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Research Article
Persistency and Seed Breaking Dormancy on Local Upland Rice of Southeast Sulawesi, Indonesia

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Abstract

Background and Objective: Rice (*Oryza sativa* L.) is a very important food crop in Indonesia. The development of upland rice becomes an alternative solution to meet the national rice needs. Upland rice productivity is lower than wetland rice, one of the causes is the use of low-vigor seeds. The seeds derived from previous harvest seed and newly harvested rice seedlings have physiological dormancy. Physiological dormancy relates to seed persistency. The study aimed to evaluate the persistence and development of invigoration technique to break seed dormancy of upland rice. **Materials and Methods:** The research was conducted at the Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University. Research was arranged in a completely randomized design (CRD) consisting of 6 treatments namely: A_0 (without treatment), A_1 (KNO_3 1%), A_2 (ground brick *matri-conditioning*+*Bacillus* sp., CKD061), A_3 (ground burned-rice husk *matri-conditioning*+*Bacillus* sp., CKD061), A_4 (ground brick *matri-conditioning*+*Bacillus* sp., CKD061+KNO_3 1%) and A_5 (ground burned-rice husk *matri-conditioning*+*Bacillus* sp., CKD061+KNO_3 1%). **Results:** The results showed that upland rice cultivars from Southeast Sulawesi have varying dormancy. The persistence of seed dormancy evaluated are Pae Parigi 2 is 8 weeks, Pae Parigi 1 is 9 weeks, Pae Kulibungka is 10 weeks, Pae Masaraha is 12 weeks and Pae Rowu is 16 weeks. **Conclusion:** Application of seed bio-invigoration treatment was able to break seed dormancy of local upland rice of cv. Pae Parigi 2 with the best treatment was using KNO_3 1%+ground brick+*Bacillus* sp., CKD061.

Key words: *Bacillus* sp., CKD061, dormancy, local upland rice, persistence, seed invigoration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryza sativa* L.) is a very important food crop in Indonesia because rice is still the staple food for most Indonesian people. Indonesia is the main rice producer but at the same time, Indonesia is also the main rice consumer[1]. The development of wetland rice as a source of rice production has not been able to meet the national rice needs, especially when every year the wetland area decreasing due to conversion to industry, housing and other non-agricultural land use. To maintain rice self-sufficiency, Indonesian government trying to increase rice production, decrease the level rice consumption[2] and promote other local sources of food such as sago[3], cassava and root crops. Therefore, the development of upland rice becomes an alternative solution to meet the national rice needs, in addition to optimizing the utilization of dry land that is large and untapped.

Southeast Sulawesi has a very potential land for the development of upland rice. Currently the land has been utilized for the development of upland rice area of 8,175 ha, while 401,096 ha of others have not been functioned[4]. This is very potential opportunity for the development of local upland rice through land extensification and intensification by optimizing existing technologies. Rice production in Southeast Sulawesi in 2015 is 14.512 t with harvested area of 5,388 ha[5], decreasing from year 2013 which reach 32,121 t with harvest area 10,243 ha.

The high potential for the development of upland rice in Southeast Sulawesi has not received a broad response. Upland rice productivity is lower than wetland rice, one of the causes is the use of low-vigor seeds in the cultivation process. Generally, the seeds are derived from previous harvest seed[6,7]. However, if using newly harvested rice seedlings generally have physiological dormancy (after ripening) which if not understood by the farmers, can lead to failure of seed germination.

Physiological dormancy of upland rice seeds is related to the persistence of seeds. The shelf period at room temperature required by seed from harvest until it reaches 80% germination percentage or more, expressed in weeks or the time it takes for seed to break its dormancy. One method which can be used to solve the dormancy of upland rice seed is soaking the seeds using KNO_3 before being planted[8]. Treatment of seed drying at 50°C followed by immersion in 3% KNO_3 solution for 48 h could break the dormancy of upland rice seeds[9]. Furthermore, seed invigoration techniques that are integrated with rhizobacteria biological agents are also one of the alternatives, which can break the dormancy of upland rice[7].

