POLISH JOURNAL OF SOIL SCIENCE
VOL. LV/2 2022 PL ISSN 0079-2985

DOI: 10.17951/pjss/2022.55.2.79

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APPLICATION TECHNIQUE AND DOSAGE OF HALOTOLERANT NITROGEN BIOFERTILIZER FOR INCREASING SOIL TOTAL N, N UPTAKE, CHLOROPHYLL CONTENT, PHOTOSYNTHATE ACCUMULATION AND GROWTH OF RICE PLANTS IN SALINE ECOSYSTEM

Received: 22.04.2021
Accepted: 06.10.2022

Abstract. Utilization of halotolerant nitrogen biofertilizer can increase N uptake and promote growth and yield of rice plants in saline ecosystem. Halotolerant nitrogen biofertilizer can be applied to seed and seedling in certain dosage. The aim of this study was to obtain the application technique and dosage that can increase N uptake and promote growth and yield of rice plants in saline ecosystem. The research was conducted in the greenhouse in Kawarang District (Indonesia) from September until November 2020. There was used randomized block design which consisted of two factors with three replications. The first factor was application techniques, i.e. seed treatment, nursery treatment, and seed + nursery treatment. The second factor was dosage of halotolerant nitrogen biofertilizer, i.e. 0, 500, 1,000, 1,500 and 2,000 g ha^{-1}. The results showed that halotolerant nitrogen biofertilizer applied in seed and nursery treatment can increase N uptake and plant height by 3.95 g plant^{-1} and 34.50 cm, respectively. Dosage of 1,500 g ha^{-1} can increase the

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total N of soil (0.26%), chlorophyll content (46.97 SPAD), photosynthate accumulation (3.33 g), and rice yield (13.40 t/ha). Application of halotolerant nitrogen biofertilizer in seed and nursery technique at a dosage of 1,500 g ha^{-1} can be further recommendation in rice cultivation on saline ecosystems.

Keywords: dosage, halotolerant nitrogen biofertilizer, nitrogen, saline ecosystem, technique

INTRODUCTION

Utilization of saline soil for rice cultivation may face various obstacles. Plants that grow on saline soils experience high osmotic stress, toxicity and disturbances in plant nutrition or nutrient balance (Wang *et al*. 2022). Saline soils interfere N cycle and are generally indeficit in the soil (Setiawati *et al*. 2022). Nitrogen deficiency in soil could lead to difficulty and limited uptaking N by rice plants, meanwhile element N is crucial for rice plants to support its growth and development (Zhang *et al*. 2018). The excessive inorganic N fertilization to meet plant N needs can cause new problems, such as triggering mineralization of soil organic matter, increasing soil salinity, plants becoming less responsive to inorganic fertilizers, surplus of elements N, P, Ca, Mg, and Na but deficiency of elements K and Si (Khumairah *et al*. 2022a). In addition, the potential of NO_3 leaching can increase where moderate to high amounts of salt remain in the soil because plants experiencing salt stress cannot efficiently absorb and/or utilize inorganic N fertilizers applied as plants that do not experience salt stress (Chen *et al*. 2021). Increased use of inorganic N fertilizers can also be a threat to the natural environment by contaminating water, air and soil (Rahman and Zhang 2018). For this reason, we need another fertilizer alternative that is environmentally friendly but can work effectively in increasing the N element in saline soils so that it can support plant growth, namely the use of nitrogen biofertilizers. Nitrogen biofertilizer can be defined as substances that contain N fixing rhizobacteria which, when applied to plants, seeds or soil can colonize the plant rhizosphere and promote plant growth by increasing the supply and availability of essential plant nutrients (Khumairah *et al*. 2022b). N-fixing rhizobacteria colonize plant roots, fix N to become available in the soil which are represented as N-total value of soil, and promote plant growth (Basu *et al*. 2021). Production of indole acetic acid, gibberellin and several other determinants, resulted in an increase in plant height, chlorophyll content, and accumulation of photosynthate leading to increased N absorption thereby improving plant health under salt stress conditions (Arifin *et al*. 2021). In order to be applied in saline soils, nitrogen fixing rhizobacteria, that act as bioactive agents in nitrogen biofertilizers, must also be salinity tolerant. They are called "halotolerant nitrogen biofertilizers" (hereinafter referred to as "HNB"). Halotolerant nitrogen biofertilizer can be applied to various phases of plant growth, but in this study it was provided at the seed and nursery stages which are considered as the most critical stages for rice plants (Hussain *et al*. 2018). The dosage of halotolerant biofertilizers also determines the effectiveness of its performance. Basically, the greater the dosage of HNB, the more numerous or dense halotolerant nitrogen fixing rhizobacteria in it, so that it may provide more N (Mącik *et al*. 2020). Yet, if the microbial density is increased in the biofertilizer, then the dosage is allowed to be reduced (Li *et al*. 2022). Based on the above problems, this study aims to obtain the best application technique with the exact dosage that can increase total N, N uptake, chlorophyll content, photosynthate accumulation and growth of rice plants applied in saline ecosystem.

