The effectiveness of preplant seed bio-invigoration techniques using *Bacillus* sp. CKD061 to improving seed viability and vigor of several local upland rice cultivars of Southeast Sulawesi

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Abstract. Research was aimed to evaluate the bio-invigoration techniques using *Bacillus* sp. CKD061 in improving seed viability and vigor of local upland rice. The research is arranged in factorial with completely randomized design (CRD). The different upland rice cultivars as first factor that consists of 11 cultivars, namely: Pae Tinangge, Pae Rowu, Pae Uwa, Pae Tanta, Pae Waburi-Buri, Pae Mornene, Pae Indalibana, Pae Lawarangka, Pae Huko, Pae Wagamba and Pae Momea. The second factor is the seed bio-invigoration technique, consists of 5 treatments, namely: without seed bio-invigoration (B0), NaCl + Bacillus sp. CKD061 (B1), KNO3 + Bacillus sp. CKD061 (B2), Ground burned-rice husk + Bacillus sp. CKD061 (B3), and Ground brick + Bacillus sp. CKD061 (B4). The results showed that seed bio-invigoration using Bacillus sp. CKD061 gave effect on the seed viability and vigor. Interaction of the seed bio-invigoration and upland rice cultivars were able to improve seed viability and vigor. Seed bio-invigoration treatment using ground brick + Bacillus sp. CKD061 was the best treatment, which could improve the viability and vigor of Pae Waburi-Buri, Pae Mornene and Pae Indalibana. The treatment increased vigor index by 133% in Pae Waburi-Buri and 127% in Pae Mornene, and Pae Indalibana compared with control.

1. Introduction
Rice (*Oryza sativa* L.) is a very important food crop because rice is still the main staple food in Indonesian. The demand of rice is increase every year. Various efforts have been done to increase rice production such as through the increasing productivity, breeding new variety [1] and development rice under the shade [2]. Another program is by the decreasing the level of rice consumption and promoting a local source of staple foods such sago [3], cassava or corn [4], but the results are not yet optimal. One effort to rice production is through the development of upland rice in dry land [4].

Constraints encountered include limited input of cultivation technology, especially in terms of the use of quality seeds and control techniques of plant-disturbing organisms. In addition, the decline in rice production is also attributed to the decrease in productive wetland due to the transfer of functions to the interests of industry, housing and other non-agricultural land use. The development of upland rice in dry land can be a solution to increase production. However, upland rice received less attention because of its low productivity. Development of upland rice (especially local upland rice) in Southeast
Sulawesi is still limited, in addition to marginal land issues, as well as the implementation of cultivation techniques of upland rice, especially in the use of quality seeds. The use of high quality seeds is an important prerequisite for generating economically profitable crop production. Therefore, preparation and treatment of seeds to improve the quality is very important to do, especially with the physiological dormancy (after ripening) problem in post-harvest upland rice seed in the field.

The alternative to overcome these problems is through seed invigoration technology integrated with biological agents of rhizobacterial groups, microorganisms capable of acting as biofertilizers and biopestisides [5]. Invigoration is a way to improve the physiological quality of seeds, especially seed vigor, through physical or chemical treatment. High-vigorous seeds are able to demonstrate good performance in germination processes under diverse environmental conditions [6]. Seed invigoration is physiological and biochemical improvements associated with synchronous germination, velocity, and increased seed germination using low-potency matrix solids or low osmotic potential solutions. This treatment is known as seed matriconditioning or osmoconditioning treatments can be integrated with rhizobacterial applications, called bio-matriconditioning [7-8] or bio-osmoconditioning. This treatment aims to improve the viability and vigor of seeds, growth and yield of plants [5,9], also proved able to protect the seed from seedborne and soilborne fungi at an important phase at the beginning of its growth [10]. Treatment can be recommended as a growth promoting of local rice crops of Southeast Sulawesi.

