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The Effect of Seed Bio-invigoration Using Indigenous Rhizobacteria to Improve Viability and Vigor of Upland Rice (*Oryza sativa* L.) Seeds

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Abstract : Seed vigor and germination ability directly affect seedling emergence and yield. Seed bio-invigoration using indigenous rhizobacteria was studied to improve viability and vigor of upland rice seeds. The research design using completely randomize design (CRD) with eighteen treatment ie. Control, Dithane, Hydration, KNO_3 + *Bacillus* sp. CKD061, KNO_3 + *P. fluorescens* TBT214, KNO_3 + *Serratia* sp. CMN175, NaCl + *Bacillus* sp. CKD061, NaCl + *P. fluorescens* TBT214, NaCl + *Serratia* sp. CMN175, matriconditioning using ground burned-rice husk + *Bacillus* sp. CKD061, matriconditioning using ground burned-rice husk + *P. fluorescens* TBT214, matriconditioning using ground *burned-rice* husk + *Serratia* sp. CMN175, matriconditioning using ground brick + *Bacillus* sp. CKD061, matriconditioning using ground brick + *P. fluorescens* TBT214, matriconditioning using ground brick + *Serratia* sp. CMN175, *Bacillus* sp. CKD061 + *P. fluorescens* TBT214, *Bacillus* sp. CKD061 + *Serratia* sp. CMN175, *P. fluorescens* TBT214 + *Serratia* sp. CMN175, with three replication. Research showed that seed bio-invigoration with *Bacillus* spp. CKD061 integrated with ground *burned-rice* husk or ground brick give the highest maximum growth rate, germination rate, relative growth, vigor index, and T_{50}. Seed treatment with *Bacillus* spp. CKD061 integrated with ground burned rice husk increased vigor index by 63% when compared to control.

Keywords : Bio-invigoration, Indigenous rhizobacteria, Upland Rice, Seed Viability and Vigor.

Introduction

Rice (Oryza sativa L.) in Indonesia is very important crop because as main staple food. Indonesia actually is major rice producing countries together with China, India, Bangladesh and Vietnam[1], but at the same time, Indonesia also the main consumer of rice [2]. The demanding rice is expected to increase in the future, at least in line with the population growth. Although Indonesia is the third-largest country regarding global rice production, it is still a rice importer sometimes. Indonesia has the largest rice consumption per capita that reached 140 kilograms and population reached 250 million people. Considering that Indonesia has a population that consumes large quantities of rice, and facing the risks involved being a rice importer when food prices rise.

Indonesia places top priority on reaching self-sufficiency in rice. Therefore rice production must be increasing at least the same level with the population growth.

The Indonesian government has two police to reach rice self-sufficiency. On the one end, it encourages farmers to increase their production by stimulating and providing innovation technology and on the other end, by trying to curb rice consumption, while promoting consumption of other source staple foods, such sago[3], cassava or corn. To overcome this problem, rice production should be increased, especially through the extension of new paddy fields and increasing rice productivity on existing wetland [2].

The promising alternative for increasing rice production is through the development upland rice on dryland. The development of upland rice on dry land is relatively cheaper when compared to the opening of new irrigated paddy fields. Upland rice can be cultivating in combining with other commodities such as peanuts, soybeans, and corn in the pattern of upland rice-based farming systems. One of the problems in cultivating upland rice is correlating with seed quality. Sometimes the use of improved and high-quality seed has not been a priority. Generally, farmers use an indigenous rice seed from the previous harvest season, with no specific treatment that can maintain the vigor seed. Poor seed quality will result in the low emergence and the seedlings less tolerant to abiotic stress, more sensitive to plant diseases, and will reduce the quality and yield of crops produced [4].

The use of high-quality seed or applied seed treatment is an important prerequisite for generating crop profitable and crop production economically. Seed treatments are commonly applied to combat seed borne diseases, and diseases and pests that may be present in soil or be airborne when seedlings emerge [4]. Specialized seed treatments such as priming [5], including osmoconditioning or matriconditioning, coating, pelleting are often used to improve seed vigor, seed germination or protect seeds against pathogens. Seed vigor is one of the most important factors affecting the seedling establishment and final production [6,7,8].

