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Effects of inoculation of plant growth promoting rhizobacteria to minimize panicle grain shattering habit for increased yield of rice (*Oryza sativa* L.)

Mohamad Halid Razak[1], Zulkarami Brahim[2] and Halimi Mohd Saud[1,2]*

[1]Department of Agriculture Technology, Faculty of Agriculture, 43400 Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[2]Laboratory of Food Crops, Institute of Tropical Agriculture, 43400 Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

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Locally isolated *Bacillus subtilis* (UPMB10) as nitrogen fixer and *B. pumillus* (GM118) as phosphate solubilizer act as plant growth promoting rhizobacteria (PGPR). The objective of this study is to determine the response of single and co-inoculation of these PGPR on the panicle breaking strength to minimize panicle grain shattering to increase rice yield. The ability to fix nitrogen and solubilize phosphate, production of indole-3-acetic acid (IAA) and grain yield production of single and co-inoculants of PGPR were studied. The result demonstrated that single and combined inoculations were able to fix nitrogen and solubilize phosphate. Besides, the ability of UPMB10 and mix inoculation to produce high concentration of IAA enables them to provide a high breaking force needed to detach grain from panicle compared to GM118 and in turn increase the rice grain yield. Hence, this study showed the potential single and combined inoculations as a biofertilizer to increase rice productivity in the granary areas of Malaysia.

Key words: Plant growth promoting rhizobacteria (PGPR), co-inoculation, *Bacillus*, indole-3-acetic acid (IAA), grain shattering, yield, *Oryza sativa* L.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) is a free living soil born bacteria that colonize the rhizosphere. The interaction between plant root exudates and bacteria to support each other in term of symbiosis interaction for growth and development also enhanced the plant growth when applied to the seed crops (Yu et al., 2012). Besides, it can increase the nutrient status of host plants through the mechanism of biological N_2 fixation, phosphate (P) and potassium (K) solubilization.

When single PGPR inoculation of rhizobacteria were applied, they showed potential ability to enhance shoot growth, root density and yield of rice. However, recent studies showed that co-inoculation of PGPR are better than single inoculation as they provide more balanced

*Corresponding author. E-mail: halimi@upm.edu.my.

nitrogen, phosphorus, and mineral nutrients (Mohammadi and Sohrabi, 2012). These improvements in growth attributes of plants caused by PGPR are due to the potential of fixing and solubilizing mineral fertilizer and nutrition for the plant and improved the absorption of production of phytohormones resulting in the increased availability of nutrients to plants and root permeability (Glick, 2012). Phytohormones produced by PGPR such as IAA is crucial as they are capable of increasing shoot growth, root hair density and length, enhance rice seed germination and improve growth (Saharan and Nehra, 2011).

Normally in wild species of rice, grain shattering habit is an adaptive trait for seed dispersal at maturity to prevent seeds from being eaten by predator and guaranteed the continuous propagation of rice seed (Lin et al*.,* 2007). However, easy shattering causes considerable yield loss in cultivated grain rice. This opinion was supported by Sadeghi et al*.* (2010), which stated that the threshing force had significant difference in different rice varieties. Breaking tensile strength force is the force required to detach a grain from the panicle which describes the grain shattering habit, assumed to be one of the effective loss-evaluating parameters during harvesting (Alizadeh and Allameh, 2011). It is an important criterion for evaluating rice variety in the interactive effect with PGPR inoculation programs. Hence, one way to combat this problem is by promoting the use plant growth promoting rhizobacteria (PGPR).

This approach of using PGPR to reduce grain shattering should be conducted in Malaysia to determine the effectiveness of locally isolated PGPR toward the increment of grain quality of local rice varieties and in turn increase the national rice yield production. Here, we report the characteristic and locally isolated effects of single and co-inoculation of PGPR namely *Bacillus subtilis* (UPMB10) and *Bacillus pumillus* (GM118) on the panicle breaking strength to minimize panicle grain shattering under controlled and field conditions.