2. Materials and Methods

The research was conducted in Agrotechnology Laboratory, Agriculture Faculty of Halu Oleo University, from September 2015 to March 2016. The research was arranged based on factorial in completely randomized design (CRD). The first factor is the local upland rice cultivar consisting of 11 local upland rice cultivar of Southeast Sulawesi: V1=Pae Tinangge, V2=Pae Rowu, V3=Pae Uwa, V4= Pae Tanta, V5=Pae Waburi-Buri, V6=Pae Mornene, V7=Pae Indalibana, V8=Pae Lawarangka, V9=Pae Huko, V10=Pae Wagamba dan V11=Pae Momea. The second factor is bio-invigoration treatment with Bacillus sp. CKD061 Consists of 5 treatments namely: B0 = without bio-invigoration treatment (as control), B1 = NaCl + Bacillus sp. CKD061, B2 = KNO3 + Bacillus sp. CKD061, B3= ground burned-rice husk + Bacillus sp. CKD061 dan B4= ground brick + Bacillus sp. CKD061. Each treatment was replicated 3 times, therefore, overall there were 165 experimental units.

The effects of seed bio-invigoration on the seed viability and vigor were evaluated by measuring their germination percentage, vigor index, relative growth rate, and growth uniformity.

1. Germination percentage (GP), depicting seed potential viability [11], 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 formula:

\[ GP = \frac{\sum \text{NS at observation 1} + \sum \text{NS observation 2}}{\sum \text{seeds planted}} \times 100\% \]

2. 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%.

3. Seed uniformity, depicting seed vigor, was measured based on the percentage of normal seedlings (NS) on the day between the first count (5 dap) and second (7 dap) i.e. at 6 dap.

4. Vigor index (VI), depicting the growth rate vigor [10], was measured based on percentage of normal seedlings at the first observation (i.e. 5 dap):

\[ VI = \frac{\sum \text{NS at observation 1}}{\sum \text{seeds planted}} \times 100\% \]
3. Results and discussion

3.1. Results

3.1.1. The effects of seed bio-invigoration treatment on germination percentage on local upland.

There is an interaction effect between seed bio-invigoration treatment and cultivars on the germination percentage (Table 1). Different cultivars show different responses to the seed bio-invigoration treatment. In Pae Uwa cultivar the best interaction is in the treatment of KNO₃ + Bacillus sp. CKD061 and ground burned rice husk + Bacillus sp. CKD061 with the value of each germination percentage is 94.67% and 93.33%. In Pae Waburi-buri cultivar the best interaction is in the treatment of NaCl + Bacillus sp. CKD061, KNO₃ + Bacillus sp. CKD061, ground burned rice husk + Bacillus sp. CKD061, ground brick + Bacillus sp. CKD061 with the value of each germination percentage is 93.33%, 93.33%, 94.67% and 93.33%.

Table 1. The effects of seed bio-invigoration treatment on germination percentage of several local upland rice cultivars of Southeast Sulawesi

| Local cultivar | Seed Bio-invigoration Treatment | B0     | B1     | B2     | B3     | B4     |
|---------------|---------------------------------|--------|--------|--------|--------|--------|
| V1            |                                 | 65.33 abQ | 64.67 bQ | 81.33 abP | 72.00 bPQ | 78.67 abPQ |
| V2            |                                 | 50.67 bQ | 83.33 abP | 54.67 cQ | 84.00 abP | 62.67 bQ |
| V3            |                                 | 69.33 aQ | 81.33 abPQ | 94.67 aP | 93.33 aP | 81.33 abPQ |
| V4            |                                 | 13.33 eR | 84.00 abP | 61.33 bcQ | 69.33 bQ | 67.33 bQ |
| V5            |                                 | 65.33 abQ | 93.33 aP | 93.33 aP | 94.67 aP | 93.33 aP |
| V6            |                                 | 61.33 abQ | 60.00 bQ | 80.00 abPQ | 73.33 bQ | 90.67 aP |
| V7            |                                 | 61.33 abQ | 60.00 bQ | 80.00 abPQ | 73.33 bQ | 90.67 aP |
| V8            |                                 | 65.33 abQ | 78.67 abPQ | 77.33 bPQ | 73.33 bPQ | 84.00 aP |
| V9            |                                 | 57.33 abQ | 78.67 abPQ | 69.33 bcPQ | 78.67 abP | 64.00 bPQ |
| V10           |                                 | 73.33 aP | 80.00 abP | 86.67 abP | 89.33 aP | 88.00 aP |
| V11           |                                 | 61.33 abQ | 73.33 bPQ | 77.33 bP | 73.33 bPQ | 84.00 aP |

