yang unggul berbanding dengan tanah paya kawalan in-situ. *Hardening* menggunakan *Trichoderma* menghasilkan pembentukan dan prestasi anak benih yang tertinggi, ditunjukkan oleh berat kering akar dan pucuk yang lebih besar daripada rawatan lain, diikuti dengan hardening dengan Methylobacterium td-J7 dan *Methylobacterium* td-tpb3.

Kata kunci: *Hardening; Methylobacterium;* padi; penubuhan anak benih; percambahan anaerobik

INTRODUCTION

Optimizing rice cultivation in tidal swamp areas is a strategic approach to boost national rice production beyond irrigated areas. Tidal swamp cultivation faces challenges such as labor scarcity, leading farmers to adopt direct seeding to conserve labor. Direct- Seeding Rice (DSR) offers several benefits over traditional transplanting methods. DSR improves water use efficiency and reduces greenhouse gas emissions (Joshi et al. 2013; Susilawati et al. 2019; Tao et al. 2016). Additionally, it minimizes seedling damage during transplanting, potentially resulting in higher yields. Direct seeding in swamps also positively impacts the cropping index by supporting shorter crop durations (Kaur & Singh 2017; Susilawati, Nusyamsi & Syakir 2016). On the other hand, flooded conditions due to tides and ebb can cause seeds to fail to germinate. Direct seeding in tidal swamps during early flooding can lead to hypoxia or anoxia, as waterlogged soil restricts oxygen availability for germinating seeds, reducing seedling emergence (Mondal et al. 2020). This lack of oxygen can hinder primary root and leaf development, inhibit nutrient absorption, and ultimately result in plant death (Miro & Ismail 2013).

When rice seeds are submerged in water for extended periods, they rely on oxygen dissolved in the water to carry out respiration and other metabolic processes necessary for growth and development. Additionally, swamp soils' high organic matter content can exacerbate hypoxic conditions (Su et al. 2017). As organic matter decomposes, it consumes oxygen while releasing carbon dioxide and other gases, which displace soil pore oxygen and reduce its availability for germinating seeds. Moreover, swampy soil has a low pH and high iron toxicity. Sulaeman et al. (2022) reported that the pH range in swampy areas ranges from 4.27 to 5.35, while iron toxicity can occur up to 1947 ppm. Iron toxicity poses a considerable predicament in tidal soil, particularly in areas where water circulation is hindered due to inadequate water management. Consequently, seeds may fail to develop into normal seedlings with low establishment rates. Seed germination in stagnant flooding led to a 44.6% decrease in seedling emergence and an up to 89.6% reduction in seedling growth compared to germination in aerobic conditions (Wahyuni et al. 2021).

Indicators to determine a seed's ability to germinate in anaerobic conditions include a faster water absorption rate (Yang et al. 2007), rapid water absorption within the first 48 h of imbibition (El Hendawy et al. 2011), higher coleoptile and radicle growth rates, α-amylase activities, and higher sucrose and glucose content in germinating seeds (Adachi et al. 2015). These indicators suggest that seeds capable of germinating under anaerobic germination (AG) conditions belong to AG-tolerant varieties or possess traits similar to AG-tolerant varieties. Conversely, several invigoration techniques, such as priming, hardening, and thermo-treatment can accelerate germination and seedling growth. Implementing appropriate invigoration techniques can enhance a seed's ability to germinate under AG conditions.

Priming is an invigoration technique with seed hydration until the metabolic process of germination begins, but the important embryonic structure (radicle) has not yet emerged (Matsushima & Sakagami 2013). Hydropriming increases root growth, rapid emergence, and seed vigor in all water conditions except extreme dry or extreme wet (Matsushima & Sakagami 2013), improves seedling growth rate, and improves seed germination parameters (Doley et al. 2018). Meanwhile, priming with GA_3 and kinetin improves vigor and seedling growth (Kamran et al. 2021; Sukifto et al. 2020). Hardening is useful for embryo physiological activity and associated structure (Kumar et al. 2019). Hardening is carried out by hydration and re-hydration to reach the initial moisture content, and this hydration and re-hydration cycle is carried out two or more times (Lee & Kim 2000).

Invigoration techniques with simple hardening increase rice seed germination, and if conducted with ascorbic acid, increase seed germination and yield (Shah et al. 2019), while hardening with *Methylobacterium* or *Trichoderma* increases the viability and vigor of seeds (Hasanuddin, Vina Maulidia & Syamsuddin 2016; Widajati, Salma & Lastiandika 2013). Thermal treatment with an initial temperature of 40 °C increases the germination index of rice seeds (Lee, Lee & Kwon 2002), while an initial temperature of 80 °C improves seed germination of pepino (Prohens, Soler & Nuez 1999). As shown by Wahyuni et al. (2021), hardening with GA_3, kinetin, ascorbic acid, P_2O_5, Methylobacteria, *Trichoderma*, and thermo-treatments increased the number of seeds that germinated, the length of the coleoptile, the dry weight of the seedlings, and the average time it took for seeds to germinate in laboratory experiments. This study was conducted to verify the effect of seed invigoration and find the best seed treatments on the germination and growth of rice seedlings under submerged conditions in tidal swamps.

MATERIALS AND METHODS

LOCATION

The study in a greenhouse and laboratories was conducted at the Indonesian Center for Rice Research, Subang, while a field experiment was carried out at a tidal swamp area in Karang Agung Experimental Station (2°35'47''S, 104°12'43''E, 17.4 m asl), Banyuasin Regency, South Sumatra Province, in 2021.