The seed invigoration improves the rice seedlings[10] and seed viability[11]. The invigoration also has a better effect on the seedling growth of sunflower[12], maize[13], sweet basil[14] and faba bean[15]. It could improve germination under water stress[16], salinity stress[17,18]and drought[19]. Bio-invigoration technique not only improve the viability and seed vigor but also increase growth and yield of the crop[6,20-23]. Therefore, seed preparation and treatment to improve seed quality is very important through the seed invigoration integrated with the applying of indigenous rhizobacteria, that has able to act as biofertilizer and biopesticides. Bio-invigoration improves seed quality in associated with the speed, uniformity and increased ability to germinate. Seed bio-invigoration can be done by using bio-matriconditioning[2]. The study aimed was to evaluate the long persistence of seed dormancy on several local upland rice cultivars from Southeast Sulawesi, Indonesia and development of invigoration technique to break seed dormancy.

MATERIALS AND METHODS

Research design: This study was conducted in Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University from May-October, 2016. The study consisted of two series of experiments, namely (1) Seed dormancy persistence of local upland rice of Southeast Sulawesi. Observation of seed dormancy persistence of five newly harvested Southeast Sulawesi rice cultivars (Pae Parigi 2, Pae Parigi 1, Pae Kulibungka, Pae Masaraha and Pae Rowu) was observed descriptively using seed germination indicator for several weeks until each cultivar tested reach percentage germination $\geq$85% indicating that the seed has entered the normal germination stage (dormancy has been broken), (2) Breaking dormancy evaluation of local upland rice seed using seed bio-invigoration technique (using Pae Parigi 2 cultivar). The experiment was arranged based on complete randomized design (CRD) consisted of 6 treatments, namely: Without treatment+water (A_0), KNO_3 1% (A_1), matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061 (A_2), matri-conditioning of ground brick+*Bacillus* sp., CKD061 (A_3), KNO_3 1%+matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061 (A_4) and KNO_3 1%+matriconditioning of ground brick+*Bacillus* sp., CKD061 (A_5). Each treatment was replicated 3 times, therefore, overall there were 18 experimental units.

Rhizobacterium isolate preparation: The rhizobacteria propagated by smearing isolates onto growth media in petri dishes. *Bacillus* sp., CKD061 was grown on media TSA (30 g tryptic soy broth (difco), 20 g agar and sterile deionized water added till its volume reached 1000 mL). The growing bacterium colony suspended in sterile deionized water[24] until a population density of 10^9 CFU mL^{-1}.

Treating seed with bio-invigoration: The method used in this seed treatment is a modification of the previous method[25]. Before being treated, the upland rice seeds were disinfected with 2% sodium hypochlorite for 5 min, washed 3 times with sterile water, then air dried in a laminar air flow cabinet for 1 h. The dried seeds are then treated. Seed treatment with matriconditioning was done by mixing the seeds with a medium of ground burned-rice husk or ground brick using ratio of seed:media:water = 20:15:10. The seed treatment with rizobacteria was done by immersing the seeds in the suspension of each rizobacteria. Seed treatment with biomatriconditioning using the same procedure with treatment of matriconditioning, but water replaced with rhizobacterial suspension. The rhizobacterial suspension is prepared by mixing 50 mL sterile water in a petri dish (f 9 cm) containing *Bacillus* CKD061 rhizobacteria. The sterile water and rizobacteria are stirred until they are mixed and ready for use in seed treatment. Seeds that have been treated are placed at room temperature for 48 h. After treatment, the seeds are cleaned from embedded medium, dried in a laminar air flow cabinet and then stored until ready to use.

Evaluation of seed dormancy persistence of some local upland rice cultivars of Southeast Sulawesi (series 1): Evaluation of seed dormancy persistence was conducted using standard test on paper media as germination medium. The newly harvested upland rice seed cultivar of Pae Parigi 2 (persistence 0 week after harvest) was immediately planted. A total of 25 seeds were planted to a moist CD paper using rolled paper test established with 3 replications. If the seed has not germinated then the test is repeated the following week until the seed reaches at least 85% germination percentage.

