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Effectiveness of halotolerant diazotrophic bacteria in increasing nitrogen and potassium uptake of rice in high salinity medium

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Abstract

Purwanto, Oktaviani, E., Ulinuha, Z., Tarjoko & Leana, N. W. A. (2024). Effectiveness of halotolerant diazotrophic bacteria in increasing nitrogen and potassium uptake of rice in high salinity medium. *Bulg. J. Agric. Sci., 30*(5), 888–892

This study aimed to examine the effectiveness of indigenous diazotrophic bacteria from saline paddy fields of The Northern Coastal of Pemalang in increasing N and K nutrient uptake in rice plants. This research was conducted at the Experimental Farm and Agronomy & Horticulture Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. This study was designed using a completely randomized block design with nitrogen fixing bacteria as the treatment and repeated three times. The treatment that was tried was a type of N_2-fixing bacteria consisting of control without inoculation of bacteria (P_0), *Acinetobacter junii* (P_1), *Bacillus tropicus* (P_2), *Acinetobacter schindleri* (P_3), *Pseudomonas stutzeri* (P_4), *Bacillus altitudinis* (P_5), *Bacillus cereus* (P_6), and *Bacillus subtilis* (P_7). The study used the culture solution method with the source of nutrition from AB Mix^{tm} nutrient with EC set at 5.5 dSm^{-1}, and pH 5.5–6.5. Observation variables including N uptake by rice plants were analyzed at the maximum vegetative phase using the Kjedahl method, Na and K absorption of rice plants were analyzed by diacidic extraction method and the results were measured using a flame photometer. The research data were analyzed using ANOVA, and if they were significantly different, they continued with DMRT 5%. The result showed that inoculation of rice plants with Bacillus subtilis significantly increased plant biomass, N uptake, and K uptake. Diazotrophic bacteria inoculation was able to increase the N uptake of rice plants by an average of 0.341 g/plant (104.36%), while in the control treatment it was only 0.167 g/plant. Inoculation of diazotrophic bacteria in plants under salinity stress tends to reduce Na uptake in rice plants. Inoculation of diazotropic bacteria was able to increase the average K/Na ratio of 113.31%, and this indicated that inoculation of halotolerant diazotrophic bacteria was able to increase the tolerance of rice plants to salinity stress.

Keywords: rice; diazotropic; bacteria; saline; nutrient

Introduction

Rice as the staple food of the Indonesian people, makes rice the main commodity and its availability must be maintained. Various efforts to increase production have been carried out, however, have not shown optimal results. Rice production tends to decrease. Khasanah & Astuti (2022) reported that Indonesia's rice production in 2021 was 31.36 million tons and there was a decrease in production of 0.45% compared to 2020. Java Island is the largest center of rice production, especially Central Java, East Java and West Java with a total production of 52% of national production (Wardani et al., 2019; Syarifuddin et al., 2019; Pratama & Harini, 2019). Increased production is faced with the reduction of productive land as a result of land conversion from productive rice fields to housing, industry and other purposes. Purbiyanti et al. (2017) reported that the conversion of agricultural land in Indonesia reached 10 million ha per year.

The increase in national rice production must always be increased through an extensification program by utilizing saline land. Indonesia has potential saline land for rice production of around 9 million ha. Rice fields with high salinity in Java are generally in the northern coastal area caused by seawater intrusion through rivers or canals (Erfandi & Rachman, 2011). However, the use of saline land faces problems as the effect of salinity on plants. Salinity triggers soil degradation through the accumulation of salt in the soil and tends to reduce crop productivity (Basak et al., 2022). High salt content in aqueous solutions reduces the ability of plants to absorb water, which is due to differences in osmotic pressure or water-deficit effect of salinity (Machado & Serralheiro, 2017). Saline soil has electrical conductivity above 4 dS/cm with soluble content such as NaCl, Na_2CO_3, Na_2SO_4 and some anions such as Cl^- can cause damage to cell membranes, causing leakage in cell membranes (Karolinoerita & Yusuf, 2020). Furthermore, Shrivastava & Kumar (2015) stated that salinity causes a decrease in plant growth as a result of osmotic stress, nutrient deficiency, ion toxicity, and oxidative stress, so that plants experience difficulties in absorbing water. Abd El-RheemKh & Zaki (2018) reported that an increase in salinity led to a decrease in the levels of N and P in the tissues of Peanut Plants, in addition, there was an increase in the levels of K in the plant tissues.