GREENHOUSE TESTED SCALE

A swampy rice variety, Inpara 9, was used as the planting material. Treatment consisted of two factors: germination condition (GC) and seed invigoration treatment (ST). Three GC treatments (anaerobic condition (AG), AG with Fe stressed (AG+Fe), and aerobic condition (AeG) were applied, including twenty ST treatments consisted of control, hardening, priming, and thermal treatment with several chemical and biological materials. Altogether, there were 60 treatment combinations. Detailed descriptions of each treatment in the greenhouse are shown in Table 1. The design of the experiment was a completely random block design with three replications, and each treatment involved 20 seeds per replication.

The planting medium was prepared according to the GC treatments. The container used was a plastic box measuring 50 cm in length, 30 cm in width, and 30 cm high, filled with soil to a height of 15 cm. In the AG with Fe-stressed treatment, 3000 ppm of $FeSO_4$ was added to the soil.

Before sowing, the seeds were treated according to the ST treatments. To conduct priming, seeds were soaked in a solution for a specific amount of time, drained, and dried under shade with forced air at 28 ± 3 °C until they reached their initial moisture content. Hardening was like priming, but the hydration and rehydration cycles were repeated at least twice, and the solution was used in accordance with Table 1, whereas thermal treatment was conducted at an initial temperature.

After the seed treatments were completed, some seeds were planted in a greenhouse using a water-lodged soil method with slight modifications to the method described by Manangkil et al. (2008). The seeds were sown 2 cm deep below the soil surface and then watered according to GC treatments (Table 1). Meanwhile, some other seeds were used as material to study the effect of seed invigoration treatments on the improvement of the biochemical activity of seeds. The analysis activity of the α-amylase enzyme from treated seeds was carried out following the ceralpha method referring to the AOAC Method 2002.01 using the K-CERA 06/18 kit (Megazyme 2018), and total sugar contents were determined by the phenol sulfuric method of Quero-Jiménez et al. (2019).

Data collection in the greenhouse was: (1) the number of emerged seeds, which was recorded daily according to the Association of Official Seed Analysts (1983); (2) the bioassay of seedlings in the second week after sowing (WAS): shoot length (SL in cm), root length (RL in cm), shoot dry weight (SDW in mg), and root dry weight (RDW in mg/WAS; (3) the seeds invigoration effectiveness index (EI) was calculated in each GC following DeGarmo, Sullivan and Canada (1984). On the contrary, data collection in the laboratory involved (4) activity of the α-amylase enzyme and (5) total sugar contents of treated seeds.

EVALUATION SEEDLING STAGE AT HOTSPOTS AREA OF TIDAL SWAMPS

Since waterlogging and Fe-stress often occur simultaneously in one period of time in tidal areas, it is necessary to select the best treatment suitable to overcome the challenges. A field experiment was carried out in accordance with the condition of swamp land in Karang Agung. The aim of the research was to find out the best treatment to improve seedling growth in swamp land, which is generally related to AG or AG with iron stress. The treatments were applied to find out the best treatment to improve seedling growth in swamp land so that farmers could perform direct-seed rice. While the nine best seed treatments from the greenhouse scale was used as ST treatments in field experiment at tidal swamp, a non-treated seed was used as a control. The same variety, Inpara 9, was used as the planting material with a seed rate of 40 kg ha^{-1}. The experimental design was completely randomized with three replications.

Treated seeds were sown directly in 1 m × 1 m experimental plots with a spacing of 20 cm × 10 cm, and the distance between treatments was 40 cm. The field was maintained in a flooded condition with a 10-12 cm depth for two WAS. We observed SL in cm, RL in cm, shoot dry SDW in g, and root RDW in g at two WAS.

DATA ANALYSIS

Data were analyzed using analysis of variance (ANOVA). The difference between treatments was continued analysis using the Duncan Multiple Range Test (DMRT) at $p=0.05$. In addition, the calculation of a combined EI for both AG and AG+Fe conditions on a greenhouse scale was carried out after the experiment was completed. The STs with higher EI were used as invigoration treatments in the field experiment on swampy land.

RESULTS AND DISCUSSIONS

EFFECT OF GERMINATION CONDITION AND SEED TREATMENT ON SEEDLING ESTABLISHMENT

Germination conditions (GC), seed invigoration treatment (ST), and interaction (GC*ST) significantly affected seedling establishment (SE) and all seedling growth variables (Table 2). Seedling establishment and bioassay were superior in the AeG condition compared to the AG

