https://doi.org/10.1051/e3sconf/202344404034

Proline Content, Physiological and Agronomic Characters of Rice (*Oryza sativa* cv. Inpari Unsoed 79 Agritan) under Treated with PGPR in Saline Medium.

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Abstract. Tidal areas near the coastline are prone to salinity impacts as a negative impact of seawater intrusion which causes dissolved salt levels to increase. The biological approach using PGPR is an environmentally friendly approach. The aim of the study was to examine the effectiveness of useful bacteria from saline soils to increase the resistance of rice plants to salinity stress. The research was carried out from April to October 2022 in the Lab. Agronomy & Horticulture Faperta UNSOED. This research was experimental research using liquid culture techniques for cultivating plants using AB Mix media. The design used was a randomized block design with three replications. The treatments were included Control (P0), *Acinetobacter junii (P1), Bacillus tropicus (P2), Acinetobacter schindleri (P3), Pseudomonas stutzeri (P4), Bacillus altitudinis (P5), Bacillus cereus (P6), dan Bacillus subtilis (P7).* The results of the study showed that Diazotrophic Bacteria inoculation can increase the resistance of rice plants to rice plants by increasing proline production, increasing net assimilation rates and relative growth rates. *Acinetobacter schindleri, Bacillus subtilis* and *Bacillus tropicus* are potential N_2 fixing bacterial strains to increase the growth and yield of rice under saline conditions. Treatment of *Acinetobacter schindleri* was able to provide the highest grain yields reaching 16.95 g/plant.

1 Introduction

Salinity is a global problem that threatens national rice production. Tidal areas near the coastline are prone to salinity impacts as a negative impact of seawater intrusion which causes dissolved salt levels to increase and salt accumulation in the root area will cause plants to experience a decrease in their ability to absorb water [1]. Salinity has a negative impact on various growth stages of rice plants, thereby reducing grain production. High salinity conditions had a negative impact on seed germination, plant height, tiller number, biomass accumulation, percent of sterile florets, and decreased grain production [2].

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Rice plants adapt to saline conditions, especially in response to changes in electrical conductivity in the range of 3-4 dS/m [3]. Various mechanisms have been developed for tolerant rice plants to saline conditions, including adaptation to morphological changes, physiological responses and accumulation of secondary metabolites [4]. Plants that experience salinity stress will result in an imbalance of electrons with high energy and electron conversion will transfer free electrons to oxygen resulting in the formation of ROSs which cause chlorosis of chlorophyll, lipid membranes, carbonylation of proteins, inactivation of sulfhydryls, DNA, and reduce the net assimilation rate by up to 50% percent [5]. The adaptation of rice plants to salt stress can be categorized through three mechanisms, namely tolerance, ion exclusion, and tissue ion tolerance [3,6]. Osmotic tolerant, plants will develop an osmoprotectant mechanism by accumulating osmolyte to maintain osmotic potential and balance osmotic pressure in the soil [7].

The increase in osmoprotectant production during salinity stress included an increase in proline levels in plant tissues, and an increase in salt content in the media up to 500 mM NaCl significantly increased proline levels [8,9]. Plant physiological response to high salinity conditions through the accumulation of proline and plants that are tolerant to saline will accumulate higher proline than plants that cannot tolerate saline conditions [10]. An increase in proline levels will result in an increase in cell turgor which results in an increase in stomata conductivity and transpiration and an increase in internal carbon dioxide concentration, chlorophyll levels, and an increase in the rate of assimilation of plants [11].

Technological developments in utilizing saline land have been carried out a lot, including the application of ameliorant materials and also the use of agricultural lime. However, this has not shown significant results in increasing rice production. A biological approach through the use of tolerant varieties needs to be balanced with technology that is able to provide a good plant environment and increase production. The application of biological technology through the application of saline-tolerant beneficial microbes is an alternative that is environmentally friendly. Direct influence of microbes on rice plants through the production of growth substances, ACC deaminase, secondary metabolites such as exopolysaccharides, osmolytes and antioxidants [12,13]. Various beneficial microbes have been isolated from saline fields including *B. megaterium, Bacillus sp*., and *B. megaterium* which are able to increase the growth of cucumber plants under salinity stress [14]. Bactaria that have the ability to produce phytohormones, ACC deaminase and potential as PGPR have also been isolated from locations of highly soil salinity in Taiwan [15]. PGPR are bacteria that are associated with the rhizosphere of plants and can stimulate growth, which also includes the group of nitrogen-fixing bacteria [29,30]. N_2 fixing bacteria have also been isolated from paddy saline soils on the north coast of Pemalang including *Pseudomonas stutzeri, Acinetobacter junii, Bacillus cereus, Bacillus tropicus, Bacillus altitudinis, Bacillus subtilis Bacillus pumilus, Acinetobacter baumanii,* and *Acinetobacter schindleri* [16]. However, the ability to increase the growth, yield, and physiological response of rice under conditions of high salinity stress has not yet been tested. This study aims to examine the ability of Nitrogen-fixing bacteria from the paddy soils of the North Coast of Pemalang to increase the tolerance of rice plants to high salt stress, and to increase growth and yield.