Seed invigoration or seed enhancements are “post-harvest treatments to improve germination and seedling growth or to facilitate the delivery of seeds and other materials required at the time of sowing” [9]. Seed invigoration is ascribed to beneficial treatments, applied to the seeds after harvest but prior to sowing that improves germination or seedling growth or facilitates the delivery of seeds and other materials required at the time of sowing [10,11].

The invigoration improves the growth of rice seedlings [12], seed viability [13], seed emergence and seedling growth. It also has a good effect on seedling of sunflower [14], maize[15], black seed [16], chilly [17], faba bean [18] and sweet basil [19]. It could enable the crop to give higher yields under moisture stress [20], the increasing root proliferation that enhances nutrient and water uptake [21]. It improves germination under salinity stress [22,23,24,25,26,27], water stress [28], sodic soil [29] and drought [30,31] by hastened the activities of total amylase and alcohol dehydrogenase [32], enhancing the activities of polyphenol oxidase and peroxidase activities [33] and also protein synthesis [14].

Therefore, 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 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. Bio-matriconditioning is treatment seed with low matrix potential media or matriconditioning media that is integrated with applied of indigenous rhizobacteria. Bio-invigoration technique aims to improve the viability and seed vigor, growth and also the yield of crop [34,35,36,37,38]. Studies on the seed bio-invigoration integrated with indigenous rhizobacteria that can improve the viability and vigor of upland rice are still limited so that the research activity has become very important.

Experimental

1. Preparation of Rhizobacteria

Rizobacteria used in this study included *Bacillus* sp. CKD061, *Pseudomonas fluorescens* TBT214 and *Serratia* sp. CMN175, were isolated from rhizospehere of healthy chilly pepper in the previous study [39]. During times active use *Bacillus* sp. CKD061 and *Serratia* sp. CMN175 were routinely cultivated on agar plate of Tripthic Soy Agar (TSA) medium at the room temperature, while *Pseudomonas fluorescens* TBT214 was cultivated on King's B Medium (KBM). The TSA medium composition (g/l) are Tripthic Soy Broth (difco) 30 g and agar 20 g, while KBM are peptone protease 20 g, K2HPO4 2.5 g, MgSO4.7H2O 6 g, glycerol 15 ml, and agar20 g. After 24 hours the growing bacterium colony was suspended in sterile deinonized water till a population density of 10^9cfu/ml [40].

2. Seed Bio-matriconditioning

The upland rice seeds were disinfected with natrium hypoclorit 2% for 5 minutes, rinsed 5 times with sterile water, then air dried in laminar air flow cabinet for one hour. Seed biomatriconditioning were conducted by placing 10 g of seeds into culture bottle which were then mixed with 7.5g of matriconditioning media (i.e. ground burned rice husk or ground brick) and added with 5 ml each of suspensions (10^9CFU/ml concentration) of rhizobacterium *Bacillus* sp. CKD061, *P. fluorescens* TBT214 or *Seratia* sp. CMN175. Sterile distilled water was used as control. The bottle was then covered with plastic and tied with rubber bands. To avoid the occurrence of aerobic condition, three small holes were made on the plastic cover by using a needle. The cultures were then incubated for 24 hours at room temperature (28-30 ^{o}C) after which the seeds were air dried in the laminar air flow cabinet for one hour.

3. Seed viability dan vigor test

The study were arranged in completely randomized design with eighteen bio-matriconditioning treatments including control. The treatment replicated in three times under laboratory condition. The treated seeds were sowed on sterile burned rice husk placed in a plastic box (20 cm x 15 cm x 10 cm). Twenty-five seeds were sowed per box and three boxes were prepared per treatment, and stored in growth chamber during 7 days. The seed viability and vigor were evaluated by measuring their maximum growth rate, germination rate, relative growth rate, vigor index, uniformity, and T_{50}. Maximum growth rate (MGR), was calculated in the end of observation at 7 days after planting (DAP) based on the formula developed [41] as follows:

$$MGR = \frac{\Sigma\ Seedlings}{\Sigma\ Seeds\ planted} \times 100\%$$

Germination rate (GR), was calculated at the end of observation (7 DAP) based on the formula developed [41] as follows:

$$GR = \frac{\Sigma\ Normal\ seedlings}{\Sigma\ Seeds\ planted} \times 100\%$$

Relative growth rate (GR-r), depicting seed vigor, is the ratio of GR to maximum GR. The maximum GR itself was obtained from the assumption that at the first observation, normal seedlings had reached 100%. GR was calculated based on the accumulation of daily growth rate [41]:

$$GR\text{-}r = \frac{GR}{GR\ max}$$

$$GR = \sum_0^{tn} \frac{N}{t} \times 100\%$$

$$GR\ max = \frac{100}{\Sigma\ NS\ at\ observation\ 1}$$

Where:
t = time of observation
N = % Normal seed (NS) per observation

Vigor index (VI), depicting the growth rate vigor [42], was measured based on percentage of normal seedlings at the first observation (i.e. 5 DAP):

$$VI = \frac{\Sigma\ NS\ at\ observation\ 1}{\Sigma\ Seeds\ planted} \times 100\%$$

Seeds uniformity (SU), depicting the growth rate vigor [41], was measured based on percentage of normal seedlings at the time between the first and the end of observation (i.e. 6 DAP):

T_{50} is the time required to achieve 50% of total seeds germinate, observed by counting the number of seeds that germinated every day. T_{50} describe seed vigor, calculated by the formula:

$$T_{50} = ti + \frac{(n50\% - ni)}{nj - ni}(tj - ti)$$

Where:

Ti = time before seed germination 50%

tj = time after seed germination 50%

50% = the amount of seed germination 50% of the total seed germination

nj = the amount of seed germination at the time after seed germination 50%

ni = the amount of seed germination at the time before seed germination 50%

The data were analysed by using ANOVA and when it showed significant effect, it was furtherly tested with Duncan's Multiple Range Test (DMRT) at α=0.05 All data analysis was conducted by using SAS.

Results and Discussion

Seed treatments using biomatriconditioning were more effective in improving upland rice seed maximum growth rate, germination percentage and relative growth rate compared that to control. Among the three rhizobacteria studied, *Bacillus* sp. CKD061 either integrated with ground burned rice husk or ground brick showed a higher maximum growth rate, germination percentage and relative growth rate compared to *Serratia* sp. CMN175. The lowest maximum growth rate, germination percentage and relative growth rate were found on a control which was significantly different from the other treatments except for *Serratia* sp. CMN175 (Table 1).

Table 1. The effects of biomatriconditioning on maximum growth rate (MGR), germination rate (GR) and relative growth rate (GR-r) upland rice seed.

Treatments	MGR (%)	GR (%)	GR-r (%/etmal)
Control	78.67 ce (±2.31)	70.67 c (±6.11)	66 d (±5.74)
Dithane	85.33 ae (±2.31)	70.67 c (±5.77)	70 bd (±5.58)
Hidration	84.00 ae (±4.00)	73.33 bc (±5.77)	68 cd (±4.99)
KNO_3+CKD061	84.00 ae (±6.93)	74.67 bc (±3.06)	72 ad (±1.07)
KNO_3+TBT214	89.33 ad (±2.31)	86.67 ac (±2.31)	84 ab (±1.80)
KNO_3+CMN175	77.00 de (±4.62)	72.00 bc (±6.93)	71 bd (±6.09)
NACL+CKD	84.00 ae (±4.00)	84.00 ac (±4.00)	83 ac (±3.10)
NACL+TBT214	88.00 ad (±8.00)	87.00 ac (±4.62)	85 ab (±5.43)
NACL+CMN175	80.00 ce (±2.00)	74.67 bc (±1.15)	74 ad (±2.08)
SAS+CKD061	96.00 a (±4.00)	91.00 ab (±8.33)	89 a (±7.37)
SAS+TBT214	89.33 ad (±4.62)	80.00 ac (±4.00)	77 ad (±3.91)
SAS+CMN175	80.00 ce (±2.00)	73.33 bc (±6.43)	73 ad (±2.27)
SBM+CKD061	93.33 ab (±2.31)	90.67 a (±4.62)	88 a (±3.67)
SBM+TBT214	85.33 ae (±6.11)	76.00 ac (±4.00)	74 ad (±5.01)
SBM+CMN175	90.67 ac (±2.31)	85.33 ac (±4.62)	78 ad (±4.08)
CKD061+TBT214	90.67 ac (±6.11)	84.00 ac (±8.00)	83 ac (±7.58)
CKD061+CMN17	82.67 ae (±2.31)	80.00 ac (±4.00)	79 ad (±4.67)
TBT214+CMN17	76.00 e (±6.93)	70.67 c (±4.62)	70 bd (±3.85)