TABLE 1. Treatments on greenhouse tested scale

Treatment Code	Description	
Germination Condition (GC)		
AG	Anaerobic Germination	Maintain flooding with depth 10 cm for two weeks after sowing (WAS)
AG+Fe	AG with Fe stressed	As well as AG with an addition of 3000 ppm of FeSO4 to the soil to create iron toxicity stress conditions (Unoki et al. 2020)
AeG	Aerobic condition	Maintain with field capacity condition
Seed Invigoration Treatment (ST)		
H. Water	Hardening (H)	Water (H20)
H. GA3	Hardening (H)	10-ppm GA3 solution
H. ZnSO4	Hardening (H)	ZnSO4 at a concentration of 4.7 g/kg of seeds with a seed weight to solution ratio of 1:5
H. Kinetin	Hardening (H)	15-ppm kinetin solution
H. Methylo td-J7	Hardening (H)	Methylobacterium td-J7
H. Methylo td-tpb3	Hardening (H)	Methylobacterium td-tpb3
H. Tricho	Hardening (H)	Trichoderma
H. Ascorbic acid	Hardening (H)	10 mg/L ascorbic acid solution
H. P2O5	Hardening (H)	P2O5 at a concertation of 0.5 w/v
H. IAA	Hardening (H)	15-ppm IAA solution
P. Water	Priming (P)	Water (H20)
P. GA3	Priming (P)	10-ppm GA3 solution
P. ZnSO4	Priming (P)	ZnSO4 at a concentration of 4.7 g/kg of seeds with a seed weight to solution ratio of 1:5
P. Kinetin	Priming (P)	15-ppm kinetin solution
P. Methylo td-J7	Priming (P)	Metholcaterium td-J7
T.40 °C	Thermo-treatment (T)	an initial temperature of 40 °C
T. 40 °C + NaOCl	Thermo-treatment (T)	an initial temperature of 40 °C + NaOCl
T. 80 °C	Thermo-treatment (T)	an initial temperature of 80 °C
T. 80 °C + NaOCl	Thermo-treatment (T)	an initial temperature of 80 °C + NaOCl
Control		Control (Without seed treatment)

or AG+Fe conditions (Figure 1). When compared to AeG, the decrease in SE reached 72.52% and the decrease in seedling growth reached 65.21%, 80.72%, 86,49%, and 88.15% for SL, RL, SDW, and RDW, respectively, in the AG condition (Figure 1). Similar result was shown by Wahyuni et al. (2021) whereby germination seeds under AG conditions caused the highest reduction in RDW and SDW compared to AeG conditions on the laboratory scale. This outcome is likely due to the hypoxic conditions experienced during germination in AG, inhibiting root and leaf growth (Miro & Ismail 2013).

The interaction effect between GC*ST and SE and seedling growth is shown in Table 3. The SE of untreated seeds on AG was the highest (63.3%) compared to AG (0.00%) and AG+Fe condition (6.67%). All treated seeds sown in AeG conditions had higher SE than untreated seeds, which varied between 66.7% and 100%, and the highest SE was hardened with ascorbic acid (100%). In AG+Fe condition, all treated seeds improved SE compared to control, except P. with *Methylobacterium* td-J7 and T80 °C+ NaOCl, and the highest SE was shown by H. with *Methylobacterium* td-tpb3 (86%). In AG condition, some invigoration treatments were unable to increase SE compared to the control, such as H. with water, kinetin, and P. with GA_3, $ZnSO_4$, and *Methylo* td-J7, and invigoration treatment H. *Methylo* td-J7 had the highest SE.

Seedling length (SL), RL, and bioassay of seedling growth of treated seed in different germination conditions showed a similar trend with SE. Seed invigoration treatments (ST) that increased SE also exhibited a positive impact on seedling growth and dry weight of seedlings. Hardening with *Methylo* td-J7, *Methylo* td-tbp3, *Trichoderma*, P_2O_5, and $ZnSO_4$ showed higher SE and seedling growth compared to other treatments on AG and AG+Fe (Table 3).

The SE and seedling growth parameters on AG and AG+Fe conditions of seeds invigorated by hardening (H.) with GA_3, $ZnSO_4$, kinetin, and *Methylo* td-J7 were higher than those treated by priming (P.) with the same solutions (Table 3). It is suspected that this happened due to the hydration-rehydration process in two hydration cycles of hardening compared to one hydration cycle of priming, so that embryo activation and embryo enlargement were higher in hardening.

SOLUBLE SUGAR CONTENT AND A-AMYLASE ENZYME ACTIVITY

Soluble sugar (SS) and activity of α-amylase (AA) in treated seeds varied among invigoration treatments (Table 4). All invigoration treatments increased the soluble sugar (SS) content significantly compared to the control, except for thermal treatment with an initial temperature of 80 °C. The highest SS was obtained from seeds that were treated with H. kinetin and H. Methylo td-J7 (Table 5). The same trend was shown by α-amylase activity (AA), in which all treated seeds showed higher AA than control, except for T.80 °C + NaOCl and P. GA_3, which were comparable with control. The increase in SS and AA was higher in the treatment with hardening compared to priming.

Means followed by the same letter within a column are not significantly different

Indicators of seeds that are capable of germination in AG conditions are that they have higher soluble sugar than AG-susceptible seeds (Barik et al. 2020), higher α-amylase activities (Adachi et al. 2015), and also faster coleoptile and root growth (El Hendawy et al. 2011). Nearly all treated seeds demonstrated higher seedling emergence (SE), seedling growth, and bioassay of seedlings in AG and AG+Fe conditions compared to the control (Table 3), and all treated seeds also exhibited higher soluble sugar content and α-amylase activity than untreated seeds (Table 4).