2 Materials and Method

2.1 Site of Research

The research was conducted at Agronomy and Horticulture laboratory and Experimental Farm of Agriculture faculty, Jenderal Soedirman University Purwokerto, Indonesia from May until August 2022.

2.2 Materials and Tools

The materials used in this study included rice seeds of the Inpari Unsoed 78 Agritan variety, N-fixing bacterial isolates as the tretaments, NaCl was used to regulate the salinity level of the media AB Mix media was used as a rice culture medium, distilled water, acetone, nail polish for taking samples of leaf stomata, chemicals for proline analysis (3% sulfosalicylic acid, ninhydrin acid solution, glacial acetic acid, phosphoric acid, toluene), styrofoam, filter paper, tissue, mask, gloves and label. The tools used in this study included seed boxes, lux meters, pH meters, EC meters, analytical balances, ovens, spectrophotometers, glass ware, pipettes, sample bags, and writing stationary.

2.3 Research Design

This reseach was designed by using Randomized Completely Block Design (RCBD) with 4 replications. The treatments tried included Control (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), dan Bacillus subtilis (P_7)*. Each unit consisted six rice plants. The research was used water solution culture with nutrient source from AB Mix media. The pH of medium was adjusted at 7, and the EC of medium was adjusted at 6 mmhos/cm. The rice variety was used in this research was Inpari UNSOED 79 Agritan.

The observed variables consisted of proline content [10], chlorophyl content [17], net assimilation rate, crops growth rate, number of productive tillers, panicle length, number of grains per panicle, weight of 1000 grains, and weight of grains per clump.

2.4 Data Analysis

The data was analysed by using ANOVA, and the result showed significant differences were continued by DMRT α=5%.

3 Result and Discussion

3.1 Agronomic Traits of Rice under Treated with Diazotrophic Bacteria in Saline Medium

The results showed that the treatment of Diazotrophic bacteria had a positive effect on the growth of rice plants under salt stress conditions. These results show that inoculation of Diazotrophic bacteria was able to increase the growth of rice plants in terms of plant height, number of tillers, leaf area, and plant dry weight. Plant height is a very visible growth indicator and is very responsive to changes in environmental conditions. The results showed that the inoculation of diazotrophic bacteria increased the growth of plant height by 13.63 percent on average compared to the control. The highest plant height was achieved in *Acinetobacter schindleri, Bacillus altitudinis* and *Bacillus cereus* inoculations of 80.75, 78.04 and 75.12 cm, respectively (Table 1). The control treatment only reached a plant height of 66.30 cm. *Acinetobacter schindleri* inoculation gave the highest plant height compared to the control and increased by 21.79 percent. These results were in line with Rokhbakhsh-Zamin where *Acinetobacter* sp inoculation was able to increase the growth of Pearl millet plants [31].

Diazotrophic Bacteria inoculation can significantly increase the number of tillers of rice plants. The results showed that inoculation of diazotropic bacteria was able to produce an average of 34.25 tillers in one clump or an increase of 75.64 percent compared to the control.

However, bacterial strains did not show differences in the number of tillers of rice plants. Where the number of rice tillers in the diazotropic bacteria inoculation treatment ranged from 29.50 to 39.50 tillers per clump. The positive effect of inoculation of diazotrophic bacterial strains was also seen in the variable leaf area. The average leaf area of rice plants inoculated with diazotropic bacteria was 5121.86 cm^2, an increase of 165.24 percent compared to the control treatment which only had a leaf area of 1931 cm^2. The highest leaf area was achieved in the *Acinetobacter schindleri* treatment of 7001 cm^2, although it was not significantly different from the *Acinetobacter junii, Bacillus tropicus, Bacillus altitudinis*, and *Bacillus cereus* treatments (Table 1).