MATERIALS AND METHODS

Research site

This research was done in the greenhouse in Jayamukti, Banyusari Village, Kawarang District, West Java (Indonesia) from September until November 2020 (latitude 6°8'46"S, longitude 107°33'51"E, and altitude 31 m a.s.l.). The area is the center of Indonesian rice production which is heavily affected by sea water intrusion so that the greenhouse conditions were engineered to be similar to the closest environment. An average daily temperature and humidity belong to characteristics of lowland areas, which were 30.68°C and 72.05%, respectively. Daily average temperature and humidity can be seen in Fig. 1.

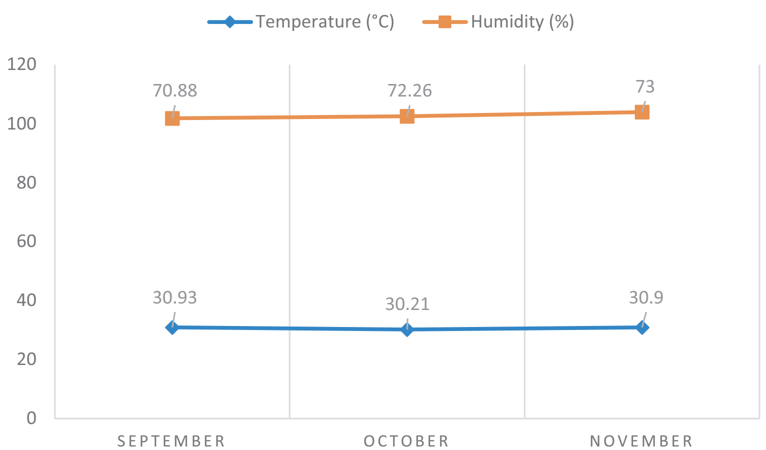

Fig. 1. Average daily temperature and humidity

The soil used was categorized as moderately saline since soil EC was 7.57 dS m^{-1} and pH 6.68. Soil texture was clay with low C/N ratio. Results of initial soil analysis used in experiment were presented in Table 1.

Table 1. Soil physical and chemical properties as initially analyzed

pH	C-org (%)	N (%)	C/N ratio	CEC ($cmol.kg^{-1}$)	Base saturation (%)	EC ($dS\ m^{-1}$)	Total bacteria ($CFU\ mL^{-1}$)	Sand (%)	Silt (%)	Clay (%)
6.68	1.36	0.21	7	45.79	30.57	7.57	8.0×10^9	37	14	49

HNB preparation

Active agents in HNB were halotolerant nitrogen fixing rhizobacteria isolates consisting of *Pseudomonas stutzeri*, *Klebsiella pneumonia*, *Bacillus cereus*, and *Delftia tsuruhatensis* which had been cultured previously in salinized nutrient agar media (6 dS m^{-1}) with a density of 10^9CFU/mL^{-1}. Then these bioagents were mixed with solid carrier materials which had been ground to a size of 0.5–1.5 mm and sterilized for 15 min using an autoclave with a temperature of 121°C, and pressure at 15 atm, with peat 50% + 17.5% compost + 17.5% biochar + 5% dolomite + 5% guano + 5% nutrition in alumunium foil. The amount of inoculant mixed into the carrier was 20% of the total carrier volume by injection. Water content and pH of HNB were 35.07% and 8.1, respectively.