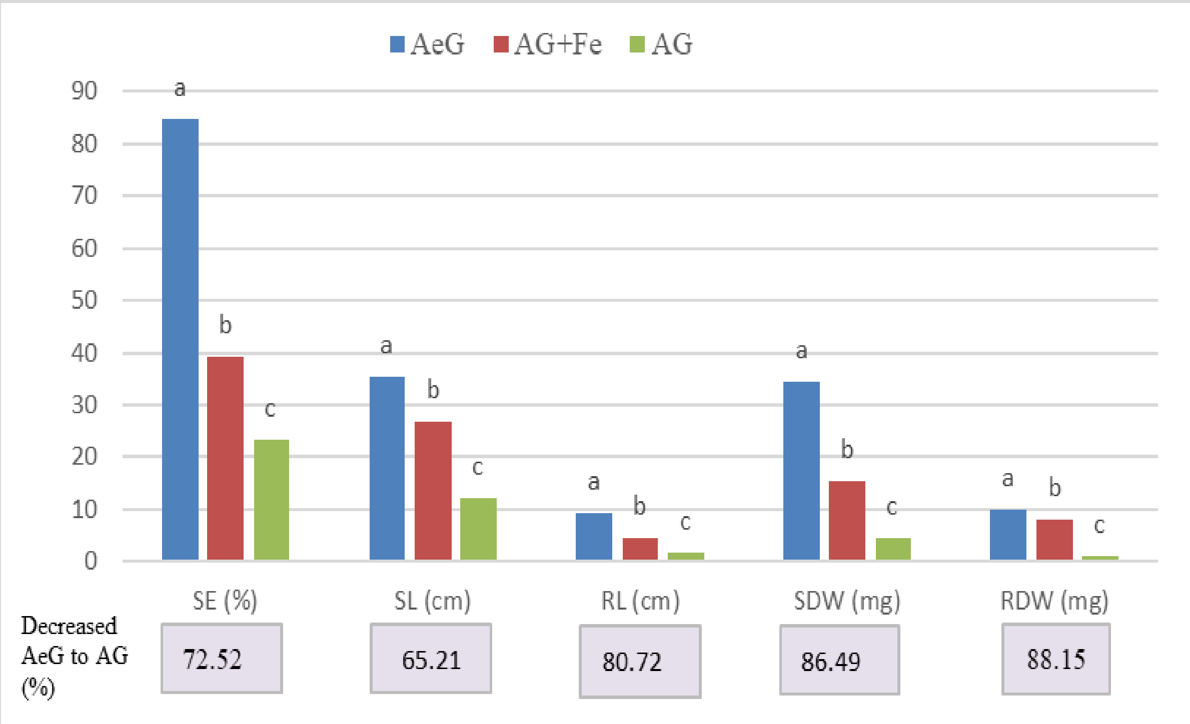
These results suggest that treated seeds could potentially increase germination tolerance in both AG conditions and AG with iron stress. High soluble sugar content and α-amylase activity were positively correlated with improved seedling establishment and performance (Table 3). According to Salleh, Nordin and Puteh (2020), higher α-amylase activity accelerates rice hydrolysis and embryonic growth, simulating germination. Meanwhile, higher shoot growth in treated seeds represents an adaptive response of water-seeded rice to acquire oxygen and serves as a major submergence avoidance or escape mechanism against low oxygen or low light situations (Manangkil et al. 2008). Seed invigoration with hardening showed better improvement than priming and thermo-treatment across all observed variables.

TABLE 2. Recapitulation F-value on analysis of variance at greenhouse experiment

Source of diversity	SE	SL	RL	SDW	RDW
Block	ns	ns	ns	ns	ns
Germination Condition (GC)	**	**	**	**	**
Seed Invigoration Treatment (ST)	**	**	**	**	**
GC*ST	**	**	**	**	**
CV (%)	14.78	14.39	11.96	14.93	10.60

** and *: significant at the 0.01 level and 0.05 level, ns: not significantly different

SE= Seedling establishment; SL= Shoot length; RL= Root length; SDW= Shoot dry weight; RDW= Root dry weight. Data RDW was transformed to SQRT (x+0.5) before analysis



Means in one variable followed by the same letter are not significantly different in the DMRT test at the 5% level

FIGURE 1. Effect of germination condition on seedling establishment (SE) and bioassay of seedling under greenhouse experiment

CORRELATION BETWEEN PARAMETERS IN GREENHOUSE EXPERIMENT

The correlation in the AG+Fe condition showed a significant positive relationship among SE, SL, RL, SDW, and RDW. The same positive correlation also happened between SS, AA with SE, and all germination growth parameters. A similar trend was shown in the AG condition, whereby relations amongst the traits studied were positive and significant, except for the correlation between SS with SE and seedling traits. Meanwhile, the correlation coefficient in the AeG condition also displayed positive association amongst parameters observed, except a negative and weak association was shown between SS with SE and RDW (Figure 2).

INDEX OF EFFECTIVENESS ON GREENHOUSE EXPERIMENT

The effectiveness index (EI) of invigoration treatment in seedling establishment and seedling growth is shown in Figure 3. In the AeG condition, all invigorated seeds had a higher EI than the control, and the three highest EIs were shown by seeds treated with H. GA_3, (1.744), followed by H. *Methylobacterium* td-tpb3 (1.684), and H. *Trichodherma* (1.672). These indicate that the three STs had the opportunity to be used as seed invigorators under field capacity conditions.

Invigoration treatment applied under AG+Fe conditions also increased EI, except for P with *Methylobacterium* td-J7 and thermotreatment at 80 °C + NaOCl. Figure 3 shows that the best three invigoration treatments that provided the highest IE were H. with *Methylobacterium* td-tpb3 (1.505), followed by H. water (1.340), and H. with *Methylobacterium* td-J7 (1.168). So, the ST had the opportunity to be used to improve SE and seedling growth under AG with iron stress, which usually occurs in tidal swamps.