MATERIALS AND METHODS

Source of inoculation, culture conditions and treatments

The two PGPR strains, *B. subtilis* (UPMB10) and *B. pumillus* (GM118) were obtained from the culture collection of the Department of Agriculture Technology Faculty of Agriculture, Universiti Putra Malaysia, Serdang. *B. subtilis* was initially isolated from oil palm rhizosphere and *B. pumillus* from a paddy soil. Bacterial single colonies from stock were prepared by touching and streaking on nutrient agar plates in a third streak pattern. The plates were then incubated for 24-48 h at 30ºC. A single colony was inoculated into 700 ml nutrient broth in an Erlenmeyer flask. Inoculated flasks were shaken (200 rpm) in a rotary shaker for 10 h at 30ºC. The final concentration of the bacterial cultures was adjusted to about 10^8 cfu/ml (Kausar et al., 2011). Four treatments were conducted for the rice plant experiment which: [1] Control (without inoculation), [2] *B. pumillus sp.* (GM118), [3] *B. subtilis sp*. (UPMB10) and [4] *B. pumillus sp*. + *B. subtilis sp*. (Mixture).

Phytohormones production

Phytohormones production or Indole-3-acetic acid (IAA) was determined by colorimetric method (Gordon and Weber, 1951). Fully grown bacterial culture were incubated in 100 mL tryptic soy broth (TSB) and shaken for 24 h. One mL of the bacteria culture was transferred into new 100 mL TSB with addition of 5 mL L-tryptophan as the pre-cursor of Indole-3-acetic Acid. TSB without bacterial inoculation served as control. 1.5 mL of the bacterial culture were transferred into sterile Eppendorf tube and centrifuged at 7000 rpm for 7 min. One mL of the supernatant was mixed with two mL of Salkowsky reagent (2% of 0.5 M $FeCl_3$ in 35% prechloric acid) and allowed to settle for 25 min for development of pink color which indicates IAA production. The absorbance values of each isolate was determined by using spectrophotometer at 535 nm and compared using the standard curve. The IAA standard curved was prepared using pure IAA stock as 0, 5, 10, 15, 20, 25, 30, 35, 50 and 45 µg/mL of IAA ($Y=0.0186x$; $R^2= 0.9802$). Supernatants of un-inoculated test tubes were used as control, where no visible color was observed.

Evaluation of PGPR application on rice

The experiment was conducted in a glasshouse, at Universiti Putra Malaysia and field study in IADA Kemasin Semerak, Kelantan, Malaysia to evaluate the effects of UPMB10 and M118 on rice grain yield MR263. For glasshouse experiment, seeds were sown in trays of 3.2 × 3.2 × 4.5 cm cell size in a medium of peat and sandy (1:1) soil. When the seedling reached 5 cm height (after two weeks), they were transplanted into buckets (4 plants per pot) 40 cm diameter (20 kg soil pot^{-1}) with four replications. The soil used in this study was collected from Kemasin Semerak, Kelantan Malaysia. The used nitrogen fertilizer was urea (46% N), potassium fertilizer was muriate of potash (60% K_2O) and triple super phosphate (50% P_2O_5) at application rate 0.76 g N $plant^{-1}$, 0.71 g K $plant^{-1}$ and 0.42 g P $plant^{-1}$. For field study, they were transplanted into micro plot (4 m × 4 m) with three replications. Total of fertilizers per hectare per season applied were: 121.1 kg nitrogen, 67.3 kg phosphorus and 45.5 kg potassium. The fertilizers were applied manually three times per season after transplant. The plants were harvested at days 105 after transplant and air dried and stored in the laboratory for analysis. Three replicates with two single inoculations treatments plus one combination inoculation and control were studied in field experiments.

Plant inoculation with *Bacillus sp*.

An appropriate dilution of 10^8/mL bacterial cell suspension of *B. pumillus* or/and *B. subtillis* were used to inoculate onto the plants by spraying with pump sprayer (ratio 1:100 ml H_2O) in all experiments at 20, 45 and 65 days after sowing.

Grains physiological analysis

The rice yield parameters which include total grain number per panicle, fill grains per panicle, 1000 g weight, total spikelet per panicle, panicle length and number of primary branch per panicle were determined after harvest. The filled grains were separated from unfilled grains by using salt solution of 1.06 specific gravity (Seizo, 1980).

