Seed treatment to improve seedling establishment in the anaerobic conditions

S Wahyuni1*, N Agustiani1, S Salma2 and M L Widiastuti1

1 Indonesian Centre for Rice Research, Indonesia
2 Indonesian Soil Research Institute, Indonesia

Corresponding author: sri_wahyuni_64@yahoo.co.id

Abstract. Limited farm labor in swamplands encourage farmers to apply direct seeding method, however land conditions with excess water cause un-germinated seeds. The purpose of this research was to obtain seed treatments to increase seedling establishment in anaerobic germination conditions. The first experiment was laboratory trial arranged in a factorial design with two factors namely seed treatments and rice varieties. A total of 32 treatments along with control were tested on three rice varieties: Inpara 9 (swampy variety), IR 42 (AG-susceptible), and KHO (Anaerobic Germination/AG tolerant). The results showed that treated seeds show faster germination and has higher germination index. The second experiment was greenhouse trial to confirm the effectiveness of 12 best seed treatments from the first experiment. Water managements were conducted in anaerobic and aerobic conditions. Seeds on anaerobic conditions decreased seedling establishment, shoot length, root length, shoot dry weight, and root dry weight for 44.6, 28.1, 34.4, 80.7, and 74.2%, respectively. KHO showed better performance in anaerobic conditions. Treated seeds had better seedling establishment and seed growth in anaerobic conditions, except for hardening Trichoderma treatment. The best three seed treatments in anaerobic conditions were hardening H2O, thermo treatment 80°C+NaOCl, and hardening ascorbic acid.

1. Introduction

The extensification of rice in swamps and drylands holds a great potential to increase the national rice production. The constraints faced in the development of rice in swamps area the limited farm labors and land conditions with excess water. One of alternatives to overcome the lack of labors is by implementing direct seeding. But direct seeding in swamps area, especially in tidal swamps with excess water, facing problem of poor germination and low seedling establishment. In submerged condition, the content of O2 in water or soil will be reduced (hypoxic) or even in a long submerge condition, it can become anoxic. In such conditions, seeds fail to germinate or fail to grow into normal seedlings, and generally seedlings have longer coleoptile, but roots and primary leaves fail to develop [1].

Germination is a complex process that includes morphological, physiological, and biochemical changes in seeds. Germination stages are comprised of imbibition, enzyme activation, embryo growth initiation, seed coat softening, and germination. The imbibition process during germination is conducted through three phases: rapid absorption of water by seeds (phase-I), followed by a lag phase where the water potential is
balanced with its environment (phase-II), and the appearance of radicles followed by rapid absorption of water (phase-III) [2]. During phase-II, there are major metabolic changes in seeds to prepare the appearance of radicles. Water absorption patterns differ between rice genotypes and this absorption patterns play an important role in determining the tolerance of rice genotypes to germinate under anaerobic conditions [3]. Almost all varieties or lines that are able to germinate in anaerobic and submerged conditions, manifest rapid water absorption in the first 48 hours of the imbibition process and germinate rapidly both in anaerobic and aerobic conditions [4]. Under hypoxic conditions, AG-tolerance rice lines exhibit faster coleoptile growth, faster radicles appearance, higher $\alpha$-amylase activity, and higher sucrose and glucose content in germinating seeds than in AG-susceptible rice lines [5]. Those studies above indicated that seeds can be used in direct seeding in swamps are seeds that are able to germinate quickly in anaerobic conditions (AG-tolerance) or seeds that are able to absorb water quickly in the first 48 hours and are able to germinate in submerged conditions.

Treatment to increase the ability of seeds to start germination quickly and to increase its germinating power and vigor, is known as invigoration. In general, invigoration treatment can be categorized as: hydration of seeds thermal treatment, and coating. Hardening (hydration-rehydration) is done by soaking seeds in a certain time and then the seeds are dried until reaching the initial water content [6]. The hydration-rehydration cycle can be repeated two or more times [7]. The benefits of hardening are increasing embryo growth, enzyme activation, and the energy for germination and also reduced mean germination time and mean emergence time [8]. Priming is a seed hydration technique until the onset of metabolic process of germination, but the essential structure of the embryo, its radicle, has not yet to emerged [9,10].