In the AG condition, five treatments (H. Water, H. Kinetin, P. GA_3, P. $ZnSO_4$, and P *Methylo* td-J7) had comparable EI to the control, but the other invigoration

TABLE 3. Interaction effect of germination condition and seed invigoration treatment on seedling establishment, shoot length, root length, and root dry weight

Seeds invigoration treatment	Germination condition											
	AG	AG+Fe	AeG	AG	AG+Fe	AeG	AG	AG+Fe	AeG	AG	AG+Fe	AeG
		SE (%)			SL (cm)			RL (cm)			RDW (mg)	
H. water	0.00 g	70.00 ab	96.67 a	0.00 f	35.05 ab	37.20 b-e	0.00 f	6.23 a-d	11.14 a	0.00 d	12.97 bcd	7.77 de
H. GA3	6.67 efg	63.33 bc	90.00 ab	9.17 de	34.23 ab	37.85 bcd	0.83 ef	5.36 a-e	10.31 abc	0.83 cd	8.58 c-g	17.09 a
H. ZnSO4	56.67 bc	60.00 bc	80.00 a-d	24.73 ab	31.79 abc	34.66 def	3.67 abc	4.48 b-f	8.39 c-f	1.10 cd	3.28 ghi	8.41 de
H. kinetin	0.00 g	43.33 cde	90.00 ab	0.00 f	33.27 abc	35.25 c-f	0.00 f	6.65 ab	9.27 a-e	0.00 d	15.32 ab	12.77 b
H. Methylo td-J7	86.67 a	50.00 bcd	83.33 a-d	28.77 a	35.88 a	40.43 ab	4.31 ab	6.50 abc	8.78 b-e	3.22 ab	13.48 abc	9.96 bcd
H. Methylo td-tpb3	46.67 cd	90.00 a	90.00 ab	22.68 ab	36.83 a	35.87 c-f	3.01 bc	6.07 a-e	9.80 a-d	2.21 abc	19.31 a	16.55 a
H. Trichoderma	43.33 d	56.67 bc	96.67 a	27.58 a	32.24 abc	41.73 a	4.23 ab	7.18 a	10.31 abc	3.55 ab	6.85 c-i	11.21 bcd
H. ascorbic acid	10.00 efg	63.33 bc	100 a	21.17 ab	32.35 abc	35.47 c-f	4.67 a	5.88 a-e	10.75 ab	3.80 a	8.32 c-g	8.00 de
H. P2O5	63.33 b	66.67 bc	96.67 a	23.97 ab	32.76 abc	38.57 abc	4.09 ab	4.31 b-f	10.15 abc	3.63 ab	8.58 c-g	9.44 bcd
H. IAA	40.00 d	50.00 bcd	83.33 a-d	18.41 bc	30.57 abc	37.65 bcd	2.42 cd	4.87 a-f	10.25 abd	0.98 c	12.22 b-e	5.71 e
P. water	16.67 ef	16.67 fg	70.00 bcd	12.89 cd	31.25 abc	32.18 fg	1.42 def	3.50 ef	8.35 c-f	1.01 cd	3.82 f-i	9.09 b-e
P. GA3	0.00 g	23.33 efg	90.00 ab	0.00 f	31.97 abc	34.98 c-f	0.00 f	4.80 a-f	9.22 a-e	0.00 d	11.69 b-e	10.35 bcd
P. ZnSO4	0.00 g	33.33 def	90.00 ab	0.00 f	23.46 bc	34.96 c-f	0.00 f	3.82 def	8.97 b-e	0.00 d	4.32 f-i	8.12 de
P. kinetin	20.00 e	23.33 efg	80.00 a-d	18.87 bc	25.72 abc	33.41 efg	2.27 cde	3.93 c-f	8.92 b-e	0.83 cd	6.72 d-i	8.86 cde
P Methylo td-J7	0.00 g	6.67 g	66.67 cd	0.00 f	3.28 d	34.37 def	0.00 f	0.58 g	7.69 ef	0.00 d	0.45 i	12.53 bc
T. 40 °C	3.33 fg	23.33 efg	86.67 abc	4.67 ef	26.08 abc	34.43 def	0.50 f	5.75 a-e	7.93 def	0.13 d	8.06 c-g	12.17 bc
T. 40 °C+NaOCL	20.00 e	16.67 fg	93.33 a	6.44 def	27.75 abc	31.97 fg	0.00 f	4.37 b-f	7.94 def	0.00 d	7.25 c-g	5.65 e
T. 80 °C	16.67 ef	13.33 fg	83.33 a-d	8.34 def	21.08 c	34.81 c-f	0.95 def	2.67 fg	9.53 a-e	0.36 cd	5.87 e-i	7.71 de
T. 80 °C+NaOCL	36.67 d	6.67 g	66.67 cd	18.81 bc	6.33 d	31.97 fg	3.08 bc	0.67 g	9.10 b-e	1.73 bcd	0.33 i	7.99 de
Control	0.00 g	6.67 g	63.33 d	0.00 f	6.92 d	30.62 g	0.00 f	1.08 g	6.75 f	0.00 d	0.97 hi	8.00 de

Means followed by the same letter within a column are not significantly different. H= hardening; P= Priming; T= thermal treatment. SE= Seedling establishment; SL= Shoot length; RL= Root length; RDW= Root dry weight

TABLE 4. Soluble sugar (SS) total and α-amylase activity (AA) in various invigoration seed treatments