Experimental design

The experimental design used in this study was randomized block design (RBD) consisting of two factors with three replications. The first factor was application techniques, i.e. seed treatment, nursery treatment, and seed + nursery treatment. The second factor was dosage of HNB, i.e. 0, 500, 1,000, 1,500 and 2,000 g ha^{-1}. The experiment was started by transfering saline soil into a bucket with a capacity of 10 kg. Soil was taken from a depth of 0–25 cm and had been cleaned from plant debris. For application in seed treatment, HNBs were mixed with rice seeds according to the respective treatment doses, namely 0, 500, 1,000, 1,500, and 2,000 g ha^{-1} and 0, 20, 40, 60, and 80 g kg^{-1} rice seeds. For application in the nursery, HNBs were spread on the nursery media. For the combination treatment between seed treatment and nursery, 20 g of HNB were mixed with 1 kg of seeds and the remaining part was spread in the nursery according to each treatment. Nursery stages were done in a pot tray of 30 × 40 × 10 cm by planting the seeds on the soil growing medium and organic fertilizer with a ratio of 1:1 v/v. Seeds were spread on the media, then stored in a place that is protected from direct sunlight. After 14 days after sowing (DAS), seeds were planted shallowly in the bucket and then the dead or damaged seedlings were sewn for one to two weeks after planting. Weeding was suggested when rice plants were 21 to 35 days old. Irrigation was carried out in an intermittent manner until the conditions were crumbled with a water level of 1 cm from the ground surface. Intermittent watering was done by controlling between dry and inundated conditions, consecutively. An inorganic fertilization used urea 300 kg ha^{-1}, SP-36 200 kg ha^{-1}, and KCl 150 kg ha^{-1}.

Plants were maintained until 58 days or at the end of vegetative period. Variables observed were some soil characteristics (N total, pH, EC of soil) and some plant characteristics (N uptake, chlorophyll content, photosynthate accumulation and plant height) at the end of vegetative period, i.e. 58 days after planting (DAP). Rice variety used was INPARI 34 which is known as saline resistant varieties.

Total N, pH, EC of soil

In order to obtain the percentage of total nitrogen in soil, the Kjeldahl method was used (Bremner 1965). 0.500 g of rhizospheric soil sample in a size of <0.5 mm was put into a digestion tube. 1 g of selen mixture and 3 ml of H_2SO_4 p.a. were added and digested at a temperature of 350°C for approximately 4 hours or until white steam comes out and a clear extract is obtained. The tube was then removed and cooled, and the diluted extract with ion-free water to exactly 50 ml, shaken until a homogeneous form, was left overnight for the particles to settle. Clear extract was used for N measurement by distillation using the following formula:

$$\text{Plant N content (\%)} = (Vc - Vb) \times N \times \text{bst N} \times 50\text{ ml } 10\text{ ml}^{-1} \times 100\text{ mg sample}^{-1} \times fk \quad \text{(Eq. 1)}$$
$$= (Vc - Vb) \times N \times 14 \times 50/10 \times 100/250 \times fk$$
$$= (Vc - Vb)\, N \times 28 \times fk$$

where:
Vc, b = ml titar sample and blank
N = normality of H_2SO_4 standard solution
14 = nitrogen equivalent weight
100 = conversion to %
fk = water content correction factor = 100/(100 - % water content)

Soil pH and EC were analized using multifunction pH and the EC meter model no. EZ-9908 (van Reeuwijk 1993). A total of 10 g rhizospheric soil sample was put into a bottle, then 50 ml of ion-free H_2O was added and shaken for 30 min. Soil suspension is measured with a pH meter that has been calibrated using a buffer solution of pH 7.0 and pH 4.0 to obtain a soil pH value, and an EC meter that has been calibrated using a standard solution of 0.010 M NaCl to obtain a soil EC value.

N uptake, chlorophyll content, photosynthate accumulation, growth and yield of rice plants

Nitrogen uptake by rice plants was measured using the wet ashing method with H_2SO_4 using the following formula (Lisle *et al*. 1990):

$$\text{N uptake (g plant}^{-1}) = \text{plant N content (\%)} \times \text{plant dry weight (kg)} \quad \text{(Eq. 2)}$$

Chlorophyll content was measured using the soil plant analysis development (Konica Minolta, Chlorophyll Meter Plus SPAD 502) by clamping sample leaf on the head of chlorophyll meter and then the results displayed on screen were read. Photosynthate accumulation was measured by oven plant samples consisting of leaves and stems at a temperature of 80°C for 48 h, then weighed using analytical scales. Growth characteristics were represented in a form of plant height value, where samples were measured from the base of the stem to the tip of the leaf or plant. Yield of rice plants was presented in grain yield (t/ha).

Data analysis

Data were collected and analyzed using analysis of variance (ANOVA) and treatment means were compared using the Duncan Multiple Range test at p = 0.05 probability.