A biological approach in an effort to increase the productivity of saline soils has been carried out by using saline tolerant rice varieties, but their productivity is still low. Therefore, it needs to be supported with technology that can increase the fertility of saline soil so that the growth and yield of rice plants can increase. Alternative environmentally friendly technology by utilizing beneficial microbes. The rhizosphere of rice plants is an area with very high microbial abundance, and the use of beneficial microbes will increase growth and suppress the effects of osmotic stress (Tedeschi, 2020). Beneficial microbes are useful for increasing plant growth and reducing salt stress by increasing the availability of nutrients and hormones or by reducing ethylene production (Gao et al., 2022). Gao et al. (2022) and Nawaz et al. (2020) reported that the application of PGPR (*P. fluorescens, B. pumilus,* and *E. aurantiacum*) significantly improved the growth, yield and application of *E. aurantiacum* increased K uptake in wheat plant tissue by 286.36%. Furthermore, Shabaan et al. (2022) reported that plants inoculated with salt-tolerant rhizobacteria in corn plants were able to increase nutrient uptake and reduce Na^+ contnets so that the K^+/Na^+ ratio increased. This study aimed to examine the effectiveness of indigenous diazotrophic bacteria from saline paddy fields of The Northern Coastal of Pemalang in increasing N and K nutrient uptake in rice plants.

Material and Methods

Experimental design

This research was conducted at the Plastic House Experimental Farm and Agronomy & Horticulture Laboratory, Faculty of Agriculture, Jenderal Sudirman University, Purwokerto, Central Java Indonesia. The research was conducted from July till October 2022. This study was designed using a completely randomized block design with nitrogen fixing bacteria as the treatment and repeated three times. The treatment that was tried was a type of N_2-fixing bacteria consisting of control without inoculation of bacteria (P_0), *Acinetobacter junii* (P_1), *Bacillus tropicus* (P_2), *Acinetobacter schindleri* (P_3), *Pseudomonas stutzeri* (P_4), *Bacillus altitudinis* (P_5), *Bacillus cereus* (P_6), and *Bacillus subtilis* (P_7). The rice variety used in this study was Inpari Unsoed 79 Agritan.

The study used the culture solution method with the source of nutrition from AB Mix^{tm} nutrient with EC set at 5.5 dSm^{-1}, and pH 5.5–6.5 (Junianti et al., 2020). Bacteria were purified using NA media (Himedia), then propagated using NB media (Himedia) as stock. Propagation of bacterial culture for applications using 5% molasses media. A total of 2 ml of bacterial culture stock was inoculated in 1000 ml of 5% molasses media, then shaken at a speed of 120 rpm for 48 h and the bacterial population reached above 10^7 cfu/ml. AB Mix solution is made concentration of 1100 ppm in 4 L of water, then treated with NaCl 5 $dScm^{-1}$ and inoculated bacteria as much as 2 mL. The concentration of the solution was maintained at 1100 ppm, with an EC of 5.5 dSm^{-1}, and a pH of 5.5–6.5. Salinity treatment and bacterial inoculation were carried out when the plants were 2 weeks after planting and renewed once a week. Rice seeds were sown using rockwool. Before sowing, rice seeds were sterilized using 0.1% mercuric chloride solution and then rinsed with sterile distilled water. Rice seeds were soaked in distilled water for 24 h, then sown in moist rockwool media. Rice seeds were transplanted after 12 days after sowing.