Evaluation breaking dormancy of upland rice seed (series 2): The newly harvested upland rice seed cultivar of Pae Parigi 2 (persistence 0 week after harvest) was immediately treated using dormancy breaking treatment and then planted. A total of 25 seeds were planted to a moist CD paper using rolled paper test established with 3 replications. If the seed has not germinated then the test is repeated the following week until the seed reaches at least 85% germination percentage. Observations were made on viability and vigor of upland rice seed using the following variables:

- Germination percentage (GP), depicting seed potential viability[26] was measured based on the percentage of normal seedlings (NS) during the first i.e., 5 days after planting (DAP) and the second (i.e., 7 DAP) observation by using the following equation:

$$GP\ (\%) = \frac{\sum \text{NS at observation 1} + \sum \text{NS at observation 2}}{\sum \text{Seeds planted}} \times 100$$

- Relative growth rate (RG-r), depicting seed vigor is the ratio of KCT to maximum RG-r. The maximum RG itself was obtained from the assumption that at the first observation, normal seedlings had reached 100%
- Vigor index (VI), depicting the growth rate vigor[27] was measured based on percentage of normal seedlings at the first observation (i.e., 5 DAP):

$$VI\ (\%) = \frac{\sum \text{NS at observation 1}}{\sum \text{Seeds planted}} \times 100$$

Statistical analysis: Statistical analyses were performed using two-way analysis of variances (ANOVA) by means using the Statistical Package of Social Sciences (SPSS) program version 20 for Windows (Chicago, IL, USA). If the test result showed a significant difference, then tests of treatment differences were performed using Duncan's multiple range test (DMRT) at $\alpha = 0.05$.

RESULTS

Seed dormancy persistence of some local upland rice cultivars of Southeast Sulawesi: Based on observations on seed germination, 5 local rice cultivars of Southeast Sulawesi have different seed dormancy persistence (Table 1). The shortest local upland rice seed dormancy persistence was found in Pae Parigi 2 cultivar for 8 weeks with germination of 90.67%, Pae Parigi 1 cultivar for 9 weeks with germination of 86.67%, Pae Kulibungka cultivar for 10 weeks with germination of 89.33%, Pae Masaraha cultivar for 12 weeks with germination of 94.67%, while the Pae Rowu cultivar has the longest dormancy persistence for 16 weeks with germination of 89.33%.

Table 1: Seed dormancy persistence of some local upland rice cultivars

	Germination (%)				
Germination time after harvested (weeks)	Pae Parigi 2	Pae Parigi 1	Pae Kulibungka	Pae Masaraha	Pae Rowu
0	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00
3	9.33	5.33	2.67	13.33	0.00
4	16.00	6.67	4.00	16.00	6.67
5	16.00	18.67	9.33	24.00	9.33
6	25.33	57.33	10.67	32.00	18.67
7	46.67	65.33	25.33	40.00	28.00
8	90.67	74.67	77.33	49.33	45.33
9	-	86.67	84.00	57.33	49.33
10	-	-	89.33	66.67	57.33
11	-	-	-	84.00	58.67
12	-	-	-	94.67	65.33
13	-	-	-	-	66.67
14	-	-	-	-	70.00
15	-	-	-	-	72.00
16	-	-	-	-	89.33
Seed dormancy persistence of local upland rice cultivars (weeks)	8.00	9.00	10.00	12.00	16.00

Table 2: Germination percentage of local upland rice seed of cultivars Pae Parigi 2 on various seed bio-invigoration treatments

	Germination (%)	
Seed bio-invigoration treatments	Week 1	Week 2
Without treatment (control)	0.00^{b}	1.33^{e}
KNO_3 1%	4.00^{b}	26.66^{d}
Matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	4.00^{b}	57.33^{c}
Matriconditioning of ground brick+*Bacillus* sp., CKD061	9.33^{b}	73.33^{b}
KNO_3 1%+matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	10.66^{b}	73.33^{b}
KNO_3 1%+matriconditioning of ground brick+*Bacillus* sp., CKD061	26.67^{a}	85.33^{a}

Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to HSD

Table 3: Vigor index of local upland rice seed of cultivars Pae Parigi 2 on various seed bio-invigoration treatments

	Vigor index (%)	
Seed bio-invigoration treatments	Week 1	Week 2
Without treatment (control)	0.00^{b}	0.00^{d}
KNO_3 1%	0.00^{b}	10.66^{c}
Matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	0.00^{b}	26.66^{b}
Matriconditioning of ground brick+*Bacillus* sp., CKD061	0.00^{b}	32.00^{b}
KNO_3 1%+matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	0.00^{b}	29.33^{b}
KNO_3 1%+matri-conditioning of ground brick+*Bacillus* sp., CKD061	9.33^{a}	57.33^{a}

Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to HSD

Breaking dormancy of upland rice seed: Seed bio-invigoration treatment was able to break the seeds dormancy of Pae Parigi 2 cultivar only in 2 weeks. Seed bio-invigoration treatment significantly increased the germination of upland rice seeds. In week 1, the germination reaches the range of 4.00-26.66%, whereas at week 2, the germination range reaches 26.67-85.33% and between treatments tested, only the application of KNO_3 1%+matriconditioning ground brick+*Bacillus* sp., CKD061 effectively breaks seed dormancy and increases viability up to 85% at 2 weeks (Table 2).

Seed bio-invigoration treatment also significantly increased vigor index of upland rice seed. In week 1, only application of KNO_3 1%+matri-conditioning ground brick+*Bacillus* sp., CKD061 was able to break the dormancy of seeds but its viability reached only 9.33%. Meanwhile, at week 2, seed bio-invigoration treatment increased the vigor index by 10.66-57.33%, KNO_3 1%+matri-conditioning ground brick+*Bacillus* sp., CKD061 depicted the best vigor index and significantly different from other treatments (Table 3).

Seed bio-invigoration treatment significantly increased the relative growth rate of upland rice seeds. In week 1, the

Table 4: Relative growth rate of local upland rice seed of cultivars Pae Parigi 2 on various seed bio-invigoration treatment

	Relative growth rate (% etmal^{-1})	
Seed bio-invigoration treatment	Week 1	Week 2
Without treatment (control)	0.00^{b}	0.95^{e}
KNO_3 1%	3.01^{b}	23.20^{d}
Matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	2.85^{b}	50.95^{c}
Matriconditioning of ground brick+*Bacillus* sp., CKD061	7.14^{b}	64.54^{b}
KNO_3 1%+matriconditioning of ground burned-rice husk+*Bacillus* sp., CKD061	7.93^{b}	63.30^{b}
KNO_3 1%+matriconditioning of ground brick+*Bacillus* sp., CKD061	22.82^{a}	79.23^{a}

Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to HSD

relative growth rate reached 3.01-22.82%, whereas at week 2, the range of relative growth rate reached 23.20-79.23%, KNO_3 1%+matri-conditioning ground brick+*Bacillus* sp., CKD061 at week 2 was able to break the dormancy of the seeds while increased relative growth rate reached 79.23% (Table 4).

DISCUSSION

The results showed that the local upland rice of Southeast Sulawesi has different seed dormancy persistence depending on cultivars. Cultivar Pae Parigi 2 has a dormancy persistence for 8 weeks, Pae Parigi 1 for 9 weeks, Pae Kulibungka for 10 weeks, Pae Masaraha for 12 weeks and Pae Rowu for 16 weeks. The differences of seed dormancy persistence are thought to be caused by genetic differences between cultivars affected by husk thickness. The thickness of lemma and palea in rice seeds can inhibit germination, which causes the dormancy of seeds[28].

Based on the persistence of dormancy, dormancy divide into three groups of short dormancy[29]: Persistence groups (<4 weeks), moderate dormancy persistence groups (4-8 weeks) and long dormancy persistence groups (>8 weeks). Based on these groupings, the Pae Parigi 1, Pae Kulibungka, Pae Masaraha and Pae Rowu cultivars belong to a long dormancy persistence group, while Pae Parigi 2 is a moderate dormancy group.

Evaluation results of dormancy breaking technique of upland rice seed of Pae Parigi 2 cultivar showed that seed bio-invigoration treatment was very effective in breaking the dormancy of tested upland rice seeds. Seed application using KNO_3 1%+matri-conditioning ground brick+*Bacillus* sp., CKD061 was able to break the dormancy of upland rice seed after 2 weeks in storage. This treatment also consistently improved the viability and vigor of upland rice seeds in all observed variables.