Invigoration treatment with hardening 2 cycles of 12 hours or 24 hours for 1 or 2 cycles improved the rice seeds vigor and germination percentage of rice seeds [8,11]. Hydropriming affects plant water absorption which increases root growth and ultimately increases rapid emergence and seed vigor in all water conditions, except in extreme dry or extreme wet conditions [12]. While priming using P, K, ascorbic acid, and hormones, or osmopriming using KCl, CaCl, NaCl, or with PEG, can accelerate germination and increase seeds vigor and growth [13,14,15]. Soaking the rice seeds in K and P decreases the average germination time, increases the number of sprouts that appear per day, and improves viability and seeds vigor [16]. Osmohardening using CaCl, simple hardening (hardening with H$_2$O, and hardening with KCl are the three best treatments in accelerating rice seeds germination [17], and in another experiment osmohardening with KCl gives better yields, followed by osmohardening treatment with CaCl, hardening, and priming with ascorbic acid [18]. Seed treatment with Methylobacterium spp. or Trichoderma able increase the growth ability, root growth, and seed storability [19–22]. Whereas, thermal treatment at 40$^\circ$C for 72 hours increased germination index [23] or seed treatments with thermotherapy (80$^\circ$C for 24 h) and dipping in sodium hypochlorite (0.8% NaOCl) for 10 min improved seed germination of pepino [24]. Although there are many seed treatment studies to stimulate early germination, those studies did not consider the seed treatment effectiveness in improve germination and early growth of rice-seedling in anaerobic condition. For that, the purpose of this study is to obtain seed treatment that increase seed germination in direct seeding planting system under anaerobic conditions.

2. Materials and methods

2.1. Laboratory experiments

2.1.1 Research materials. Three rice varieties/lines tested were (A1) Inpara 9 variety, a new-high-yielding variety of swamp rice; (A2) IR 42, a variety that sensitive in anaerobic germination (AG) conditions [5, 25]; and (A3) Khan Hlan On (KHO) line, an AG-tolerant line [26]. Prior to experiment, we tested the initial germination ability using ISTA method [27].
2.1.2. Seed treatments. This research employed seed treatments methods that could improve seed emergence or seedling vigor under optimal and sub-optimal conditions based on desk study. And we have tested these seed treatments methods to observe their germination responses under AG conditions. The first technique that we employed was thermo treatment conducted by soaking seeds into water with initial temperature of 40°C for 72 hours [23] or 80°C for 24 hours, with or without dipping the seeds in sodium hypochlorite (NaOCl 0.8% for 10 min) [24].

We tested several priming methods, such as hydropriming, priming with plant growth regulators, priming with other chemicals, priming with micro bacteria, and osmopriming. Hydropriming was conducted through soaking seeds in aerated distilled water for 48 hours [12, 17]. Priming with plant growth regulators was conducted in 10 ppm GA₃ solution or 15 ppm Kinetin solution for 24 hours [28, 29]. As for priming with other chemicals, the seeds were immersed in ZnSO₄ 4.7 g/kg seed with ratio of 1:5 seed weight to solution for 24 hours [30], in 10 mg/L ascorbic acid for 48 hours [18], in KH₂PO₄ solution with P concentration of 0.5 w/v for 24 hours [16], and in 5% PEG 6000 for 24 hours [15]. Another priming method used was by using micro bacteria, in which the rice seeds were soaked in Methylobacterium spp. and Trichoderma for 24 h [21, 22] by modifying application methods. The last priming method used was osmopriming with solution of 30 g/L KNO₃, 20.74 g/L KCl, 22.2 g/L CaCl₂ and 16.4 g/L NaCl - whereby all solutions having potential osmotic of 1.25 MPa, for 24 hours [18]. Another treatment that we used was hardening, where the seeds were soaked in water, chemical solutions, or other plant hormones, and then followed by redrying the seeds to its initial moisture content under shade with forced air at a temperature of 28 + 3°C. The cycle of hydration and rehydration was repeated twice.