Observed parameters

Observation variables including N uptake by rice plants were analyzed at the maximum vegetative phase using the Kjeldahl method (Shabaan et al., 2022). Na and K absorption of rice plants were analyzed by diacidic extraction method and the results were measured using a flame photometer (Kapadia et al., 2021).

Statistical analysis

The research data were analyzed using ANOVA, and if they were significantly different, they continued with DMRT 5%.

Results and Discussion

Effect of Halotolerant Diazotrophic Bacteria on Shoot Dry Weight

The results showed that inoculation of diazotrophic bacteria had a significant effect on the shoot dry weight of the rice (0.0355 > p). Diazotropic bacteria inoculation was able to increase the dry weight of rice plants up to 104.21% compared to control. The results of plant dry weight showed that the *Bacillus substilis* bacterial treatment was able to produce the highest plant dry weight of 13.89 g/plant, followed by *Acinetobacter schindleri*, *Bacillus altitudinis, Acinetobacter junii*, *Bacillus cereus* and *Bacillus tropicus* with 13.113, 12.966, 12.804, 12.71 and 12.291 g respectively (Figure 1).

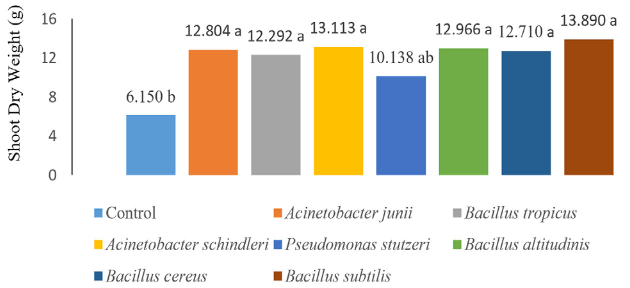

Fig. 1. Effect of halotolerant diazotrophic bacteria on shoot dry weight

Bacterial inoculation was able to increase the dry weight of rice plants positively under saline conditions. The ability of bacteria to increase plant growth is supported by the ability of bacteria to produce growth substances, such as growth regulators and their ability to fix N_2. The ability to fix N_2 by bacteria is able to provide nitrogen nutrients and is well absorbed by plants so that plant vegetative growth increases. The total plant dry matter increases with the availability of N nutrients, and the availability of sufficient nitrogen in high salinity conditions can reduce the negative impact of high salt content in the growing medium (Irshad et al., 2009).

These results are in line with several previous studies on several plants. Jha & Subramanian (2013) reported that inoculation of *B. pumilus* and *P. pseudoalcaligenes* was able to increase rice plant growth and effectively reduce the negative impact of any increase in salinity above 1 percent. Furthermore, Nawaz et al. (2020) reported that the application of PGPR to *P. fluorescens, B. pumilus*, and salt-tolerant *E. aurantiacum* strains significantly increased the growth and yield of wheat plants grown in saline conditions, where PGPR has the ability to produce auxin (IAA), Fixing N_2, solubilizing of P and was able to produce ACC deaminase.

Nutrient Uptake of Rice

Nutrient levels of nitrogen, potassium and Na in plant tissues are shown in Table 1. Inoculation of diazotrophic bacteria in medium with high salinity was able to increase nitrogen uptake of rice plants. Diazotrophic bacteria inoculation was able to increase the N uptake of rice plants by an average of 0.341 g/plant (104.36%), while in the control treatment it was only 0.167 g/plant. Inoculation with *Bacillus subtilis* showed the highest N uptake of 0.381 g/plant, however this result was not different from other diazotrophic bacteria treatments (*Acinetobacter junii, Bacillus tropicus, Acinetobacter schindleri, Pseudomonas stutzeri, Bacillus altitudinis* and *Bacillus cereus*). The results of this study proved that the application of rhizobacteria, especially N-fixers, was able to improve the ability of plants to absorb nutrients. The ability of bacteria to fix N and produce IAA stimulates root growth, resulting in a wider root surface area and PGPB promotes nutrient acquisition and survival under stress conditions (Kumar et al., 2020).