RESULTS AND DISCUSSION

Total N, pH, and EC of soil

As can be seen in Table 2, application techniques, whether seed treatment, nursery treatment, or combination of each treatment, present the same N total content, while HNB dosage of 1,000, 1,500 and 2,000 g ha^{-1} showed that N total content was greater than dosage of 0 and 500 g ha^{-1}, significantly. Halotolerant nitrogen fixing rhizobacteria as bioactive agents in HNBs help provide N in the early stages of plant growth, where this phase is the most critical in plant formation that determines the success of plant production (Khumairah *et al*. 2022c). The greater the dosage of HNB, the greater halotolerant nitrogen fixing rhizobacteria population in it, which affects the N total content of the soil even more. Wakelin *et al*. (2010) reported that inputs from nitrogen fixing rhizobacteria are an important contributor to soil quality because it can provide N in soil and support decomposition of crop residues which typically have a wide C:N ratio. The application technique and dosage of HNB also did not have a significant effect on soil pH and EC values, the average soil pH value after application of HNB was 6.64 and soil EC ranged from 7 to 8 dS m^{-1}. It is generally considered that rice plants can adapt to a wide range of pH, the growth of rice plants was retarded only when the pH was lower than about 5 (Doni *et al*. 2021). The pH level in this study was then considered to be in a good pH range for rice plant growth. In contrast, the EC value in this study was at the level of 7–8 dS/m which is classified as moderately saline where the crop yields were limited (FAO 1988). Balkan *et al*. (2015) stated that the level of tolerance of rice plants to moderately saline can reduce yields by 20 to 50%. The EC measured in this study increased on average from the EC initial soil analysis. The causes of saline soils are the high input of water containing salt, for example, seawater intrusion that occurs either periodically or simultaneously due to tsunamis, higher evaporation or evapotranspiration than precipitation, and soil parent material containing salt deposits (Rachman *et al*. 2018).

Table 2. Total N of soil due to different application technique and dosage of HNB on rice plants in saline ecosystem

Treatments	N total (%)	pH	EC (dS/m)
Application techniques (t)			
t_1 = seed treatment	0.24	6.65	7.52
t_2 = nursery treatment	0.25^a	6.64	8.15
t_3 = seed and nursery treatment	0.27^a	6.64	7.95
Dosage of HNB (d)			
d_0 = 0 g ha^{-1}	0.20^a	6.64	8.44
d_1 = 500 g ha^{-1}	0.25^{ab}	6.64	7.68
d_2 =1,000 g ha^{-1}	0.29^b	6.64	8.00
d_3 = 1,500 g ha^{-1}	0.26^b	6.65	7.53
d_4 = 2,000 g ha^{-1}	0.28^b	6.64	7.69

Note: The mean score followed by the same letter is not significantly different according to the Duncan's Multiple Range Advanced Test at the 5% level.

N uptake, chlorophyll content, photosynthate accumulation, growth and yield of rice plants

Table 3 shows that the interaction of the application technique and the dosage of HNB could increase the N uptake by rice plants. Combination of seed and nursery treatment with 1,500 g ha^{-1} of halotolerant nitrogen biofertilizer can increase N uptake significantly compared to other treatments, namely 3.95 g $plant^{-1}$. This is presumably because in each important stage of early plant growth, N needs are always met. Bioactive agents provide and fulfill N needs at seed germination phase and continue to support the availability of N until the germination enlargement stage or the nursery stage where N in larger amounts is required more than other elements (Larimi *et al*. 2014). In addition, at a higher dosage of biofertilizer which in this experiment was 1,500 g ha^{-1}, the bioactive agent contained in HNB was more numerous so that it can provide more N to be used by plants to support their growth (de Mesquita Alves *et al*. 2018). Rice plants that had been given N-fixing rhizobacteria were reported. There were observed a 95% increase in total N content and a 99% increase in harvest index under gnotobiotic and field conditions (Yanni *et al*. 2001, Biswas *et al*. 2000). Mutalib *et al*. (2012) also reported that rice plants inoculated with N-fixing rhizobacteria could increase N nutrient uptake, leaf chlorophyll content, photosynthetic rate and plant dry weight. This was related to N total soil status which stated that the availability of nitrogen in the soil through the N fixing activity will increase the uptake of N nutrients in plant and leaf tissue (Etesami *et al*. 2014).