Means in the same column (a-c) or in the same line (P-Q) suffixed with different letters are different at 5% levels of significance according to DMRT. V1=Pae Tinangge, V2=Pae Rowu, V3=Pae Uwa, V4=Pae Tanta, V5=Pae Waburi-Buri, V6=Pae Mornene, V7=Pae Indalibana, V8=Pae Lawarangka, V9=Pae Huko, V10=Pae Wagamba, V11=Pae Momea, B0 = control, B1 = NaCl + Bacillus sp. CKD061, B2 = KNO₃ + Bacillus sp. CKD061, B3 = ground burned rice husk + Bacillus sp. CKD061, B4 = ground brick + Bacillus sp. CKD061.

In Pae Wagamba cultivar the best interaction is in the treatment of ground burned rice husk + Bacillus sp. CKD061, ground brick + Bacillus sp. CKD061 with the value of each germination percentage is 89.33% dan 88.00%. Meanwhile, Pae Mornene, Pae Indalibana, Pae Lawarangka, and Pae Momea, provides the best response to the treatment of ground brick + Bacillus sp. CKD061 with the value of each germination percentage is 90.67%, 90.67, 84.00% and 84.00%.

3.1.2. The effects of seed bio-invigoration treatment on germination growth rate on local upland rice.

Pae Waburi-Buri, Pae Mornene, and Pae Indalibana cultivars provide a better response to the treatment of ground brick + Bacillus sp. CKD061 with the value of each germination percentage is 93.33%, 90.67% dan 90.67%. Among all of seed bio-invigoration treatments tested, Pae Waburi-Buri cultivar was able to respond all of seed bio-invigoration treatments provided. Among all of seed bio-invigoration treatments tested, only NaCl + Bacillus sp. CKD061 was able to solve the dormancy of Pae Tanta cultivars by increasing the relative growth rate by 565% (Table 2).
Table 2. The effects of seed bio-invigoration treatment on relative growth rate of several local upland rice cultivars of Southeast Sulawesi

| Local upland rice cultivar | Seed Bio-invigoration Treatment B0 | B1 | B2 | B3 | B4 |
|----------------------------|------------------------------------|----|----|----|----|
| V1                         | 59.90 abQ 64.35 bPQ 78.22 abP 67.56 bPQ 66.67 bcPQ |
| V2                         | 43.11 bQ 81.27 aP 47.08 cQ 74.03 bP 54.70 cQ |
| V3                         | 67.17 abQ 79.68 abPQ 91.68 aP 88.92 abP 77.46 bPQ |
| V4                         | 12.29 cR 81.75 aP 59.52 cQ 68.35 bPQ 65.56 bcQ |
| V5                         | 58.51 abQ 86.54 aP 92.95 aP 94.29 aP 93.33 aP |
| V6                         | 57.40 abQ 60.41 bQ 80.00 abPQ 70.06 bQ 90.67 aP |
| V7                         | 57.40 abQ 60.41 bQ 80.00 abPQ 70.06 bQ 90.67 aP |
| V8                         | 62.05 abQ 76.38 abP 76.79 bP 72.38 bPQ 80.83 abP |
| V9                         | 49.56 bQ 76.48 abP 62.86 bcPQ 76.29 bP 63.17 bcPQ |
| V10                        | 70.67 aQ 77.59 abPQ 82.51 abPQ 88.73 abP 85.68 abP |
| V11                        | 60.19 abQ 75.24 abP 74.79 bP 72.38 bPQ 80.83 abP |

Notes: Means in the same column (a-c) or in the same line (P-Q) suffixed with different letters are different at 5% levels of significance according to DMRT.