Inoculation of diazotrophic bacteria was able to increase the nutrient uptake of potassium by rice plants. It can be seen that the K uptake of rice plants increased significantly in the treatment of various diazotrophic bacteria, compared to the control. This was in contrast to Na uptake, where Na uptake decreased in various treatments of Diazotrophic bacteria. The highest Na uptake was seen in the control treatment of 0.124 g/plant (Table 1).

Table 1. Effect of halotolerant diazotrophic bacteria on N, K and Na uptake

Treatments	N, g/plant	K, g/plant	Na, g/plant
Control	0.167 b	0.049 c	0.124 a
Acinetobacter junii	0.332 a	0.102 a	0.078 b
Bacillus tropicus	0.322 a	0.087 a	0.075 b
Acinetobacter schindleri	0.346 a	0.067 bc	0.095 ab
Pseudomonas stutzeri	0.297 a	0.055 c	0.092 ab
Bacillus altitudinis	0.364 a	0.070 bc	0.089 ab
Bacillus cereus	0.347 a	0.072 bc	0.107 ab
Bacillus subtilis	0.381 a	0.073 bc	0.105 ab

Note: the numbers followed by the same letter in the same column are not significantly different according to DMRT 5%

Inoculation of rice plants with *Acinetobacter junii* bacteria had the highest K absorption of 0.102 g/plant, and Na uptake of 0.078. The lowest Na uptake was achieved at *Bacillus tropicus* inoculation of 0.075 g/plant. This phenomenon indicated that inoculation of various diazotrophic bacteria was able to increase nutrient uptake of both nitrogen and potassium, and also suppress the accumulation of Na in the rice shoot. Microbes are able to alter the uptake of toxic ions and nutrients by roots by altering host physiology or by directly reducing the accumulation of toxic ions in leaves in addition to increasing macro and micro nutrients (Paul & Lade, 2015). Upadhyay & Singh (2014) reported reported that inoculation of wheat plants with *B. pumilus, B. cereus* and *B. subtilis* was able to increase K uptake in the shoot tissue, and decrease Na uptake.

K/Na Ratio

The results showed that inoculation of diazotrophic bacteria increased the K/Na ratio in rice plants in media with high salinity. The increase of K/Na ratio in the inoculation treatment of diazotropic bacteria averaged 0.86 or an increase of 113.31% compared to the control (0.403). The highest value of K/Na ratio was achieved in the inoculation treatment of *Acinetobacter junii* and *Bacillus tropicus* of 1.328 and 1.209, respectively. Inoculation with bacteria *Acinetobacter junii* and *Bacillus tropicus* was able to increase the value of K/Na very large by 229.83% and 200.28%, respectively.

In general, inoculation of diazotrophic bacteria increased the value of the K/Na ratio, and these results indicated that inoculation of diazotrophic bacteria was able to increase the tolerance of rice plants in high salinity conditions. The key for rice plants to be able to survive in conditions of salinity stress is to maintain the K/Na ratio in cells (Jha & Subramanian, 2013; Reddy et al., 2017). The results of this study are