Table 1. Agronomy traits of rice under treated with Diazotrophic Bacteria in Saline medium

Treatments	Plant Height (cm)	Number of Tiller	Leaf Area (cm^2)	Plant Dry Weight (g)
Control (P_0)	66.30 c	19.50 b	1931 c	7.46 d
Acinetobacter junii (P_1)	73.77 b	33.25 a	4834 ab	12.81 bc
Bacillus tropicus (P_2)	73.83 b	35.25 a	4793 ab	15.53 b
Acinetobacter schindleri (P_3)	80.75 a	39.50 a	7001 a	19.63 a
Pseudomonas stutzeri (P_4)	73.51 b	33.75 a	4329 b	10.14 cd
Bacillus altitudinis (P_5)	78.04 ab	35.00 a	5878 ab	12.97 bc
Bacillus cereus (P_6)	75.12 ab	33.50 a	5055 ab	15.99 b
Bacillus subtilis (P_7)	72.34 b	29.50 a	3963 bc	20.33 a

Notes : number followed by same latter in the same coloum is not significant different according DMRT 5%.

Increasing the character of plant height, the number of tillers of rice plants inoculated with Diazotropic bacteria had an impact on increasing the leaf area of rice plants. Leaves are the center of photosynthesis, so by increasing the leaf area it will result in an increase in the ability of the leaves to capture sunlight for the photosynthesis process. The results of photosynthesis will be translocated to various tissues and accumulated in the form of plant dry matter. It can be seen that inoculation of dizotropic bacteria can increase the dry weight of rice plants compared to control. Application of diazotrophic bacteria can significantly increase plant dry weight up to 106.67 percent. The results of this study indicated that the highest plant dry weight was achieved in the *Bacillus subtilis* treatment, although it was not different from the *Acinetobacter schindleri* treatment, respectively 20.33 g and 19.63 g (Table 1). Rajer reported that B. subtilis was able to increase the growth of rice plants by increasing the fresh weight and dry weight of the plant compared to rice plants that were not inoculated [32]

Diazotrophic bacteria are able to support the vegetative growth of rice plants through their ability to fix nitrogen so that nitrogen availability increases. This is supported by the resistance of all strains in environmental conditions with high salinity so that they are able to colonize the roots of rice plants and fix nitrogen properly [16]. Rice plants in the vegetative phase will absorb nitrogen both in the form of nitrate and ammonia, then processed through nitrogen metabolism and converted into nucleic acids and amino acids for protein biosynthesis and vegetative growth [18]. Several previous researchers have also reported that inoculation of Diazotropic Bacteria can increase vegetative growth of plants, such as inoculation of rice IR72 which was inoculated with isolate IRBG500 can increase root length and plant dry weight [19]. Inoculation of *A. amazonense* strain AR3122 on rice variety IR42 has been reported to increase plant dry weight by up to 21 percent [20].

3.2 Proline Content

Plant tolerance to salinity stress conditions is through the accumulation of proline so that plants are able to survive in these conditions. Proline is a class of amino acids that is synthesized and accumulated as an endogenous osmolyte under various abiotic stress conditions, including salt stress [33]. The proline content of rice plants inoculated with diazotrophic bacteria significantly increased compared to the control treatment. The results of this study indicated that proline levels increased, although between bacterial strains there was no difference in proline levels in rice plants. Proline levels in rice plants inoculated with diazotropic bacteria ranged from 3.98 to 6.24 µmol proline g^{-1} or an average increase of 46.73 percent compared to control (2.47 µmol proline g^{-1}) (Table 2).

The increase in proline levels in the treatment of diazotrophic bacteria strains indicated that inoculation of these bacterial strains increased plant resistance to saline conditions. Proline is one of the amino acids synthesized by plants that experience stress such as drought stress and salt stress and functions as an osmoregulator to increase cell turgor [21]. The results of this study are in line with several previous researchers where *B. pumilus, E. aurantiacum, P. fluorescens* and *P. fluorescens* S3 significantly increased proline levels in wheat plants under salt stress conditions [22,23].

Table 2. Proline content of rice in saline medium with Diazotrophic Bacteria inoculation

Treatments	Proline Contents (µmol proline g^{-1})
Control (P_0)	2.47 b
Acinetobacter junii (P_1)	3.98 ab
Bacillus tropicus (P_2)	5.00 ab
Acinetobacter schindleri (P_3)	5.46 a
Pseudomonas stutzeri (P_4)	6.16 a
Bacillus altitudinis (P_5)	6.24 a
Bacillus cereus (P_6)	6.14 a
Bacillus subtilis (P_7)	6.04 a

AllNotes: number followed by same latter in the same coloum is not significant different according DMRT 5%.