Table 3. N uptake by plants due to different application technique and dosage of HNB on rice plants in saline ecosystem

Application techniques	dosage of HNB (g ha^{-1})				
	d_0 = 0	d_1 = 500	d_2 = 1,000	d_3 = 1,500	d_4 = 2,000
	-----g $plant^{-1}$-----				
t_1 = seed treatment	2.79^a A	2.77^a A	2.67^a A	2.15^a A	2.05^a A
t_2 = nursery treatment	2.44^a A	2.06^a A	1.97^a A	2.18^a A	2.04^a A
t_3 = seed and nursery treatment	2.04^a A	1.95^a A	1.88^a A	3.95^b B	2.65^b A

Note: Mean with same letter was not significantly different according to the Duncan's Multiple Range Advanced Test at the 5% level. Lowercase letters were read vertically, uppercase letters were read horizontally.

In Table 4, application of HNB at a dosage of 1,500 g ha^{-1} significantly increased chlorophyll content and photosynthate accumulation of rice plants compared to other treatments, although different application technique of HNB to rice plants showed the same chlorophyll content and photosynthate accumulation results. Chauhan *et al.* (2013) stated that nitrogen is the main structure constituent of chlorophyll, so chlorophyll content can be an indicator of the adequacy of nitrogen in plants. Poor photosynthate accumulation causes leaf chlorite, premature flowering and shortening of the growth cycle. It must be accompanied by providing large amounts of N to promote plant growth and development of plant organs by increasing chlorophyll content which can be achieved with nitrogen abundance (Lincoln and Edvardo 2006). Halotolerant nitrogen fixing rhizobacteria functioned to provide N for plant on wsaline soils (Bano and Fatima 2009). Halotolerant bacteria have the ability to balance osmotic pressure in order to avoid denaturation caused by salt in their environment by accumulating salt and osmolites (organic molecules) in their cytoplasm (Oren and Rodríguez-Valera 2001). It can be expected that there exists a positive and significant relation between chlorophyll content and photosynthate accumulation with N availibility, where N availibility through N_2 fixation process could increase N content in rice straw (Aon *et al*. 2015).

Table 4. Chlorophyll content and photosynthate accumulation due to different application technique and dosage of HNB on rice plants in saline ecosystem

Treatments	Chlorophyll content (SPAD unit)	Photosynthate accumulation (g)
Application technique (t)		
t_1 = seed treatment	44.82	2.74^{a}
t_2 = nursery treatment	43.22	2.74^{a}
t_3 = seed and nursery treatment	45.31	2.94^{a}
Dosage of HNB (d)		
d_0 = 0 g ha^{-1}	44.00	2.83^{ab}
d_1 = 500 g ha^{-1}	43.98	2.76^{a}
d_2 =1,000 g ha^{-1}	43.33	2.61^{a}
d_3 = 1,500 g ha^{-1}	46.97	3.33^{b}
d_4 = 2,000 g ha^{-1}	43.98	2.50^{a}

Note: Mean score followed by the same letter was not significantly different according to the Duncan's Multiple Range Advanced Test at the 5% level.

The interaction of the combination of seed and nursery application techniques with a dosage of HNB of 1,500 g ha^{-1} significantly increased plant growth, reflected by the plant height value (34.50 cm) which was greater than the control 0 g ha^{-1} (Table 5).

Table 5. Plant height due to different application technique and dosage of HNB on rice plants in saline ecosystem

Application technique	dosage of HNB (g ha^{-1})				
	d_0 = 0	d_1 = 500	d_2 = 1,000	d_3 = 1,500	d_4 = 2,000
	----- cm -----				
t_1 = seed treatment	30.67^{a} A	31.83^{a} A	29.87^{a} A	34.13^{b} A	29.27^{a} A
t_2 = nursery treatment	29.10^{a} A	30.63^{a} A	30.97^{a} A	29.30^{a} A	32.47^{a} A
t_3 = seed and nursery treatment	28.87^{a} A	32.80^{a} B	28.70^{a} A	34.50^{b} C	33.07^{a} B

Note: Mean with same letter was not significantly different according to the Duncan's Multiple Range Advanced Test at the 5% level. Lowercase letters were read vertically, uppercase letters were read horizontally.