Grain panicle strength analysis

Determination of panicle strength was done according to Thurber et al*.* (2011) and Alizadeh and Allameh (2011) methods. Six panicles

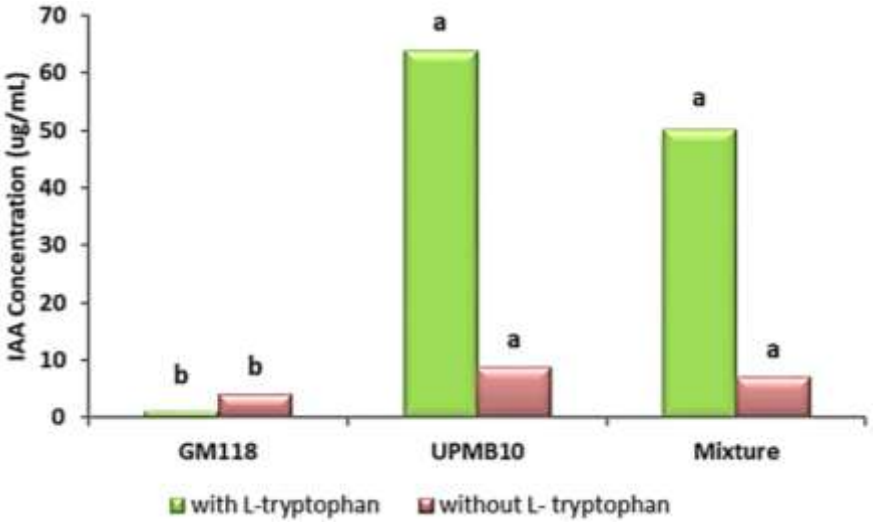

Figure 1. IAA production of single and combine inoculation with and without addition of L- tryptophan.

Table 1. Total grain number per panicle, fill grains per panicle, 1000 g weight, total spikelets per panicle, panicle length and number of primary branch per panicle at 100 DAT.

Treatments	Total Grain Panicle^{-1} (No.)	Filled Grain Panicle^{-1} (No.)	1000 Grain Weight (g)	Total Spikelets Panicle^{-1} (No.)	Panicle Length (cm)	Primary Branch (No.)
Control	79.58^{b}	79.58^{b}	16.68^{b}	102.54^{b}	22.4^{b}	11.5^{b}
GM118	**95.04^{a}**	**95.04^{a}**	18.75^{b}	**119.46^{a}**	**23.7^{a}**	**12.9^{a}**
UPMB10	**98.83^{a}**	**97.92^{a}**	18.16^{b}	**129.04^{a}**	**23.7^{a}**	12.2^{ab}
Mixture	89.71^{ab}	90.63^{ab}	**22.53^{a}**	**119.58^{a}**	23.6^{ab}	11.7^{b}
CV	25.7	23.1	32.7	17.2	7.1	8.7

Reported data are the mean of three replications and values in each column with different letters are significantly different according to Duncan`s Multiple Range Test at $p \leq 0.05$.

were randomly selected and analyzed for breaking tensile strength (BTS), or shattering level. BTS is a measure of the maximum amount of weight, in grams; a single grain can hold or attached to pedicle before being detached (Thurber et al., 2011). The shattering level was measure for each panicle by separating the panicle into three portions; upper, middle and lower. An electronic force device (FGP-1 Nidec SHIMPON) with the resolution of ± 0.01 N was used for measurement of the shattering force. The measurement of the maximum force was recorded when the applied force breaks the grain from the panicle. Average BTS values for the measurements were recorded for each sample.

Statistical analysis

All data were statistically analyzed using the SAS Software Program (Version 9.3), and treatment means were compared using Duncan Multiple Range Test ($P < 0.05$).

RESULTS

The interaction effects of single inoculation, *B. subtilis* (UPMB10) and *B. pumillus* (GM118) and also combined inoculations (Mixture) on production of IAA is given in Figure 1. In the presence of L-tryptophan, application of UPMB10 and Mixture indicated the highest IAA production which were 63.8 and 50.1 µg/mL, respectively, while GM118 produced only 1.3 µg/mL (Figure 1a). Besides, UPMB10 and Mixture still showed the highest IAA production which were 8.74 and 7.04 µg/mL, respectively even without the addition of L-tryptophan as compared to GM118 (4.07 µg/mL) (Figure 1b). The result showed that IAA production by bacteria inocula were significantly increased by the single inoculation of UPMB10 and Mixture compared to single inoculation of GM118.