3.3 Physiological Plant Attributes

The results showed that the density of stomata and the width of the stomata opening increased with the inoculation of Diazotropic bacteria compared to the controls. The application of Dizotrophic Bacteria strain was significantly able to increase stomatal density and stomatal opening width by 37.31 percent and 59.78 percent, respectively. The density of stomata in the inoculation treatment of diazotrophic bacteria strain was 62.82 units/mm^2, higher than the control which was 45.75 units/mm^2. Inoculation of rice plants with *Acinetobacter junii* had the highest stomata density of 69.50 units/mm^2, although it was not different from that of *Bacillus tropicus, Acinetobacter schindleri, Bacillus altitudinis*, and *Bacillus cereus*. Inoculation with *Bacillus subtilis* gave the lowest stomatal density compared to other treatments, namely 55.75 units/mm^2 (Table 3). The average stomatal opening width variable reached 5.19 µm, which was greater than the control which was only 3,25 µm. However, from the results of this study, there was no significant difference in the width of the stomata opening between the bacterial strains.

Stomata are plant organs that are very sensitive to salinity stress both in terms of number/density and width of openings in response to reduced transpiration rates. Based on the results of this study indicate that inoculation of diazotrophic bacteria has a positive impact on plant resistance to salt stress. In general, plants that experience salt stress will have smaller stomata than leaves without salt stress or drought stress [24]. Inoculation of Diazotrophic Bacteria strains has a positive impact on plants through their ability to fix N_2 and produce phytohormones. Bacteria used as inoculants have the ability to bind N_2 and produce IAA [16]. This stimulates root growth, and root proliferation and than stimulates root elongation so that nutrient and water absorption becomes better (Mahmood et al., 2016). Under salt stress conditions, inoculation will increase nutrient uptake, especially K, where K plays an important role in the process of opening and closing stomata [25].

An increase in the width of the stomata opening will have an impact on the amount of CO_2 that can be absorbed by the leaves so that the rate of plant photosynthesis increases. The results of this study indicate that inoculation of diazotrophic bacteria strains is very significant in increasing the net assimilation rate (NAR) of rice plants. Inoculation of Bacillus subtilis bacteria gave the highest NAR value of 0.30 g dm^{-2} week^{-1}. The increase in NAR was followed by an increase in the relative growth rate of plants. Inoculation of Diazotrophic Bacteria strains had an average RGR of 15.04 g week^{-1} or an increase of 103.26 percent compared to controls. *Bacillus subtilis* inoculation had the highest RGR value of 19.90 g week^{-1}, although it was not different from *Acinetobacter schindleri* inoculation which had an RGR value of 19.25 g week^{-1} (Table 3).

Based on the results of this study, inoculation of Diazotrophic bacteria strains showed a positive effect on the carbon assimilation process in rice plants under salt stress conditions. Diazotrophic bacteria can increase the NAR and RGR values. The ability of bacteria to produce phytohormones can facilitate roots in absorbing water and nutrients, thereby stabilizing stomatal conductance and transpiration rates [26]. PGPR saline conditions are able to increase K uptake and suppress Na uptake so as to maintain cell turgor and protect chloroplast damage due to salinity stress so that the rate of photosynthesis increases and improves plant growth [27].

Table 3. Physiological response of rice under treated with Diazotrophic Bacteria in saline medium

Treatments	Stomata density (unit mm^{-1})	Wide opening of stomata (µm)	NAR (g dm^{-2} week^{-1})	RGR (g week^{-1})
Control (P_0)	45.75 c	3.25 b	0.15 b	7.40 d
Acinetobacter junii (P_1)	69.50 a	4.88 ab	0.14 b	12.61 bc
Bacillus tropicus (P_2)	58.75 ab	5.19 a	0.19 b	15.15 b
Acinetobacter schindleri (P_3)	67.50 ab	4.94 a	0.18 b	19.25 a
Pseudomonas stutzeri (P_4)	66.00 ab	5.44 a	0.13 b	10.00 cd
Bacillus altitudinis (P_5)	62.75 ab	5.50 a	0.11 b	12.81 bc
Bacillus cereus (P_6)	59.50 ab	5.06 a	0.16 b	15.57 b
Bacillus subtilis (P_7)	55.75 bc	5.34 a	0.30 a	19.90 a

Notes: number followed by same latter in the same coloum is not significant different according DMRT 5%.