This can be perceived as the conclusion of the high N total soil value, the amount of plant N uptake, chlorophyll content and photosynthate accumulation in the plant body due to the mechanism of N fixation by bioactive agents in HNBs so that overall it can support plant height growth. Nitrogen fixation process is considered as one of the biological processes that play a very important role in the soil. The processes that occur during the N cycle, for example, ammonification, nitrification and denitrification, are drastically influenced by environmental factors such as temperature, pH, oxygen and mineral nutrients. Soil salinity can affect soil fertility by inhibiting the activity of microorganisms that mediate nitrogen turnover (Syed *et al*. 2021). Salt stress causes several disturbances in plants such as nutritional imbalance, decreased stomatal conductance, low photosynthetic activity, which causes a decrease in plant growth and yield (Ivanova *et al*. 2015). Munns and Tester (2008) also added that salt stress in plants can cause morphological changes in plants such as a reduction in plant height, number of leaves, plant size, root length and fruit production, as well as changes in secondary metabolites, such as hormones and oxidative compounds. Salinity affects almost all phases of plant growth, namely germination, vegetative growth and generative (reproductive) growth. Excessive sodium salt accumulation on the cell walls causes osmotic stress which makes it difficult for plants to absorb water, resulting in physiological drought and cell death (Munns 2002). Salinity affects the photosynthetic process primarily through a reduction in leaf area, chlorophyll content and stomatal conductance, and, to a lesser extent, through a decrease in the efficiency of photosystem II (Netondo *et al*. 2004). Reproductive development was also affected by interfering with the microsporogenesis and elongation of stamens filaments, increasing cell death in several types of plant tissue, ovular abortion and senescence of fertilized embryos (Shrivastava and Kumar 2015). However, because this study used halotolerant rhizobacteria that can survive at high salinity, it can still carry out its function of providing N for plants very well. Rhizobacteria that colonize locations that are often exposed to high salt levels tend to be more adaptive or tolerant and can serve as better plant growth promoters (Shrivastava and Kumar 2015). As can be seen in Table 6, application technique and dosage of HNB gave independent effects.

Table 6. Grain yield of rice plants due to different application technique and dosage of HNB on rice plants in saline ecosystem

Treatments	Grain yield (t/ha)
Application technique (t)	
t_1 = seed treatment	4.97^{a}
t_2 = nursery treatment	5.77^{a}
t_3 = seed and nursery treatment	8.95^{a}
Dosage of HNB (d)	
d_0 = 0 g ha^{-1}	7.32^{a}
d_1 = 500 g ha^{-1}	8.32^{a}
d_2 = 1,000 g ha^{-1}	10.34^{ab}
d_3 = 1,500 g ha^{-1}	13.40^{b}
d_4 = 2,000 g ha^{-1}	12.89^{b}

Note: Mean score followed by the same letter was not significantly different according to the Duncan's Multiple Range Advanced Test at the 5% level.

Application of HNB technique did not give significant effect on grain yield of rice plants in saline ecosystem. Yet, HNB applied in seed and nursery tended to have greater yield than applied individually. Dosage of HNB showed a significant effect on each treatment. 1,500–2,000 g ha^{-1} HNB can significantly increase rice yield when compared with 0–500 g ha^{-1}. This indicated that HNB contributes to the increase in rice yield when the dosage is at the of 1,500 g ha^{-1} level. Zhou *et al*. (2015) reported that grain yields increased with increasing levels of nitrogen fertilization to some extent, and a further increase in dosage actually decreased yields. It proves that the application of HNB at a dosage of 2,000 g ha^{-1} tended to decrease yields even though it was not statistically significant.

CONCLUSIONS

Interaction between the combination of seed and nursery application techniques with dosage of 1,500 g ha^{-1} of HNB for rice plants in saline ecosystem can increase N uptake and plant height. The dosage of 1,500 g $ha^{-1}$of HNB can increase the total N of the soil so that the chlorophyll content and photosynthate accumulation in plants body were greater when compared with the lower dosage. Application of HNB in seed and nursery technique at a dosage of 1,500 g ha^{-1} can be further recommended in rice cultivation on saline ecosystems in order to increase growth and yield of rice by fulfilling the N need for plants in the early stages of its growth.

ACKNOWLEDGEMENT

This research was supported by grants received from the University of Padjadjaran within the framework of the Academic Leadership Grant.

REFERENCES

[1] Aon, M., Khalid, M., Hussain, S., Naveed, M., Akhtar, M.J., 2015. *Diazotrophic inoculation supplemented nitrogen demand of flooded rice under field conditions*. Pakistan Journal of Agricultural Sciences, 52(1), 145–150.

[2] Arifin, M.S., Rion, M.S.I., Rahman, A., Zakir, H.M., Quadir, Q.F., 2021. *Salt tolerant rhizobacteria from coastal region of Bangladesh portrayed the potential for plant growth promotion*. Journal of Applied Life Sciences International, 24: 58–70.