3.1.3. The effects of seed bio-invigoration treatment on vigor index of several local upland rice cultivars of Southeast Sulawesi.

Cultivars of Pae Waburi-Burt, Pae Mornene, and Pae Indalibana provide a better response to the treatment of ground brick + Bacillus sp. CKD061 with value vigor index respectively 93.33%, 90.67% and 90.67%. Consistently, seed bio-invigoration treatment using NaCl + Bacillus sp. CKD061 was able to solve the dormancy of Pae Tanta cultivars by increasing the vigor index by 817% (Table 3).

Table 3. Effect of interaction bio-invigoration technique interaction on seeds vigor several cultivars of local rice seedlings of Southeast Sulawesi

| Local upland rice cultivar | Bio-invigoration B0 | B1 | B2 | B3 | B4 |
|----------------------------|---------------------|----|----|----|----|
| V1                         | 41.33 aQ 41.33 bQ 69.33 bP 45.33 cQ 40.00 cQ |
| V2                         | 18.67 bQ 60.00 aP 32.00 cQ 34.67 cQ 32.00 cQ |
| V3                         | 57.33 aQ 66.67 aPQ 78.67 abP 70.67 bPQ 60.00 bQ |
| V4                         | 8.00 bR 73.33 aP 53.33 bcQ 65.33 bPQ 60.00 bPQ |
| V5                         | 38.67 aR 64.00 aQ 92.00 aP 93.33 aP 93.33 aP |
| V6                         | 45.33 aQ 58.67 abQ 80.00 abP 61.33 bcQ 90.67 aP |
| V7                         | 45.33 aQ 58.67 abQ 80.00 abP 61.33 bcQ 90.67 aP |
| V8                         | 53.33 aQ 70.00 aPQ 76.00 abP 66.67 bPQ 60.00 bPQ |
| V9                         | 17.33 bR 69.33 aP 40.00 cQ 65.33 bP 60.00 bP |
| V10                        | 57.33 aQ 62.67 aQ 69.33 bPQ 86.67 abP 76.00 abPQ |
| V11                        | 57.33 aQ 70.67 aQ 75.33 abP 66.67 bPQ 70.67 bPQ |

Notes: Means in the same column (a-c) or in the same line (P-R) suffixed with different letters are different at 5% levels of significance according to DMRT.
3.2. Discussion
Seed bio-invigoration treatment using Bacillus sp. CKD061 integrated with ground burned rice husk or ground brick or KNO₃ gives better results in enhancing the viability and vigor of local upland rice seed compared with NaCl and control. Bacillus spp. is a group of PGPR (Plant Growth Promoting Rhizobacteria) which has been shown to be effective in increasing plant growth and yield [12]. The role of PGPR in increasing plant growth and production was presumably caused by the ability of rhizobacteria to produce IAA [9], gibberellins [13] and to dissolve phosphate [5,14]. In general, the utilization of Bacillus sp.CKD061 integrated with matriconditioning of ground burned rice husk or ground brick resulted in more effective in increasing the viability and vigor of local upland rice seed.

4. Conclusions
It is concluded that seeds bio-invigoration treatment using Bacillus sp. CKD061 which is integrated with a medium of ground burned rice husk or ground brick or KNO₃ solution, is better able to increase the viability and vigor of local upland rice seed compared to other treatments and controls. Cultivars Pae Waburi-Buri, Pae Mornene, and Pae Indalibana responded better to the treatment of ground brick + Bacillus sp. CKD061 through increased vigor index respectively 131%, 100% and 100%. Integration of bio-invigoration seed treatment using NaCl + Bacillus sp. CKD061 was able to overcome the dormancy of local upland rice seed cv. Pae Tanta.

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