Generally, all of the single inoculations, GM118 and UPMB10 and combined inoculation (Mixture) treatments indicated significant higher result in total number of grain per panicle, fill grains per panicle, 1000 grains weight, total spikelets per panicle, panicle length and number of primary branch per panicle at 100 DAT compared to control as represented in Table 1. The application of

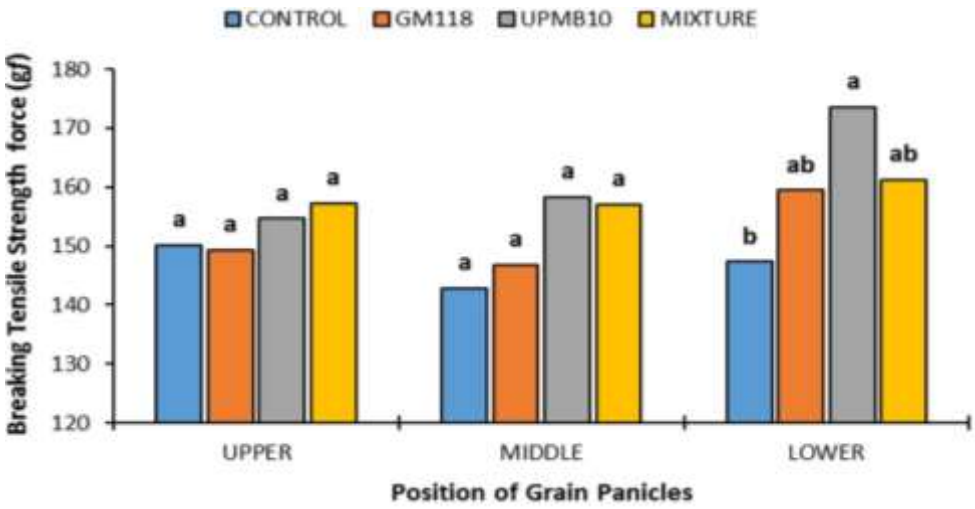

Figure 2. The average shattering force value measured after application single inoculation of GM118 and UPMB10, and Mixture (GM118 + UPMB10) inoculation with different position of grain panicles. Bar graphs with different letters are significantly different according to Duncan`s Multiple Range Test at $p \leq 0.05$

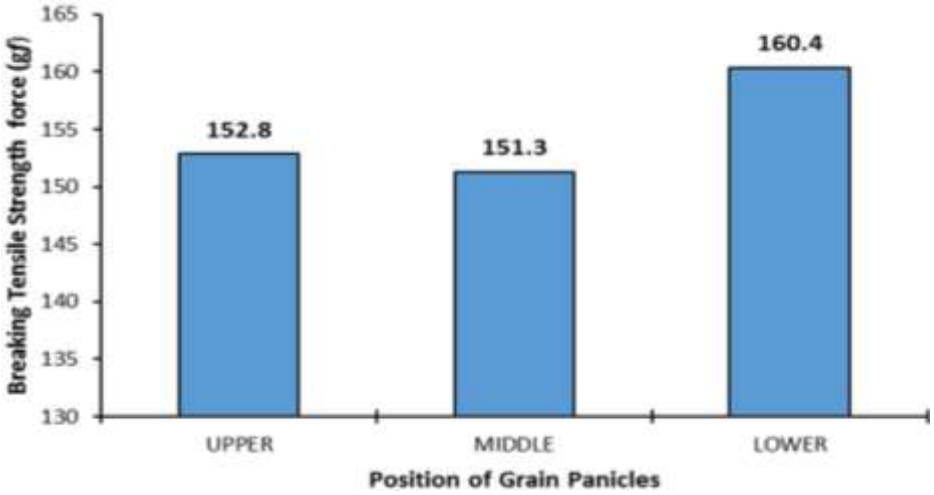

Figure 3. Comparison between shattering force values at three positions of grain on the panicle.

UPMB10 showed the highest total number of grain per panicle (98.83), number of filled grain per panicle (97.92), total number of spikelets per panicle (129.04) and panicle length (23.7 cm). However, GM118 indicated the highest number of primary branch (12.9) and showed same panicle length (23.7 cm) result with UPMB10, while Mixture showed the highest 1000 grains weight which was 22.53 g. Besides, most of parameter data showed insignificant difference between inoculations treatment except on data of 1000 grains weight and number of primary branch.