Seed treatments	SS (mg/g)	AA (CU/g)
H. water	40.04 b	12.54 h
H. GA_3	35.84 cd	32.11 c
H. $ZnSO_4$	34.36 de	20.58 f
H. kinetin	41.13 ab	23.14 e
H. *Methylo* td-J7	42.60 a	22.34 ef
H. *Methylo* td-tpb3	30.53 f	47.42 a
H. *Trichoderma*	23.18 g	40.90 b
H. ascorbic acid	20.09 h	23.09 e
H. P_2O_5	33.18 e	26.92 d
H. IAA	37.28 c	16.79 g
P. water	12.23 i	9.33 ij
P. GA_3	9.69 k	6.64 k
P. $ZnSO_4$	19.63 h	8.13 ijk
P. kinetin	29.94 f	7.20 ijk
P. *Methylo* td-J7	10.29 jk	15.38 g
T. 40 °C	10.77 ijk	9.48 i
T. 40 °C+ NaOCL	11.60 ij	9.14 ijk
T. 80 °C	4.21 m	8.60 ijk
T. 80 °C+ NaOCL	9.69 k	6.72 ijk
Control	7.89 l	7.28 ijk

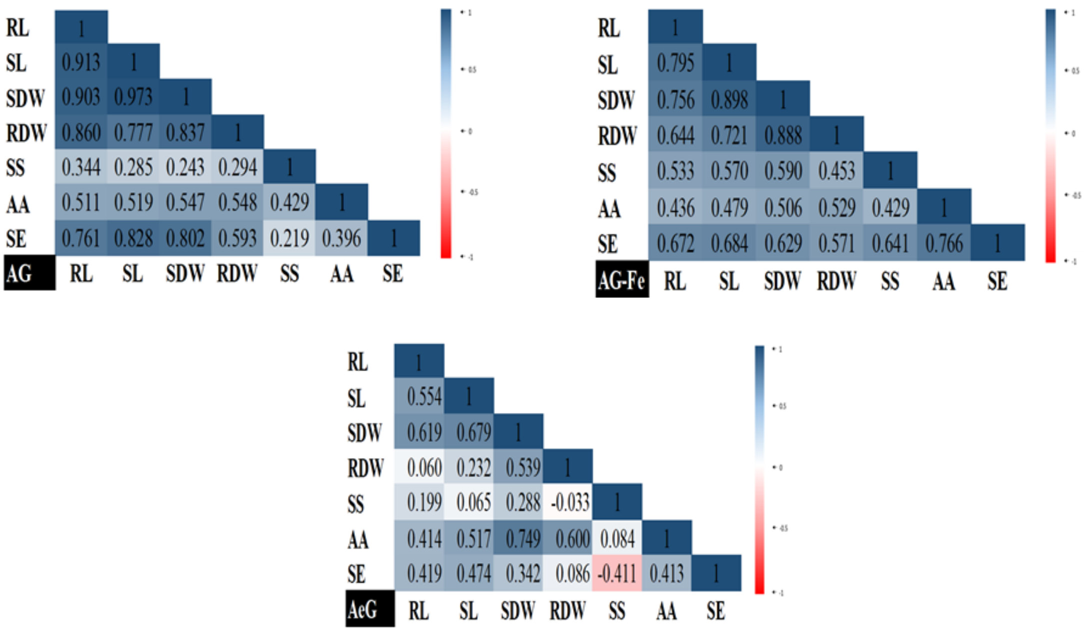


FIGURE 2. Pearson's correlation coefficients in AG, AG+Fe, and AeG conditions

treatments showed better EI than the control. The three highest EI in AG condition were H. *Methylo* td-J7 (0.965), followed by H.P_2O_5 (0.794), and H. *Trichoderma* (0.708), so the three STs had the possibility of being used as invigoration treatments in direct seeding on swampy land.

The index effectiveness indicates the relative effects of treatments on all observed variables (DeGarmo, Sullivan & Canada 1984). The index in both AG and AG+Fe conditions (Figure 3, bottom) indicated the effectiveness of invigoration treatments in improving seedling traits in anaerobic conditions in swampy areas. The nine best seed treatments, which had a higher effectiveness index, were: H. *Methylo* td-J7, followed by H. *Methylo* td-tpb3, H. P_2O_5, H. *Trichoderma*, H. $ZnSO_4$, H. Ascorbic acid, H. IAA, T.40 °C, and H. GA_3. These nine ST treatments were then used for invigoration treatments in direct seeding in the field experiment in a swampy area.

EFFECT OF SEED TREATMENT ON DIRECT SEEDING AT AG CONDITIONS IN A SWAMPY AREA AT KARANG AGUNG, BANYUASIN, SOUTH SUMATERA

Seed invigoration treatments (ST) had a significant effect on SE and seedling growth of Inpara 9 at AG conditions in a swampy area (Table 5). The result of the direct seeding experiment showed that invigoration treatments improved SE and were significantly higher compared to the control, except for the H. GA_3 and H. P_2O_5 treatments, which were comparable to the control (Table 6). Hardening with *Trichoderma, Methylo* td-J7, and *Methylo* td-tpb3 showed a high increase in SE, which reached up to 46.4% compared to the untreated seeds.

The effect of seed treatment on seedling growth of direct seeding rice in AG conditions was more obvious in improving the SDW and RDW compared with the SL and RL. Even though the SL of treated seeds was not significantly different compared to the control, the treated seeds had a heavier SDW than the control, except for H. GA_3. The same response occurred in the increase in RDW; all treated seeds showed a higher RDW than the untreated ones. Hardening with Zn SO4, *Trichoderma*, and *Methylo* td-J7 were the three top seed treatments for improving SDW, while H. with *Trichoderma, Methylo* td-J7, and P_2O_5 showed the highest increase in RDW compared to other treatments.