[3] Balkan, A., Gençtan, T., Bilgin, O., Ulukan, H., 2015. *Response of rice (Oryza sativa L.) to salinity stress at germination and early seedling stages*. Pakistan Journal of Agricultural Sciences, 52(2): 455–461.

[4] Bano, A., Fatima, M., 2009. *Salt tolerance in Zea mays (L) following inoculation with Rhizobium and Pseudomonas*. Biology and Fertility of Soils, 45(4): 405–413.

[5] Basu, A., Prasad, P., Das, S.N., Kalam, S., Sayyed, R.Z., Reddy, M.S., El Enshasy, H., 2021. *Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: Recent developments, constraints, and prospects*. Sustainability, 13(3): 1140.

[6] Biswas, J.C., Ladha, J.K., Dazzo, F.B., 2000. *Rhizobia inoculation improves nutrient uptake and growth of lowland rice*. Soil Science Society of America Journal, 64(5): 1644–1650.

[7] Bremner, J.M., 1965. *Total nitrogen. Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, 9: 1149–1178. https://doi.org/10.2134/agronmonogr9.2.c32.

[8] Chauhan, H., Bagyaraj, D.J., Sharma, A., 2013. *Plant growth-promoting bacterial endophytes from sugarcane and their potential in promoting growth of the host under field conditions*. Experimental Agriculture, 49(1): 43.

[9] Chen, P., Liu, Y., Mo, C., Jiang, Z., Yang, J., Lin, J., 2021. *Microbial mechanism of biochar addition on nitrogen leaching and retention in tea soils from different plantation ages*. Science of The Total Environment, 757: 143817.

[10] de Mesquita Alves, J., de Lima, A.S., de Mesquita, E.F., Junior, S.D.O.M., de Sousa Ferreira, R., da Silva, F.L., da Mota Santos, J., 2018. *Gas exchange and chlorophyll content in tomato grown under different organic fertilizers and biofertilizer doses*. African Journal of Agricultural Research, 13(41): 2256–2262.

[11] Doni, F., Suhaimi, N.S.M., Irawan, B., Mohamed, Z., Mispan, M.S., 2021. *Associations of Pantoea with rice plants: As friends or foes?* Agriculture, 11(12): 1278.

[12] Etesami, H., Mirsyed Hosseini, H., Alikhani, H.A., 2014. *In planta selection of plant growth promoting endophytic bacteria for rice (Oryza sativa L.)*. Journal of Soil Science and Plant Nutrition, 14(2): 491–503.

[13] FAO, 1988. *Salt-Affected Soils and their Management*.

[14] Hussain, M., Ahmad, S., Hussain, S., Lal, R., Ul-Allah, S., Nawaz, A., 2018. *Rice in saline soils: Physiology, biochemistry, genetics, and management*. Advances in Agronomy, 148: 231–287.

[15] Ivanova, K., Anev, S., Tzvetkova, N., Georgieva, T., Markovska, Y., 2015. *Influence of salt stress on stomatal, biochemical and morphological factors limiting photosynthetic gas exchange in Paulownia elongata × fortunei*. Comptes rendus de l'Académie bulgare des Sciences, 68(2): 217–224.

[16] Khumairah, F.H., Azwari, F., Setiawati, M.R., Fitriatin, B.N., Simarmata, T., 2022a. *Effect of different carrier composition on viability of halotolerant N-fixing rhizobacteria, water content and pH level in nitrogen biofertilizer for saline ecosystem*. Kultivasi, 21(1).

[17] Khumairah, F.H., Setiawati, M.R., Fitriatin, B.N., Simarmata, T., Alfarraj, S., Ansari, M.J., El Enshasy, H.A., Sayyed, R.Z., 2022b. *Halotolerant plant growth promoting rhizobacteria isolated from saline soil improve nitrogen fixation and alleviate salt stress in rice plants*. Frontiers in Microbiology, 1607.

[18] Khumairah, F.H., Ulfah, T., Setiawati, M.R., Fitriatin, B.N., Simarmata, T., 2022c. *Population and biochemical characteristics of halotolerant N2 fixing rhizobacteria isolated from saline soil ecosystem*. Jurnal AgroBiogen, 18(1): 1–10.

[19] Larimi, S.B., Shakiba, M., Mohammadinasab, A.D., Vahed, M.M., 2014. *Changes in nitrogen and chlorophyll density and leaf area of sweet basil (Ocimum basilicum L.) affected by biofertilizer and nitrogen application*. International Journal of Biosciences, 5(9): 256–265.