2.1.3. Test tube method. Seeds that had been treated using previous methods then germinated in anaerobic conditions using a test tube method [25] for 5 days. The seeds were placed in a test-tube glass that filled with water until it reaches 10 cm, and then incubated in germinator with an intermittent temperature of 20-30°C for 5 days. For each day, we observed the number of seeds that emerged/germinated. On the fifth day, we counted the total of germinated seeds, measured its radicle and coleoptile length, weighed its dry mass, and then calculated the Mean Germination Time (MGT) and Germination Index (GI) [29, 32].

2.1.4. Experimental design and data analysis. Factorial design experiment was employed that consisted of two factors namely rice varieties and seed treatments, in a completely randomized design with three replicates. Each trial unit consisted of 10 seeds planted in 2 test tubes. We tested the data variety using F-test and then compared its median value using Duncan’s Multiple Range test. If the $P$-value $\leq 0.05$, then it was considered significant. The best treatment was determined based on variables observed by index of effectiveness [33].

2.2. Greenhouse experiments

2.2.1. Seed treatments. The first experiment chose 12 best seed treatments and a control (without seed treatment) to be tested in a greenhouse using a modified water-lodged soil method [25]. Each treatment consisted of 20 seeds with 3 replications for a total of 60 seeds per treatment combination. Treated seeds were planted in a plastic box with a height of 27.5 cm and given a soil as high as 15 cm. Seeds were sown in 2 cm deep below the soil surface and were watered with two conditions: (i) anaerobic: the nursery was continually flooded in 10 cm of water level for 11 days, and (ii) aerobic: with conditions of water field capacity for 11 days. We observed seedling establishment, ASL, ARL, shoot dry weight (SDW), and root dry weight (RDW).
2.2.2. Experimental design and data analysis. Experimental design was compiled factorially using three factors: a) rice varieties, b) seed treatments and c) germination conditions, in a completely randomized design with three replications. If germination conditions produced a distinct difference, then the data were processed separately between anaerobic and aerobic germination conditions. Analysis of diversity was based on F-test and then followed by comparison of its median value using Duncan’s Multiple Range Test. If the P-value < 0.05, then it was considered significant. The best treatment was determined based on variables observed by index of effectiveness [33].

3. Results

3.1. Laboratory experiments

The germination abilities of Inpara 9, IR 42 and KHO line were 97, 92, and 88%, respectively. In testing with the test tube method, the effect of varieties was significant on all observed variables, except for average radicle length (ARL) and seedling dry weight (SDW). Inpara 9 seeds showed better ability to emerge in anaerobic conditions (SEm), germination index (GI), average coleoptile length (ACL) and also faster emergence, shown by the lower MGT, compared to KHO (AG-tolerant) (Figure 1).

Seedling emergence for all treated seeds was more than 90%, but there was no significant difference between seed treatments. The results also showed that all treated seeds had faster emergence and higher daily emergence of seedlings compared to the control seeds, except for the seeds that were given hardening treatment with CaCl₂ (Table 1). The table also showed that longest time to germinate was the control seeds, which is around 4.01 days, while all treated seeds required shorter time to germinate, ranging from 1.69 days to 3.90 days.

In the AG test in the laboratory, as many as 15 of 32 seed treatments showed an increase in coleoptile length, 14 seed treatments increase the seedling dry weight, and 6 seed treatments promoted radicle growth compared to the untreated seeds (Table 1). The best seed treatments in the laboratory experiments was determined by calculating the De Garmo’s index of effectiveness [33] and 12 best seed treatments were selected for further testing in the greenhouse under two germination conditions.