Figure 2 represent the average shattering force value measured after application of single inoculation, GM118 and UPMB10 and combined inoculation (Mixture) with different positions of grain panicle; upper, middle and lower. A significant difference was shown in lower position of grain panicle. Detaching the grain from the lower position of the panicle required a force of 160.4 g*f* but the forces required by those of the upper and middle positions were 152.8 and 151.3 g*f,* respectively (Figure 3). Meanwhile, single inoculation of UPMB10 indicated the highest breaking force to detach grain from pedicle (162.2 g*f*), followed by mixture (158.6 g*f*) and GM118 (151.9 g*f*) and control treatments required only 146.7 g*f* to detach grain from pedicle (Figure 4). The effect of single and combine inoculations on the rice grain yield in

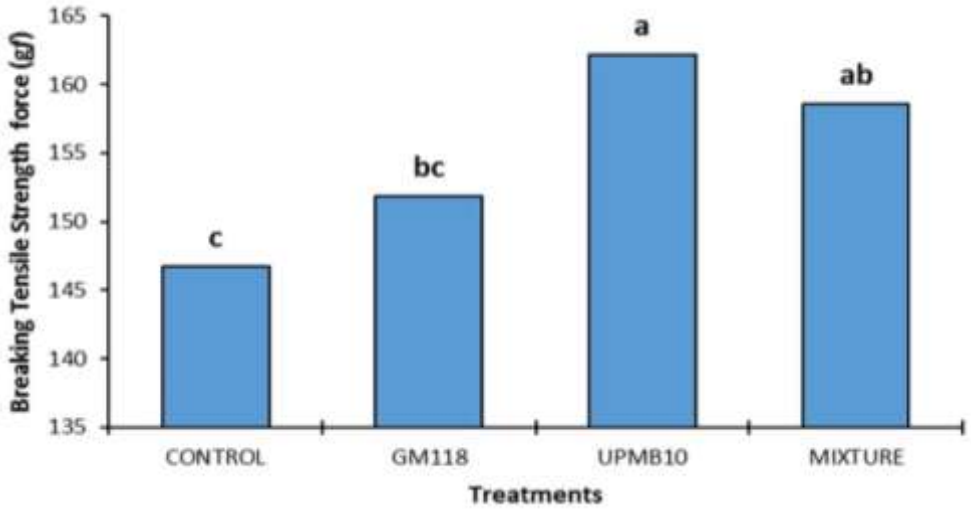

Figure 4. The average shattering force value measured after application single inoculation of GM118 and UPMB10, and Mixture (GM118 + UPMB10) inoculation of the grain panicle; Bar graphs with different letters are significantly different according to Duncan`s Multiple Range Test at $p \leq 0.05$.

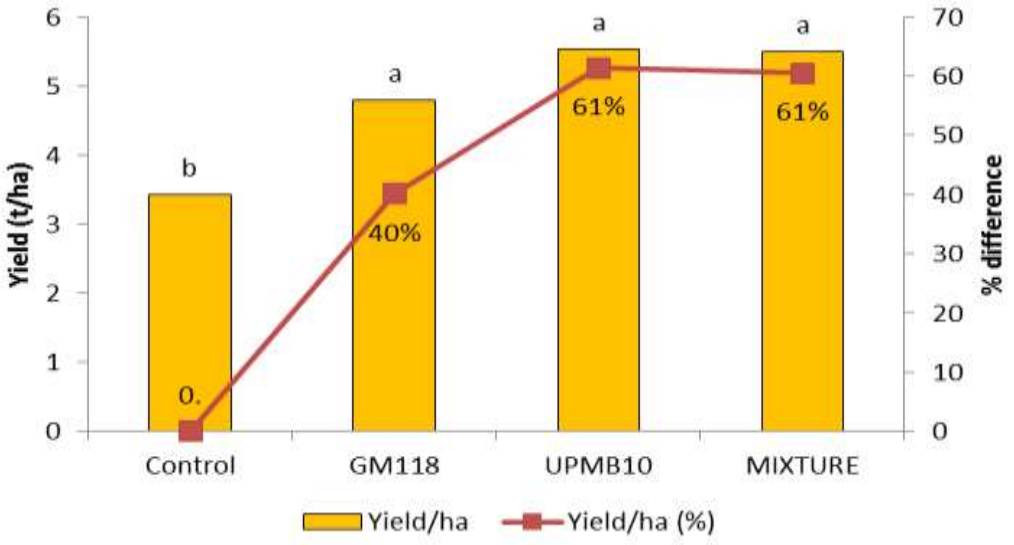

Figure 5. Grains yield (t/ha) in the field of experiment in IADA Kemasin Semerak granary measured after application of single inoculation of GM118 and UPMB10, and Mixture (GM118 + UPMB10) inoculation. Bar graphs with different letters are significantly different according to Duncan`s Multiple Range Test at $p \leq 0.05$