EFFECTIVENESS INDEX OF SEED TREATMENT ON DIRECT SEEDING IN SWAMPY AREAS ON AG CONDITION

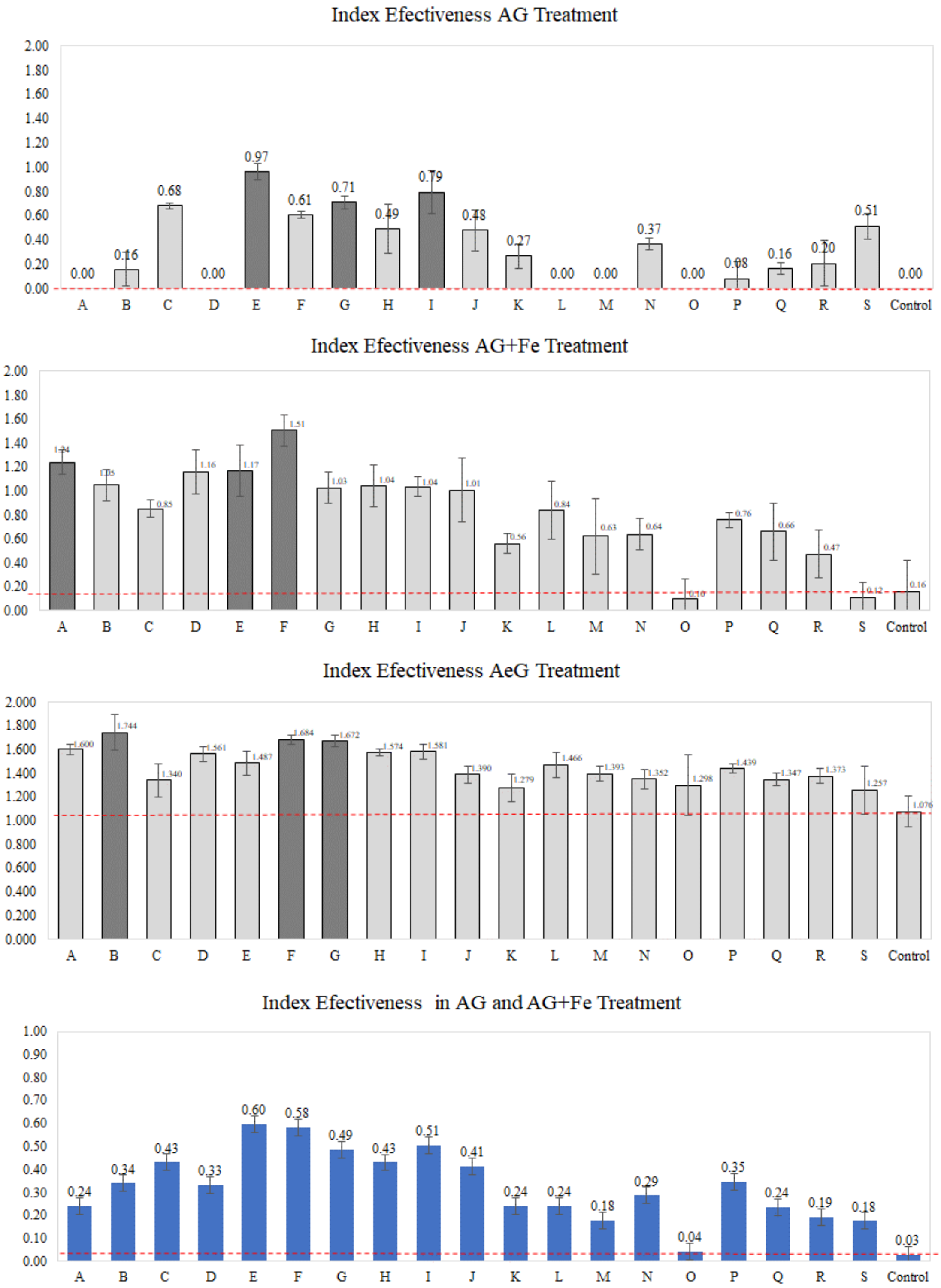
Index effectiveness results showed that all treated seeds had a higher effectiveness index than the control, except for hardening with GA_3. The higher effectiveness index indicated the more effective treatment in improving SE and seedling performance of direct seeding in swamps under AG or AG+Fe conditions. Based on the effectiveness index, the three most effective STs were hardening with *Trichoderma, Methylobacterium* td-J7, followed by H. *Methlobacterium* td-tpb3 (Figure 4).

Hardening seeds with *Trichoderma* was found to be the best treatment for improving seedling emergence and growth in an *in-situ* experiment within a swampy area under AG conditions (Table 6, Figure 4). Other studies have demonstrated that under optimal germination conditions, seed treatment with *Trichoderma* significantly increased seedling growth, seedling rate, vigor index, germination speed of rice seeds (Doni et al. 2014; Hasanuddin, Vina Maulidia & Syamsuddin 2016), root growth (Cai et al. 2013), and induced phenolic production in seeds during germination, leading to improved germination rate and seed vigor (Zheng & Shetty 2000). In the present study, seed hardening with *Trichoderma* under AG conditions also exhibited the highest SE and RDW, along with a high SDW (Table 6).

Trichoderma produces numerous essential, volatile secondary metabolites that function against various plant pathogens and promote plant growth (Bhardwaj & Khumar 2017). Additionally, Swain et al. (2021) reported that seeds treated with Trichoderma showed higher levels of defense enzymes, such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, and total phenolics, compared to untreated seeds, indicating an enhanced stress tolerance ability in rice crops.