![Figure 1](image_url) Seedling emergence (SEm), germination index (GI), coleoptile length (ACL), radicle length (ARL), seedling dry weight (SDW) and mean germination time (MGT) under AG conditions in the lab
Table 1. Seedling emergence (SEm), mean germination time (MGT), germination index (GI), average coleoptile length (ACL), average radicle length (ARL) and seedling dry weight (SDW) of 33 seed treatments under submerge conditions using the test tube method in the laboratory.

| Treatment                        | SEm (%) | MGT (day) | GI (%/day) | ACL (cm) | ARL (cm) | SDW (mg) |
|----------------------------------|---------|-----------|------------|----------|----------|-----------|
| Thermo treatment 40°C            | 96.7    | 2.78 b    | 36.9 gb    | 2.88 a-d | 0.13 b   | 4.74 cde  |
| Thermotreat 40°C, NaOCL          | 96.7    | 2.52 k    | 40.5 fg    | 3.26 a   | 0.09 c   | 5.86 e    |
| Thermo treatment 80°C            | 97.8    | 2.96 h    | 34.9 hij   | 2.51 l-p | 0 e      | 2.68 f-m  |
| Thermotreat 80°C, NaOCL          | 98.9    | 2.51 k    | 41.3 f     | 2.56 c-f | 0 e      | 5.54 ab   |
| Hydropriming                     | 96.7    | 1.69 m    | 65.9 a     | 2.97 abc | 0 e      | 2.71 a    |
| Priming GA₃                      | 94.4    | 2.79 b    | 36.0 bi    | 2.61 c-f | 0 e      | 3.98 ef   |
| Priming Kinetin                  | 96.7    | 3.29 a    | 31.0 j-m   | 1.90 h-i | 0 e      | 4.18 f-g   |
| Priming P₃O₅                     | 92.2    | 3.90 a-b  | 24.5 p     | 1.29 q   | 0 e      | 2.03 i-j   |
| Priming ascorbic acid            | 93.3    | 3.44 d-f  | 29.3 k-o   | 1.77 j-n | 0 e      | 2.56 j-m   |
| Priming Methylo td-J7            | 91.1    | 3.31 g-h  | 28.7 k-p   | 1.48 l-p | 0 e      | 2.02 l-p   |
| Priming Methylo td-tpb3          | 96.7    | 3.34 g    | 30.2 k-l   | 1.40 m-p | 0 e      | 2.01 l-p   |
| Priming Trichoderma,             | 97.8    | 3.21 f    | 32.6 ijk   | 1.53 p   | 0 e      | 2.59 f-m   |
| Priming KNO₃                     | 97.8    | 3.98 a    | 25.2 opq   | 0.89 q   | 0 e      | 1.41 p     |
| Priming KCl                      | 95.6    | 3.74 b-c  | 26.3 n-p   | 1.34 q   | 0 e      | 1.74 n-p   |
| Priming CaCl₂                    | 98.9    | 3.63 cde  | 27.9 i-j   | 1.24 q   | 0 e      | 2.02 f-p   |
| Priming NaCl                     | 95.6    | 3.60 cde  | 27.3 m-p   | 1.55 k-p | 0 e      | 2.27 k-o   |
| Hardening H₂O                    | 95.6    | 2.16 l    | 49.4 e     | 2.68 cde | 0.06 c   | 4.16 def   |
| Hardening GA₃                    | 96.7    | 2.14 l    | 56.6 bc    | 3.12 ab  | 0.26 a   | 5.18 abc   |
| Hardening Kinetin                | 98.9    | 2.57 j-k  | 41.0 f     | 2.29 e-h | 0 e      | 4.01 f-r   |
| Hardening P₃O₅                   | 95.6    | 2.58 j-k  | 41.5 f     | 2.47 d-g | 0 e      | 3.96 f-r   |
| Hardening ascorbic acid          | 94.4    | 1.98 l    | 54.1 hbd   | 2.77 hbd | 0.06 c   | 4.17 def   |
| Hardening Methylo td-J7          | 94.4    | 2.09 l    | 52.8 cde   | 2.25 d-e | 0 e      | 3.17 e-j   |
| Hardening Methylo td-tpb3        | 93.3    | 2.10 l    | 50.3 d-e   | 2.26 d-f | 0 e      | 2.89 hij   |
| Hardening Trichoderma,           | 96.7    | 2.17 l    | 57.7 b     | 2.08 ghi | 0 e      | 3.62 f-g   |
| Hardening KNO₃                   | 90.0    | 3.67 cde  | 25.2 pqq   | 1.37 n-o | 0 e      | 2.29 f-o   |
| Hardening KCl                    | 96.7    | 3.44 cde  | 29.7 k-a   | 1.87 i-j | 0 e      | 2.69 j-m   |
| Hardening CaCl₂                  | 91.1    | 3.97 a    | 24.0 q     | 1.26 opq | 0 e      | 2.03 i-p   |
| Hardening NaCl                   | 96.7    | 3.58 cde  | 27.9 i-j   | 1.16 p   | 0.01 d   | 1.86 h-m   |
| Control (un-treated)             | 96.7    | 4.01 a    | 24.8 p     | 1.82 j-m | 0 e      | 2.70 f-m   |