IADA Kemasin Semerak granary is given in Figure 5. The highest grain yield (t/ha) was shown by single inoculation, UPMB10 and combined inoculation (mixture), which 5.53 and 5.50 t/ha, respectively, and followed by single inoculation, GM118 (4.80 t/ha) as compared to control treatment. Both of UPMB10 and mixture indicated 61% difference yield/ha from control, meanwhile, GM118 indicated only 40% difference yield/ha from control. both

DISCUSSION

Plant growth promoting rhizobacteria (PGPR) used in this study were single inoculation; *Bacillus subtilis* (UPMB10) and *B. pumillus* (GM118) and also combine inoculation of UPMB10 and GM118 (Mixture). UPMB10 act as nitrogen fixer while GM118 act as phosphate solubilizer (Supplementary result: Plates 1 and 2). The presence of

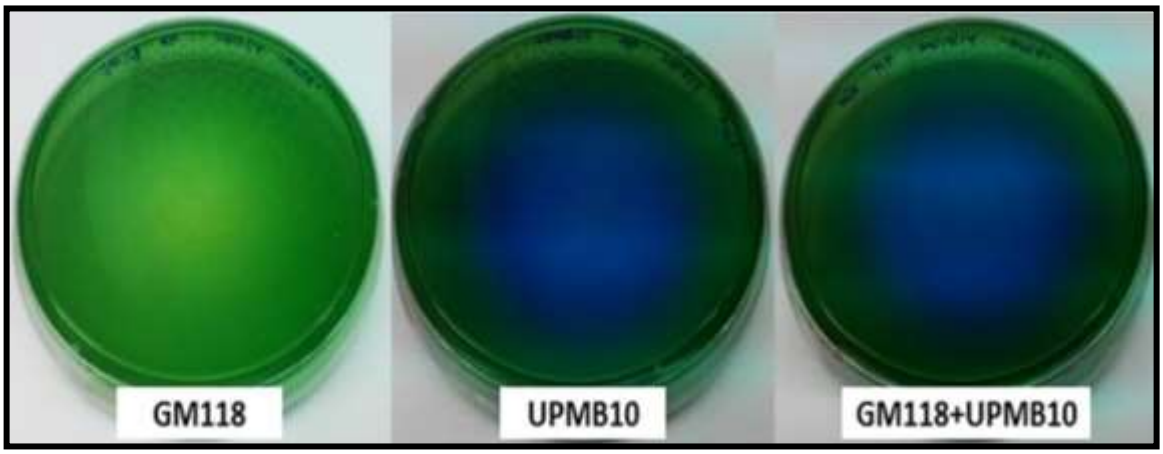

Plate 1. Nitrogen fixing ability of single and combine inoculation on N-free solid malate (Nfb) medium agar plates. Source: Day and Döberenier (1976).

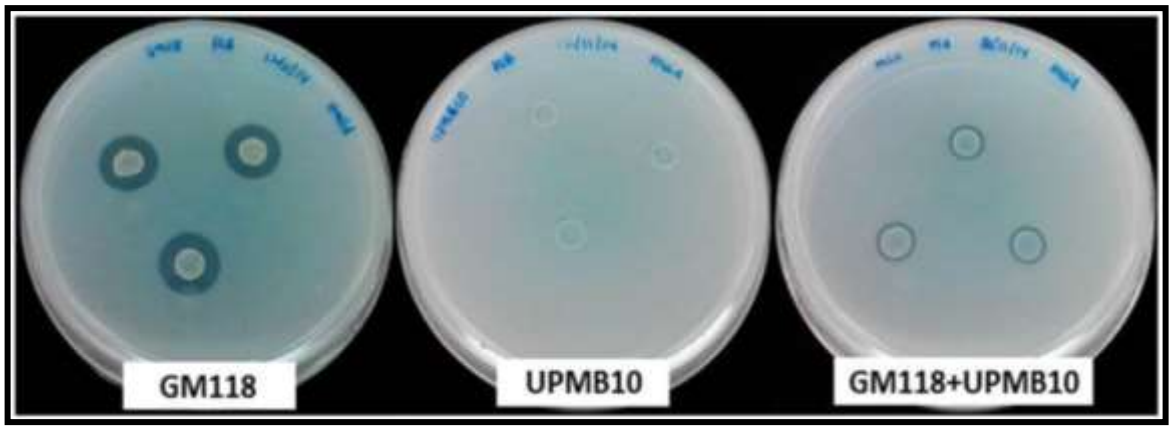

Plate 2. P solubilization of single and combine inoculation on Pikovskaya agar media plates. Source: Pikovskay (1948).