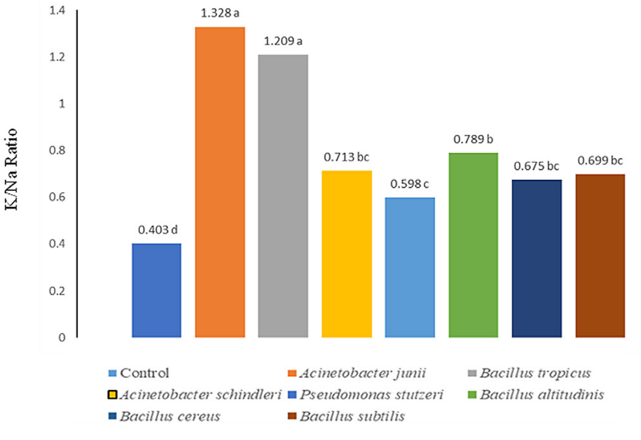

Fig. 2. Effect of diazotrophic bacteria inoculation on K/Na ratio

in line with Jha & Subramanian (2013) where inoculation with PGPR under saline stress resulted in a high K/Na ratio. Shultana et al. (2020a) stated that bacteria capable of producing EPS and high populations in saline conditions were able to reduce the concentration of available Na^+ to plants so as to increase plant tolerance in saline environmental conditions. Further more Shultana et al. (2020b) explained that the inoculation of rice plants with PGPR causes the binding of Na^+ ions in the rhizosphere through the production of exopolysaccharides and biofilms which act as a barrier for Na^+ to be translocated to the canopy resulting in a higher K/Na ratio in the plant canopy.

Conclusion

Based on the results of this study, it can be concluded that inoculation of rice plants with Bacillus subtilis significantly increased plant biomass, N uptake, and K uptake. Diazotrophic bacteria inoculation was able to increase the N uptake of rice plants by an average of 0.341 g/plant (104.36%), while in the control treatment it was only 0.167 g/plant. Inoculation of diazotrophic bacteria in plants under salinity stress tends to reduce Na uptake in rice plants. Inoculation of diazotropic bacteria was able to increase the average K/Na ratio of 113.31%, and this indicated that inoculation of halotolerant diazotrophic bacteria was able to increase the tolerance of rice plants to salinity stress.

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References

Abd El-Rheem, M. & Zaki, S. S. (2018). Effect of soil salinity on growth, yield and nutrient balance of peanut plants. *International Journal of ChemTech Research*, *8*(12), 564–568.

Basak, N., Rai, A. K., Sundha, P., Meena, R. L., Bedwal, S., Yadav, R. K. & Sharma, P. C. (2022). Assessing soil quality for rehabilitation of salt-affected agroecosystem : a comprehensive review. *Front. Environ. Sci.*, *10,* 935785, 1–15. https://doi.org/10.3389/fenvs.2022.935785.

Erfandi, D. & Rachman, A. (2011). Identification of soil salinity due to seawater intrusion on rice field in the Northern Coast of Indramayu, West Java. *J. Trop. Soils*, *16*(2), 115–121. https://

doi.org/10.5400/jts.2011.16.2.115.

Gao, Y., Zou, H., Wang, B. & Yuan, F. (2022). Progress and applications of plant growth-promoting bacteria in salt tolerance of crops. *Int. J. Mol. Sci.*, *23*(7036), 2–20.

Irshad, M., Eneji, A. E., Khattak, R. A. & Khan, A. (2009). Influence of nitrogen and saline water on the growth and partitioning of mineral content in maize. *Journal of Plant Nutrition*, *32*, 37–41. https://doi.org/10.1080/01904160802660768.

Jha, Y. & Subramanian, R. B. (2013). Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline conditions. *Chilean Journal of Agricultural Research*, *73*(3), 213–219. https://doi.org/10.4067/S0718-58392013000300002.

Junianti, E., Proklamasiningsih, E. & Purwanto. (2020). The effect of PGPR inoculation on the rice growth at vegetative phase. *Jurnal Agro*, *7*(2), 193–202.

Kapadia, C., Sayyed, R. Z., Ali, H., Enshasy, E., Vaidya, H., Sharma, D., Patel, N., Malek, R. A., Syed, A., Elgorban, A. M., Ahmad, K., Tan, A. & Zuan, K. (2021). Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrient enrichment. *Sustainability*, *13*(8369), 2–14.

Karolinoerita, V. & Annisa, W. (2020). Land salinization and its problems in Indonesia *Jurnal Sumberdaya Lahan*, *14*(2), 91–99.