Hardening with *Methylobacterium* td-J7 and *Methylobacterium* td-tpb3 were ranked as the second and third best treatments to enhance the performance of direct seeded rice plants grown in swampy areas under AG conditions (Table 6, Figure 4). It is suspected that the ability of methylobacteria to use single carbon compounds as a carbon source and generate Pyrrolo Quinoline Quinone (PQQ) (Hapsari et al. 2016), a superoxide capable of scavenging free radicals (He et al. 2003) contributes to these positive effects.

Methylobacterium's capacity to produce IAA, GA_3, trans-zeatin, and antioxidant tocopherol (Hapsari et al. 2016; Kosmiatin, Husni & Salma 2020; Subhaswaraj et al. 2017) and cytokinins (Palberg et al. 2022) is thought to be the reason these treatments can promote germination and stimulate seedling growth under anaerobic conditions. Other studies have also found that *Methylobacterium* stimulated early growth of shoot and root, improved germination, and increased root radicle length of *C. pumila* seeds under cadmium-stress conditions (Sanchez-Lopez et al. 2018).



A: H. Water, B: H. GA3, C: H. ZnSO4, D: H. Kinetin, E: H. Methylo td-J7, F: H. Methylo td-tpb3, G: H. Trichoderma, H: H. Ascorbic acid, I: H. P2O5, J; H. IAA, K: P. Water, L: P. GA3, M: P. ZnSO4, N: P. Kinetin, O: P. Methylo td-J7, P: T.40 °C, Q: T.40 °C+NaOCl, R: T.80 °C, S: T.80 °C+NaOCl

FIGURE 3. Effectiveness index of seed treatments in the greenhouse under three germination condition and combination of AG and AG+Fe treatment

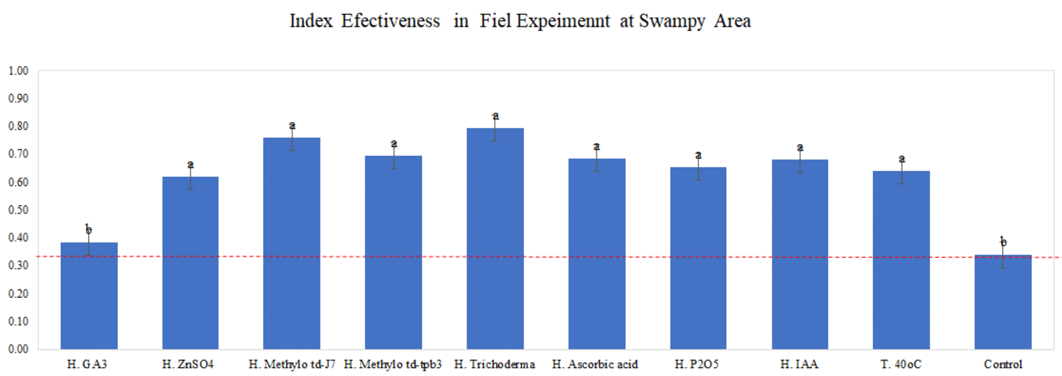
TABLE 7. F-value of seed treatment at AG condition in swampy area at Karang Agung Experimental Farm, Banyuasin Regency, South Sumatra Province

Source of diversity	SE	SL	RL	SDW	RDW
Block	ns	ns	ns	ns	ns
Seed invigoration treatment	*	*	**	**	**
CV (%)	12.23	3.39	7.66	11.40	6.15

** and *: significant at level ($p \leq 0.01$) and ($p \leq 0.05$), ns: not significantly different

TABLE 8. Effect seed treatment on field establishment and seedling growth in swamps of Karang Agung

Seed treatment	SE (%)	SL (cm)	RL (cm)	SDW (g)	RDW (g)
H. GA_3	75.2 ab	20.29 bc	4.05 b	4.129 cd	2.347 c
H. $ZnSO_4$	94.6 a	20.13 c	7.26 a	5.320 a	2.922 abc
H. *Methylo* td-J7	95.6 a	20.08 c	7.50 a	5.062 abc	3.419 ab
H. *Methylo* td-tpb3	92.7 a	21.68 a	6.04 a	4.701 abc	2.835 abc
H. *Trichoderma*	95.8 a	21.52 ab	6.25 a	5.190 ab	3.659 a
H. Ascorbic acid	92.9 a	21.16 abc	5.74 ab	4.901 abc	3.003 abc
H. P_2O_5	77.7 ab	21.73 a	7.30 a	4.195 bcd	3.624 a
H. IAA	90.0 a	21.58 ab	6.05 a	4.644 abc	3.037 abc
Thermal 40 °C	87.3 a	21.25 abc	6.67 a	4.427 abc	2.698 bc
Control	65.4 b	21.13 abc	6.41 a	3.359 d	1.499 d



Means followed by the same letter are not significantly different

FIGURE 4. Index effectiveness of seed treatments in swamps area in South Sumatra

CONCLUSIONS

Almost all seed treatments using hardening, priming, and thermal treatment increased seedling establishment, bioassay of seedlings, total sugar content, and α-amylase enzyme activity compared to the control in direct seeded at greenhouse in three GC conditions. Hardening with *Methylobacterium* td-tpb3 was the most effective treatment for improving seed germination and seedling growth in the AeG and AG+Fe conditions, whereas in the AG condition, hardening with *Methylobacterium* td-J7. Applying seed invigoration treatment *in-situ* within swampy areas that are related to AG and AG+ Fe conditions enhanced seedling establishment and performance compared to the control. Hardening with *Trichoderma* outperformed all other treatments, followed by hardening with *Methylobacterium* td-J7 and *Methylobacterium* td-tpb3, in improving seedling establishment and performance of rice in AG conditions.

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