Note: Means in the same column suffixed with different letters are different at 5% levels of significance according to DMRT. CKD061 (*Bacillus* sp. CKD061), TBT214 (*P. fluorescens* TBT214), CMN175 (*Serratia* sp. CMN175), SAS (ground burned rice husk), SBM (ground brick), ± (standard error of mean).

Compared to the control, seed treatments using bio-matriconditioning were more effective in improving upland rice seed vigor index, uniformity, and T_{50}. Seed bio-matriconditioning using *Bacillus* sp. CKD061 either integrated with ground burned rice husk or ground brick still showed a higher vigor index, uniformity and T_{50} compared to *P. fluorescens* TBT214 and *Serratia* sp. CMN175. The lowest vigor index, uniformity, and T_{50} was found on the control which was significantly different from the other treatments (Table 2).

Table 2. The effects of upland rice seed biomatriconditioning on vigor index (VI), seed uniformity (SU) and T_{50}

Treatments	VI (%)	SU (%)	T_{50} (day)
Kontrol	50.67 de (±6.11)	61.33 cd (±4.62)	3.45 a (±0.14)
Dithane	66.67 ad (±5.77)	69.33 ac (±5.03)	2.91 bc (±0.08)
Hidration	55.00 ce (±1.15)	59.00 d (±9.24)	2.93 b (±0.22)
KNO3+CKD061	65.33 ae (±8.33)	66.67 bd (±6.11)	2.73 bc (±0.05)
KNO3+TBT214	76 .00 ab (±0.00)	81.33 ab (±2.31)	2.56 bd (±0.11)
KNO3+CMN175	65.33 ae (±4.62)	69.33 ac (±6.11)	2.56 bd (±0.10)
NACL+CKD	80.00 ab (±0.00)	81.33 ab (±2.31)	2.43 cd (±0.05)
NACL+TBT214	78.67 ab (±9.24)	82.67 a (±1.15)	2.41 cd (±0.04)
NACL+CMN175	70.67 ac (±9.24)	73.33 ac (±8.33)	2.45 bd (±0.07)
SAS+CKD061	82.67 a (±6.11)	84.00 a (±8.00)	1.71 f (±0.03)
SAS+TBT214	65.33 ae (±2.31)	72.00 ac (±6.93)	2.54 bd (±0.03)
SAS+CMN175	70.67 ac (±8.08)	73.33 ac (±6.43)	2.17 de (±0.25)
SBM+CKD061	83.00 a (±2.31)	83.00 a (±2.31)	1.88 ef (±0.08)
SBM+TBT214	64.00 be (±3.46)	73.33 ac (±4.62)	2.55 bd (±0.11)
SBM+CMN175	49.33 e (±3.06)	73.33 ac (±6.11)	2.96 bc (±0.39)
CKD061+TBT21	77.33 ab (±1.15)	81.33 ab (±6.11)	2.76 bc (±0.03)
CKD061+CMN17	76.00 ab (±8.00)	78.67 ab (±4.62)	2.94 bc (±0.06)
TBT214+CMN17	68.00 ac (±0.00)	70.67 ac (±4.62)	2.91 bc (±0.02)

Note: Means in the same column suffixed with different letters are different at 5% levels of significance according to DMRT. CKD061 (*Bacillus* sp. CKD061), TBT214 (*P. fluorescens* TBT214), CMN175 (*Serratia* sp. CMN175), SAS (ground burned rice husk), SBM (ground brick), ± (standard error of mean).