3.4 Yield Atributes

Based on the results of the study, it was shown that inoculation of Diazotrophic Bacteria strains under saline conditions had an effect on the variables of panicle length, number of grain per panicle, percentage of empty grain, and grain weight per clump, however these results had no effect on the number of productive tillers and the weight of 1000 seeds. Inoculation of diazotrophic bacteria was able to increase panicle length, the number of grains per panicle, and decreased grain loss compared to the control. Grain weight per plant in the Diazotrophic Bacteria strain inoculation treatment was able to increase grain weight in the

Acinetobacter schindleri treatment to produce 16.95 g/clump of grain. This result is greater than other bacterial strains. *Acinetobacter schindleri* inoculation was able to increase grain yield per clump by 243.12 percent (Table 4).

The results of this study indicate that inoculation of Diazotrophic Bacteria can increase rice yields under salt stress conditions. Based on the various variables in this study, almost all Diazotrophic Bacteria have a positive effect on the resistance of rice plants to drought stress. The highest yield in *Acinetobacter schindleri* inoculation. This is inseparable from the support of the yield component in this treatment which shows a fairly high value, although in general it does not differ between bacterial strains. Based on these results it can be seen that apart from *Acinetobacter schindleri*, inoculation with *Bacillus subtilis* and *Bacillus tropicus* showed quite high results. This shows the potential for development as a biofertilizer inoculant. The ability to fix N_2 and produce IAA greatly supports the growth and yield of rice plants, besides that under saline conditions it can induce rice plants to increase resistance through high proline production.

Several researchers reported that PGPR inoculation in wheat under salt stress conditions was able to increase resistance by increasing the rate of photosynthesis, transpiration and proline production thereby reducing antioxidant levels [22]. Rhizobacteria inoculation greatly contributes to increasing the uptake of nutrients N, P, K, and on the other hand reducing the uptake of Ca and Na [28,22]. Rhizobacteria of the genus Bacillus have been reported to be tolerant to salt stress thereby increasing carbon assimilation so as to produce high seed yields [28,26].

Table 4. Yield and yield components of rice

Treatments	Number of Productive Tillers	Panicle Length (cm)	Number of Grains per Panicle	Percentage of Empty Grains (%)	Yield (g/clump)	Weight of 1000 grains (g)
Control (P_0)	9.25 a	20.26 ab	41.34 b	50.46 a	4.94 b	20.82 a
Acinetobacter junii (P_1)	17.50 a	18.67 b	62.71 ab	28.43 b	7.89 b	19.52 a
Bacillus tropicus (P_2)	17.13a	19.28 ab	69.79 a	21.99 b	10.30 b	20.65 a
Acinetobacter schindleri (P_3)	16.88 a	19.85 ab	60.12 ab	30.29 b	16.95 a	19.76 a
Pseudomonas stutzeri (P_4)	16.00 a	18.85 ab	59.04 ab	18.27 b	7.26 b	20.28 a
Bacillus altitudinis (P_5)	14.50 a	18.67 b	62.08 ab	26.34 b	7.95 b	20.74 a
Bacillus cereus (P_6)	14.00 a	19.50 ab	57.67 ab	20.60 b	8.39 b	20.87 a
Bacillus subtilis (P_7)	14.00 a	20.44 a	67.37 a	27.01 b	7.68 b	20.50 a

Notes: number followed by same latter in the same coloum is not significant different according DMRT 5%

4 Conclusion

Based on the results of this study it can be concluded that Diazotrophic Bacteria inoculation of rice plants under saline conditions was able to increase the vegetative growth of rice plants. Diazotrophic Bacteria inoculation can increase the resistance of rice plants to rice plants by increasing proline production, increasing net assimilation rates and relative growth rates. *Acinetobacter schindleri, Bacillus subtilis* and *Bacillus tropicus* are potential N_2 fixing bacterial strains to increase the growth and yield of rice under saline conditions. Treatment of *Acinetobacter schindleri* was able to provide the highest grain yields reaching 16.95 g/plant.

Acknowledgements. The authors would like to thank LPPM UNSOED for funding this research through the 2022 Unsoed Basic Research Scheme (RDU). The authors also thank the Agronomy & Horticulture Laboratory for providing research equipment. Metri Widya Pangetsika and Martina Lestari would not forget to thank them for helping with data collection and analysis in the laboratory.

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