Note: numbers in the same column followed by different letters mean significantly different according to the 5% of Duncan’s Multiple Range Test (DMRT). Methylo = Methylobacterium

3.2 Greenhouse experiments

3.2.1 Effect of germination conditions. Conditions of germination significantly influenced on all observed variables. The anaerobic germination condition caused the seedling establishment to decrease by 44.6% compared to the aerobic condition. Seedling growth in anaerobic condition also decreased by 28.1% for average shoot length (ASL), 34.4% for average root length (ARL), 80.7% for shoot dry weight (SDW), and 74.2% for root dry weight (RDW) compared to aerobic condition (Table 2).
Table 2. Seedling establishment and seedling growth in aerobic and anaerobic condition in greenhouse

| Germination condition | Seedling establishment (%) | Shoot Length (cm) | Root length (cm) | Shoot dry weight (mg) | Root dry weight (mg) |
|-----------------------|---------------------------|------------------|------------------|----------------------|---------------------|
| Aerobic               | 92.4 a                    | 33.8 a           | 6.4 a            | 284.2                | 92.5 a              |
| Anaerobic             | 51.2 b                    | 24.3 b           | 4.2 b            | 54.8                 | 23.9 b              |
| Decreased (%)         | 44.6                      | 28.1             | 34.4             | 80.7                 | 74.2                |

3.2.2. Effect of varieties and germination conditions. Seedling establishment and seedling growth of all varieties in aerobic condition are better than in anaerobic condition (Table 3). In the aerobic germination condition, Inpara 9 showed the best seedling establishment with high ARL, SDW and RDW, while KHO showed the highest ASL. Under submerged condition, Inpara 9 showed comparable seedling establishment, ARL and RDW; but higher SDW and shorter ASL compared to KHO (AG-tolerant). When compared with IR 42 (AG-intolerant), ASL and SDW of Inpara 9 were higher, while other variables were comparable (Table 3). This shows that Inpara 9 has the ability to germinate better than IR 42 in AG condition.

Table 3. Seedling establishment and seedling growth (shoot length, root length, shoot dry weight, and root dry weight) of three varieties in two germination conditions.

| Varieties | Seedling establishment (%) | Shoot length (cm) | Root length (cm) | Shoot dry weight (mg) | Root dry weight (mg) |
|-----------|---------------------------|------------------|-----------------|----------------------|---------------------|
|           | Aerobic | Anaerobic | Aerobic | Anaerobic | Aerobic | Anaerobic |
| Inpara 9  | 94.6 a | 53.8 a  | 34.0 b | 25.2 b  | 7.6 a  | 4.1 ab   |
| IR 42     | 92.5 ab| 51.0 a  | 28.5 c | 20.4 c  | 6.1 b  | 3.8 b    |
| KHO       | 90.0 b | 48.7 a  | 38.9 a | 27.3 a  | 5.6 c  | 4.7 a    |

3.2.3. Effect of seed treatments and germination conditions. Under aerobic germination condition, all seed treatments improved the seedling establishment to 85% or more. However, not all seed treatments had a positive effect on the seed growth (Table 4). Under AG condition, all seed treatments significantly increased seedling establishment compared to untreated, except for hardening with Trichoderma. Two seed treatments, hardening with ascorbic acid and thermo treatment 80ºC+NaOCl, were able to produce the highest increase in seedling establishment, from 15.6% in untreated seeds to 75.6%. Hardening with H2O were also able to produce a good result, showed by the increase of seedling establishment to 72.2%.