UPMB10 and GM118 in Mixture that caused it has the potential to fix nitrogen and solubilize phosphate. Both characteristics are very useful in ensuring that these isolates have potential to promote plant growth.

From Table 1, total number of grain per panicle, fill grains per panicle, 1000 grains weight, total spikelets per panicle, panicle length and number of primary branch per panicle were significantly different between inoculated treatments and control. With presence of bacteria inoculation its improvement grain physiological quality and showed positive symbiosis interaction between plant and bacteria. Rice growth performance is exposed to environmental factors which affect the physiological processes inside rice plant cells. Improving rice physiological characteristic is considered to be desirable because of its agronomic importance towards the high rice yield achievement (Li et al*.,* 2012). This finding is in agreement with Alam et al. (2008) and Islam et al. (2012) who obtained increased rice grain yield in bacteria inoculation over the uninoculated control.

UPMB10 produced the highest breaking force to detach grain from panicle and this finding was complementary to the in-vitro assay, with showed that UPMB10 produce the highest concentration of IAA either with or without L-tryptophan as represented in Figure 1. The presence of UPMB10 in Mixture who contributed to the higher concentration of IAA compared to GM118 and in turn requires high breaking force to detach grain from panicle. IAA, one of the phytohormones, can be considered as the most physiologically active auxin in plants that influences root and shoot dry matter partitioning, root and stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants (Cleland, 1990; Subba, 1999). Biswas et al. (2000) suggested a possibility of interrelation between increased tiller production and yield of rice with a change in

hormonal equilibrium, particularly IAA, due to rhizobial inoculation. Hence, it can be assumed that IAA inhibits or prolongs abscission process in the panicle via reduction of sensitivity of abscission zone (AZ). This abscission process is triggered by ethylene hormone and interplay with IAA. Meir et al. (2010), showed the role of IAA in early inhibition of organ abscission. A basipetal through the AZ inhibited abscission by rendering the AZ insensitive to ethylene. Several researchers have reported that in abscission process, the interplay between IAA and ethylene is well established (Roberts et al., 2002). Furthermore, it has been reported that elevated levels of IAA can "protect" AZ cell from abscission inducing stimulate on Arabidopsis (Basu et al., 2013). This finding can be assumed that the UPMB10 was the key trigger of hard detachment of grain panicle.

Besides, previous studies also indicated that IAA production could be a practical characteristic to choose endophytic and rhizosphere competent bacteria for rice growth promoting agents (Etesami and Alikhani, 2016; Etesami et al., 2015). The dynamic responsive pattern of IAA distribution within PGPR is a key factor to support plant growth, its reaction toward root rhizosphere, and specifically for stimulating development of plant organs.

It is clearly shown that the application of both single and combined inoculations of PGPR significantly increased the non-shattering of grain panicle (Figure 2). The result also indicated a significant difference between the lower position of grain panicle but not in the upper and middle positions and in turn showed that the lower position requires a higher force to detach grain from the panicle (Figure 3). Besides, only lower grain position of the panicle showed significant effect toward non-shattering habit. This may be due to non-uniform grain maturity across the panicle, which affects the required detaching force of a grain from the panicle (Lee and Huh, 1984; Szot et al., 1998).

This shattering habit result was also supplementary with the enhancement of total grain number and total spikelet number per panicle pattern in a time. So, the number of grains remain on the panicle can be preserved until harvesting time and reduce post-harvest losses. Hence, the national SSL percentage target to achieve yield in average 6 ton/ha can be done by applying the bacteria PGPR.

Conclusion

The data in this study showed that the positive effect of applying single inoculation; *B. subtilis* (UPMB10) and *B. pumillus* (GM118) along with combined inoculations (Mixture) of PGPR give significant increase in yield production compared to control. Besides that, the ability of UPMB10 and Mixture inoculation produce indole-3-acetic acid (IAA) and gives dissimilar result in grain shattering habit compared to GM118 inoculation. Again, single inoculation of UPMB10 and Mixture showed that both of them produced high breaking force energy to prevent detachment of grain from panicle. Moreover, increased number of total spikelet per panicle and total grain per panicle data also can be observed. Hence, PGPR that produce IAA can increase non-shattering of grain on panicle and in turn increase rice yield production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Alam MS, Talukder NM, Islam MT, Sarkar A, Hossain MM (2008). Phosphate solubilizing rhizoplane bacteria on growth and yield of transplant aman rice. Journal of Agroforestry and Environment 2(1):1-6.