[20] Li, Y., Li, H., Han, X., Han, G., Xi, J., Liu, Y., Zhang, Y., Xue, Q., Guo, Q., Lai, H., 2022. *Actinobacterial biofertilizer improves the yields of different plants and alters the assembly processes of rhizosphere microbial communities*. Applied Soil Ecology, 171: 104345.

[21] Lincoln, T., Edvardo, Z., 2006. *Assimilation of Mineral Nutrition. Plant Physiology* (4th ed.). Sinauer Associates, Sunderland MA.

[22] Lisle, L., Gaudron, J., Lefroy, R., 1990. *Laboratory Techniques for Plant and Soil Analysis*. UNE-ACIAR-Crawford Fund. Department of Agronomy and Soil Science, University of New England, Armidale. Australian Centre for International Agricultural Research.

[23] Mącik, M., Gryta, A., Frąc, M., 2020. *Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms*. Advances in Agronomy, 162: 31–87.

[24] Munns, R., 2002. *Comparative physiology of salt and water stress*. Plant, Cell & Environment, 25(2): 239–250.

[25] Munns, R., Tester, M., 2008. *Mechanisms of salinity tolerance*. Annual Review of Plant Biology, 59: 651–681.

[26] Mutalib, A.A., Radziah, O., Shukor, Y., Naher, U.A., 2012. *Effect of nitrogen fertilizer on hydrolytic enzyme production, root colonisation, N metabolism, leaf physiology and growth of rice inoculated with 'Bacillus' sp. (Sb42)*. Australian Journal of Crop Science, 6(9): 1383–1389.

[27] Netondo, G.W., Onyango, J.C., Beck, E., 2004. *Sorghum and salinity*: II. *Gas exchange and chlorophyll fluorescence of sorghum under salt stress*. Crop Science, 44(3): 806–811.

[28] Oren, A., Rodríguez-Valera, F., 2001. *The contribution of halophilic bacteria to the red coloration of saltern crystallizer ponds*. FEMS Microbiology Ecology, 36(2–3): 123–130.

[29] Rachman, A., Dariah, A., Sutono, S., 2018. *Management of High Saline Rice Fields*. IAARD Press, Jakarta.

[30] Rahman, K.M., Zhang, D., 2018. *Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability*. Sustainability, 10(3): 759.

[31] Setiawati, M.R., Al-Azmiya, N.U., Khumairah, F.H., Simarmata, T., 2022. *Halotolerant n-fixing bacteria isolates for increasing the biochemical activity, total bacteria population, N-uptake and rice seedling growth*. KnE Life Sciences, 1–12.

[32] 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.

[33] Syed, A., Sarwar, G., Shah, S.H., Muhammad, S., 2021. *Soil salinity research in the 21st century in Pakistan: Its impact on availability of plant nutrients, growth and yield of crops*. Communications in Soil Science and Plant Analysis, 52(3): 183–200.

[34] Van Reeuwijk, L.P., 1993. *Procedures for Soil Analysis* (4th ed.). Technical Paper, International Soil Reference and Information Centre, Wageningen.

[35] Wakelin, S.A., Gupta, V.V.S.R., Forrester, S.T., 2010. *Regional and local factors affecting diversity, abundance and activity of free-living, N2-fixing bacteria in Australian agricultural soils*. Pedobiologia, 53(6): 391–399.

[36] Wang, C.F., Han, G.L., Yang, Z.R., Li, Y.X., Wang, B.S., 2022. *Plant salinity sensors: Current understanding and future directions*. Frontiers in Plant Science, 13.

[37] Yanni, Y.G., Rizk, R.Y., Abd El-Fattah, F.K., Squartini, A., Corich, V., Giacomini, A., de Bruijn, F., Rademaker, J., Maya-Flores, J., Ostrom, P., Vega-Hernandez, M., 2001. *The beneficial plant growth-promoting association of Rhizobium leguminosarum bv. trifolii with rice roots*. Functional Plant Biology, 28(9): 845–870.

[38] Zhang, W., Wang, C., Lu, T., Zheng, Y., 2018. *Cooperation between arbuscular mycorrhizal fungi and earthworms promotes the physiological adaptation of maize under a high salt stress*. Plant and Soil, 423(1): 125–140.

[39] Zhou, J., Guiyuan, M.E.N.G., Chenzhong, J.I.N., Guohui, M.A., Jirui, L.O.N.G., 2015. *Effects of nitrogen rate on blast resistance and grain yield of super early hybrid rice Luliangyou 996*. Agricultural Science & Technology, 16(9).