Results from the calculation of index of effectiveness showed that not all seed treatments increase the index of effectiveness in aerobic germination condition, or in other words, some treatments were not effective at increasing germination and early growth of rice seedling. In contrasts, in AG condition, all seed treatments increased the index of effectiveness compared to untreated seeds. Three best seed treatments in
promoting seedling establishment and seedling growth in AG condition were hardening with H₂O, thermo treatment 80°C+NaOCl, and hardening with ascorbic acid (Table 5).

**Table 4. Effect of seed treatment on seedling establishment and seedling growth in two germination conditions.**

| Seed treatment          | Seedling establishment (%) | Shoot length (cm) | Root length (cm) |
|-------------------------|----------------------------|------------------|------------------|
|                         | Aerobic | Anaerobic | Aerobic | Anaerobic | Aerobic | Anaerobic |
| T1: Hydropriming        | 99.0 a  | 56.7 bc  | 33.6 bcd | 27.3 be  | 6.8 b   | 4.4 ab    |
| T2: Hardening GA₃       | 95.6 abc| 60.0 abc | 33.9 bc  | 24.7 e   | 8.4 a   | 4.9 ab    |
| T3: Hardening ZnSO₄     | 91.1 b-e| 58.9 bc  | 36.4 a   | 30.2 a   | 6.0 c   | 4.3 ab    |
| T4: Thermo treatment 40°C+NaOCl | 86.7 ef | 57.8 bc  | 32.8 cd  | 25.6 cde | 5.6 f   | 4.5 ab    |
| T5: Hardening H₂O       | 88.9 c-f| 72.2 ab  | 33.4 cde | 29.9 ab  | 6.5 c   | 5.0 a     |
| T6: Thermo treatment 80°C+NaOCl | 97.8 ab | 75.6 a   | 26.7 e   | 30.2 a   | 5.1 d   | 4.4 ab    |
| T7: Hardening Trichoderma | 87.8 def| 12.2 f   | 34.5 b   | 6.7 f    | 6.0 c   | 3.4 b     |
| T8: Hardening Kinetin   | 95.6 abc| 61.1 abc | 32.7 d   | 27.4 cde | 5.6 f   | 4.3 ab    |
| T9: Hardening ascorbic acid | 92.2 ac | 75.6 a   | 33.9 bc  | 27.7 cde | 6.1 de  | 4.4 ab    |
| T10: Hardening Methylo td-J7 | 94.4 a-d| 37.8 d   | 34.4 b   | 24.9 de  | 6.6 bc  | 4.3 ab    |
| T11: Hardening P₂O₅     | 93.8 a-e| 28.9 de  | 34.5 b   | 27.9 abc  | 6.1 de  | 4.3 ab    |
| T12: Hardening Methylo td-tpb3 | 94.4 a-d| 53.3 c   | 36.9 b   | 25.7 cde | 6.4 cd  | 4.5 ab    |
| T13: Control (un-treated) | 83.3 f  | 15.6 ef  | 35.8 a   | 7.7 f    | 8.5 a   | 1.9 c     |

Note: numbers in the same column followed by different letters mean significantly different according to the 5% of Duncan’s Multiple Range Test (DMRT), Methylo = Methylobacterium.

**Table 5. Index of effectiveness of seed treatments in two germination conditions**

| Seed treatment          | Index of effectiveness | Aerobic | Anaerobic | Aerobic | Index of effectiveness | Aerobic | Anaerobic |
|-------------------------|------------------------|---------|-----------|---------|------------------------|---------|-----------|
| Hydropriming            | 0.806                  | 0.639   | 0.162     |         | 0.531                  | 0.162   | 0.731     |
| Hardening GA₃           | 0.599                  | 0.677   | 0.314     |         | 0.505                  | 0.731   |           |
| Hardening ZnSO₄         | 1.393                  | 0.874   | 0.314     |         | 0.612                  | 0.879   |           |
| Thermo 40°C+NaOCL       | 0.789                  | 0.738   | 0.532     |         | 0.685                  | 0.532   |           |
| Hardening H₂O           | 0.610                  | 0.919   | 0.489     |         | 0.977                  | 0.489   |           |
| Thermo 80°C+NaOCL       | 0.286                  | 0.904   | 0.634     |         | 0.611                  | 0.634   |           |
|                         |                        | Control (un-treated) | 0.728   | 0.020 |                          |         |           |
4. Discussions