Alizadeh MR, Allameh A. (2011). Threshing force of paddy as affected by loading manner and grain position on the panicle. Research in Agricultural Engineering 57:8-12.

Basu MM, González-Carranza ZH, Azam-Ali S, Tang S, Shahid AA, Roberts JR (2013). The manipulation of auxin in the abscission zone cells of arabidopsis flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding. Plant Physiology 162:96-106.

Biswas JC, Ladha JK, Dazzo FB (2000). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Science Society of America Journal 64:1644-1650.

Cleland RE (1987). Auxin and cell elongation. In Plant hormones and their role in plant growth and development. Springer Netherlands pp. 132-148.

Day JM, Döbereiner J (1976). Physiological aspects of N_2-fixation by a Spirillum from *Digitaria* roots. Soil Biology and Biochemistry 8(1):45-50.

Etesami H, Alikhani HA, Hosseini HM (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX 2:72-78.

Etesami H, Alikhani HA (2016). Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects. Biological Control 94:11-24.

Glick BR (2012). Plant growth-promoting bacteria: Mechanisms and applications. Scientifica 2012.

Gordon SA, Weber RP (1951). Colorimetric estimation of indoleacetic acid. Plant Physiology 26(1):192-195.

Islam MZ, Sattar MA, Ashrafuzzaman M, Saud HM, Uddin MK (2012). Improvement of yield potential of rice through combined application of biofertilizer and chemical nitrogen. African Journal of Microbiology Research 6(4):745-750.

Kausar H, Sariah M, Mohd Saud H, Zahangir Alam M, Razi Ismail M (2011). Isolation and screening of potential *actinobacteria* for rapid composting of rice straw. Biodegradation 22:367-375.

Li X, Bu N, Li Y, Ma L, Xin S, Zhang L (2012). Growth, photosynthesis and antioxidant responses of endophyte infected and non-infected rice under lead stress conditions. Journal of Hazardous Materials

213:55-61.
Lin Z, Griffith ME, Li X, Zhu Z, Tan L, Fu Y, Sun C (2007). Origin of seed shattering in rice (*Oryza sativa* L.). Planta 226(1):11-20.
Meir S, Philosoph-Hadas S, Sundaresan S, Selvaraj KV. Burd S, Ophir R, Lers A (2010). Microarray analysis of the abscission-related transcriptome in the tomato flower abscission zone in response to auxin depletion. Plant Physiology 154(4):1929-1956.
Mohammadi K, Sohrabi Y (2012). Bacterial biofertilizers for sustainable crop production: a review. ARPN Journal of Agricultural and Biological Science 7(5):307-316.
Pikovskaya RI (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362-370.
Roberts JA, Whitelaw CA, Gonzalez-Carranza ZH, McManus MT (2000). Cell separation processes in plants-models, mechanisms and manipulation. Annals of Botany 86(2):223-235.
Sadeghi M, Araghi HA, Hemmat A (2010). Physico-mechanical properties of rough rice *(Oryza sativa* L.) grain as affected by variety and moisture content. Agricultural Engineering International: CIGR Journal 12(3-4):129-136.
Saharan BS, Nehra V (2011). Plant growth promoting rhizobacteria: a critical review. Life Sciences and Medical Research 21(1):30.
Seizo M (1980). Easy diagnosis of rice cultivation. Rice Cultivation for the Million; Japan Scientific Societies Press: Tokyo, Japan pp. 30-31.
Subba Rao NS (1999). The rhizosphere and the phyllosphere. Science Publishers, Inc., Enfield, NH. P 85.
Szot B, Ferrero A, Molenda M (1998). Binding force and mechanical strength of rice grain. International Agrophysics 12:227-230.
Thurber CS, Hepler PK, Caicedo AL (2011). Timing is everything: early degradation of abscission layer is associated with increased seed shattering in US weedy rice. BMC Plant Biology 11(1):14.
Yu X, Liu X, Zhu TH, Liu GH, Mao C (2012). Co-inoculation with phosphate-solubilzing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. European Journal of Soil Biology 50:112-117.