The results showed that bio-invigoration seed through bio-matriconditioning using indigenous rhizobacteria *Bacillus* sp. CKD061 were significantly improving upland rice seed viability and vigor compared that to control. The results were in accordance with those of the previous studies. The use *Bacillus* sp. CKD061 as PGPR can also significantly improving cocoa seed viability and vigor [4,37]. Seed bioinvigoration integrated with *Bacillus* sp. CKD061 is also reported to improve sorghum seed viability and vigor compared to those untreated ones [36].

Observation on the several seed viability and vigor parameters showed that *Bacillus* sp. CKD061 were more responsive to upland rice seeds than *P. fluorescens* TBT214 and *Serratia* CMN175. Rhizobacterium colonization into a host plant is started when a seed is germinating. At the same time, the rhizobacteria also require adequate nutrition for their growth and development. Generally, the nutrition is derived from organic acids exuded by the host plant and the type of the organic acid is different from one host to another. Therefore, a reduced contribution of rhizobacteria may be caused by a limited nutrition provided by the host plant.

The utilization of *Bacillus* sp.CKD061 integrated with matriconditioning of ground burned rice husk or ground brick resulted in a higher yield and more effective in improving viability and vigor of upland rice seed. *Bacillus* sp.CKD061 was compatible to both ground brick whose basic material was from clay mineral or ground burned rice husk. *Bacillus* sp.CKD061 belongs to *gram-positive* bacteria that possess a thicker cell wall than those of *gram-negative*. Generally, clay mineral with its high water holding capacity and adhering property is more capable of protecting microorganisms. The drying rate of clay is slower, due to its high water holding capacity and adhering property [43], therefore microorganisms will always be at an ideal condition for their growth and development. An improved and increased viability and vigor of upland rice seed resulted from the utilization of *Bacillus* sp. CKD061 integrated with seed matriconditioning using ground burned rice husk or ground brick was presumably caused by the ability of the rhizobacteria to produce IAA, to fix Nitrogen and to dissolve phosphate. *Bacillus* sp. CKD061 produced 346,97 ppm of IAA [44]. Several previous studies also showed that the role of *Bacillus* spp. as PGPR (*Plant Growth Promoting Rhizobacteria*) was correlated with the ability to synthesize plant growth regulator substances, to fix nitrogen or to dissolve phosphate [39,45]. *Bacillus* spp. also, can fix nitrogen and dissolve phosphate [46]. *Bacillus* spp. can also synthesize IAA [47,48], gibberellins [49], and cytokinins [50].

The main contribution of rhizobacterium *Bacillus* spp. associated with host plants was to increase the availability of regulator growth substances [51], such as, IAA that functions to promote plant growth and increase the availability of plant nutrition such as P that is highly required during the plant growth and development. The utilization of P-dissolving rhizobacteria that can substitute a part or all plant P-requirement results in an increased plant growth and yield. This P-dissolving is brought about by bacteria that produce phosphates that can release bound P from organic substances, and therefore, it can fulfill plant requirement[52,53].

Besides an improvement brought about by the utilization of rhizobacterium alone, the application of invigoration techniques as rhizobacterium inoculating media, it also provides a great positive role on seeds. As discussed previously, invigoration techniques are treatments for seeds (*seed conditioning*) intended to improve seed viability and vigor. *Seed conditioning* is a physiological and biochemical improvement related to the rate and uniformity, improvement, and increase of germinating potential during their delayed germination by media having a low matrix potential (*matriconditioning*) [4]. The utilization of seed invigoration techniques has been proven effective in improving seed viability and vigor [34].

Conclusion

It can be concluded that seed bio-invigoration with *Bacillus* spp. CKD061 in combination with ground burned rice husk or ground brick resulted in the highest germination rate, vigor index, relative growth rate and normal seedling dry weight. Seed treatment with *Bacillus* spp. CKD061 in combination with ground burned rice husk increased vigor index of upland rice seeds by 63% when compared to control.

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