Integration of KNO_3, ground brick matriconditioning and *Bacillus* sp., CKD061 plays a complementary role in supporting the acceleration of seed germination processes. The KNO_3 solution is known to have a stimulant effect on seed germination through its role as an electron-accepting ion. In addition, increased seed germination is due to the dormancy of skin impermeability to oxygen could be overcome by immersion in KNO_3 solution[30]. The KNO_3 plays a role to accelerate the acceptance of seeds to oxygen and soften the skin of seeds to facilitate the germination process and break the dormancy of the seeds[31]. The effectiveness of KNO_3 in dealing with seed dormancy is related to increase and availability of oxygen to support the activity of the phosphate pentose pathway and inhibit oxygen for respiration, inhibit catalase activity; so that dormancy damage of seeds is stimulated and normal seeds are formed[32].

Bacillus sp. is a group of PGPR bacteria (plant growth promoting rhizobacteria) that effectively trigger plant growth. The role of PGPR in triggering plant growth is related to their ability to synthesize growth hormone, fixing nitrogen or dissolving phosphate. *Bacillus* sp. able to synthesize indole acetic acid (IAA)[33], gibberellins and cytokines[34,35], in addition to their ability to dissolve phosphates and fix nitrogen[36]. The major contribution of *Bacillus* sp. that is associated with the plant is to increase the availability of growth hormones such as IAA[37] that serves to trigger plant growth and increase the availability of nutrients such as P. The use of phosphate solvent rhizobacteria may substitute part or all of the plants need for fertilizer P. Phosphate dissolution is caused by bacteria which produce phosphatase enzymes which could be severing the phosphate bound by organic compounds into available forms until P is available for plants[38,39].

In addition to the improvements caused by the independent use of rizobacteria, the application of seed invigoration as a rhizobacterial inoculation medium to seeds also provides a positive and non-neglected role. As mentioned earlier, seed invigoration is a seed treatment which aims to accelerate and uniform growth and increase the percentage of sprouts and seeds emerging. The principle is to mobilize resources owned by seed (internal) plus external resources to maximize the improvement of plant growth and yield. Seed conditioning is a physiological and biochemical improvement associated with speed and uniformity, improvement and

increase of seed germination potential during germination delay by lowering matrix potential media (matriconditioning) or by lowering osmotic potential medium (osmoconditioning)[40]. The use of seed invigoration techniques has been shown to increase seed viability and vigor[19,41]. The use of seed invigoration incorporated with rhizobacteria also effective to protect the seedling from the seedborne diseases and soil infestation[42].

Ground brick as a medium of seed invigoration proved to be effective in improving seed germination. Ground brick as a media matriconditioning able to increase seed germination potential and improvement of early plant growth. The seeds treatment using *Bacillus* sp. CKD061 integrated with ground brick matriconditioning could provide better results and effectively improve physiological quality of sorghum seeds[43].

The breakage of dormancy and improvement of the physical quality of the local upland rice seedlings of Pae Parigi 2 cultivars during the 1st and 2nd week was highest in the treatment of KNO_3+matriconditioning of ground brick+*Bacillus* sp., CKD061. This suggests that the use of *Bacillus* CKD061 rhizobacteria, integrated with invigoration techniques using ground brick matriconditioning, was more effective in increasing the viability and vigor of local upland rice seeds compared with other treatments. Based on the results of the research[7], seed invigoration techniques integrated with rizobacteria could overcome the physiological dormancy problems that occur when the local upland rice seed was harvested, as well as increase seed viability and vigor.

CONCLUSION

All of the local Southeast Sulawesi rice seeds tested have different dormancy persistence. Persistence dormancy 5 local upland rice cultivars Southeast Sulawesi namely: Pae Parigi 2 is 8 weeks, Pae Parigi 1 is 9 weeks, Pae Kulibungka is 10 weeks, Pae Masaraha is 12 weeks and Pae Rowu is 16 weeks. Application of seed invigoration technique able to break dormancy of local upland rice seed cultivar Pae Parigi 2 with best treatment is integration of KNO_3 1%+matriconditioning ground brick+*Bacillus* sp., CKD061.

SIGNIFICANCE STATEMENT

This study discovers the possible application of seed treatment to facilitate the delivery of seeds and other materials required at the time of sowing. This study will help the researcher to uncover the critical area of seed persistence and dormancy that many researchers were not able to explore. The finding revealed that preparation and seed treatment to improve seed quality is very important through the seed invigoration integrated with the applying of indigenous rhizobacteria, that has able to act as biofertilizer and biopesticide. Bio-invigoration improves seed quality in associated with the speed, uniformity and increased ability to germinate.

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