Khasanah, I. N. & Astuti, K. (2022). Harvest Area and Rice Production in Indonesia 2021. Badan Pusat Statistik Indonesia.

Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S. & Verma, J. P. (2020). Plant growth-promoting bacteria : biological tools for the mitigation of salinity stress in plants. *Front. Microbiol.*, *11*(1216), 1–15. https://doi.org/10.3389/fmicb.2020.01216.

Machado, R. M. A. & Serralheiro, R. P. (2017). Soil Salinity: Effect on vegetable crop growth . management practices to prevent and mitigate soil salinization. *Horticulturae*, *3*(30), 2–13. https://doi.org/10.3390/horticulturae3020030.

Nawaz, A., Shahbaz, M., Asadullah, Imran, A., Marghoob, M. U., Imtiaz, M. & Mubeen, F. (2020). Potential of salt tolerant PGPR in growth and yield augmentation of wheat (*Triticum aestivum* L.) under saline conditions. *Front. Microbiol.*, *11*(2019), 1–12. https://doi.org/10.3389/fmicb.2020.02019.

Paul, D. & Lade, H. (2015). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils : a review. *Agron. Sustain. Dev.*, *34*(4), 737–752. https://doi.org/10.1007/s13593-014-0233-6.

Pratama, A. R. & Harini, R. (2019). Analysis of the availability and demand for rice in Indonesia in 2018. *Media Komunikas Geografi*, *20*(2), 101–114.

Purbiyanti, E., Yazid, M. & Januarti, I. (2017). The conversion of rice fields in Indonesia has an influence on the government's grain/rice purchasing price policy. *Jurnal Manajemen Dan Agribisnis*, *14*(3), 209–217.

Reddy, I. N. B. L., Kim, S., Kim, B., Yoon, I. & Kwon, T. (2017). Identification of rice accessions associated with K^+/Na^+ ratio and salt tolerance based on physiological and molecular responses. *Rice Science*, *24*(6), 360–364. https://doi.org/10.1016/j.rsci.2017.10.002.

Shabaan, M., Asghar, H. N., Zahir, Z. A., Zhang, X., Sardar, M. F. & Li, H. (2022). Salt-tolerant PGPR confer salt tolerance to maize through enhanced soil biological health, enzymatic activities, nutrient uptake and antioxidant defense. *Front. Microbiol.*, *13*(901865), 1–13. https://doi.org/10.3389/fmicb.2022.901865.

Shrivastava, P. & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, *22*(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001.

Shultana, R., Tan, A., Zuan, K., Yusop, M. R. & Saud, H. M. (2020a). Characterization of salt-tolerant plant growth- promoting rhizobacteria and the effect on growth and yield of saline-affected rice. *Plos One*, *15*(9), 1–16. https://doi.org/10.1371/journal.pone.0238537.

Shultana, R., Zuan, A. T. K., Yusop, M. R., Saud, H. M. & Ayanda, A. F. (2020b). Effect of salt-tolerant bacterial inoculations on rice seedlings differing in salt-tolerance under saline. *Agronomy*, *10*(1030), 2–22.

Syarifuddin, M., Nuraini, I. & Wahyudi, S. (2019). Analysis of rice production for each province in Indonesia 2011-2016. *Jurnal Ilmu Ekonomi (JIE)*, *3*(4), 517–531.

Tedeschi, A. (2020). Irrigated Agriculture on saline soils: a perspective. *Agronomy*, *10*(1630), 4–9.

Upadhyay, S. K. & Singh, D. P. (2014). Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. *Plant Biology*, *17*(1), 288–293. https://doi.org/10.1111/plb.12173.

Wardani, C., Jamhari, Hardyastuti, S. & Suryantini, A. (2019). Rice resilience performance in Indonesia: comparison of Java and Outside Java for the 2005-2017 period. *Jurnal Ketahanan Nasional*, *25*(1), 107–130.

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