Inpara 9 rice seeds showed higher seedling emergence, seedling growth, and GI, and had faster germination compared to KHO (AG-tolerant) in the laboratory experiments (Figure 1). For the greenhouse experiments under AG condition, Inpara 9 also showed seedling establishment and growth that comparable to KHO but had shorter shoot length (Figure 2). Rapid shoot growth is one of the indicators of AG tolerant [25] whereby the rapid elongation of the shoot will encourage the seedlings to reach the surface water and the seedlings can be aerobic respiration. KHO performance was the best under AG condition among tested varieties. On the other hand, although there is no prior information regarding to Inpara 9’s tolerance under AG condition, the results of this study indicate that Inpara 9 is able to germinate and grow in AG condition. Its performance in showing the emergence of leaf tips above the water surface after 11 days of direct seeding in 10 cm of water depth, can be indicated as having AG-tolerant potential.

One of the indicators for AG-tolerant is the ability to germinate quickly [4]. All seed treatments tested were led to faster germinate, or shorter MGT, compared to untreated seeds under anaerobic condition in the laboratory test (Table 1). Several seed treatments: hardening with GA3, hardening with H2O, hardening with ascorbic acid, hardening with ZnSO4, and thermo-treatment increased the germination index compared to untreated seeds. This indicates that these seed treatments have the potential to increase AG-tolerant.

Direct seeding in submerged conditions is associated with low O2 supply and low light intensity, hence low photosynthesis and low respiration [34], which causes a 44.6% reduction in seedling establishment and 80.7% reduction in seedling growth compared to aerobic condition (Table 2). Seed treatments resulted in higher seedling establishment and longer coleoptile growth compared to control seeds (Table 3), where rapid shoot growth indicated adaptive response of water-seeded rice for acquiring [35], and can be considered as major submergence avoidance or escape mechanism [25, 36, 37].

Seed treatment with hot water treatment was able to produce an effect of eradicating seed borne fungi in tomato [38] and in maize [39] with improved seed germination. In contrast to [38], where thermotherapy at a temperature of 56ºC and 60ºC had a detrimental effect in tomato germination, in this study hot water treatment at temperature of 80ºC showed a better seedling establishment and seedling growth than at 40ºC under AG condition.

Hardening with H2O, hardening with ascorbic acid, and hydropriming accelerate water absorption in seeds and increase the α-amylase enzyme activity in kernel [18] that further increase the availability of soluble carbohydrate for embryo growth and germination [12]. Hydropriming is known to be effective in enhancing anaerobic germination, both in AG-tolerant and intolerant genotypes of rice [15]. However, in present study hardening with H2O was better in improving seedling establishment than hydropriming in AG condition. This is presumably because hardening with H2O undergone twice hydration processes compared to hydropriming that only has one hydration cycle, thus in turn enhancing the enzyme activation process and embryo enlargement. Whereas exogenous ascorbic acid as seed treatment will increase the antioxidant content in seeds, thus reducing the oxidative damage in plants under anaerobic or submerged condition [37].

5. Conclusions

KHO lines showed the best performance in AG condition, while Inpara 9 variety had the ability to germinate and grow in AG condition better than IR 42. Seedling establishment and seedling growth in aerobic conditions were better than in anaerobic condition. All tested seed treatments were effective in improving seedling establishment and early growth of seedlings under AG condition in greenhouse, except for hardening with Trichoderma. The three best seed treatments for increasing germination in AG condition were hardening with H2O, thermo treatment at temperature of 80°C followed by dipping in NaOCl and hardening with ascorbic acid. Further studies can be done to observe their impacts on grain yields in swamp conditions.
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