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# The role of halotolerant N-fixing bacteria on rice agronomic traits on saline soils by path analysis

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**Abstract:** Nitrogen-fixing bacteria (NFB) play a significant role in saline soil ecosystems. However, little is known about the correlation between NFB application on growth and yield components of rice plants on saline soils. Exploration and experimental methods were performed to obtain the potential of NFB from a rice field in saline soil and reinoculated in a pot experiment. The experiment was arranged as a randomised block design consisting of 8 treatments, namely inoculation application (control and seed treatments with 20 g inoculant/kg of seed) combined with soil application dosage (0, 500, 1 000 and 1 500 g/ha). The results showed that grain yield increased by 43.8–130.6% with seed treatment of 20 g inoculant/kg of seed combined with soil application 500–1 500 g inoculant/ha. Rice yield was affected by multiple variables NFB population, plant height, number of tillers, and grain straw ratio ($R^2$ = 0.926). Path analysis findings showed that the greatest effective contribution (45.45%) yield of rice in saline soil was contributed NFB population. This finding concludes that the application of NFB inoculants as seed treatments and soil applications can serve as an effective as well as the environmentally friendly microbial-based strategy of rice cultivation on saline soil ecosystems.

**Keywords:** food security; growth substances; indole acetic acid; halotolerant nitrogen fixer-phytohormone producing rhizobacteria

Agricultural practices are forced to the extent and intensify rice cultivation on saline soils along the coastal area to boost and produce enough rice for the rapidly growing population in Indonesia. The potential area that soils prone the salinity is estimated to be about 12.020 million ha or 6.20% of the total land area of Indonesia (Karolinoerita and Yusuf 2020). Consequently, an integrated and comprehensive adaptation and mitigation strategy are needed to improve the soil health/quality and alleviate plant salinity stress. The use of a selected, adapted rice cultivar or saline-tolerant rice cultivar combined with the proper rhizomicrobiome engineering is applied to increase the abundance and biodiversity of beneficial microbes, soil health and nutrient availability for crop growth and development (Saghafi et al. 2019, Simarmata et al. 2020, Hakim et al. 2021, Prayoga et al. 2021).

Nitrogen-fixing bacteria play a major role in maintaining soil fertility and sustainable rice production. The nitrogen (N) accumulation in paddy soil was increased by 32 kg/ha per year through the soil biological nitrogen fixation process (Greenland 1998). Biological nitrogen fixation by soil bacteria that converts nitrogen gas ($N_2$) into ammonia is an important process in maintaining the supply of N

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for rice growth in paddy fields (Wang et al. 2012). The accumulation of N in paddy fields increased through the process of biological $N_2$ fixation in the soil. This soil nitrogenase activity came from $N_2$-fixing bacteria under anaerobic flooded conditions (Lalucat et al. 2006).

Salt tolerance facilitated by beneficial microbes has been demonstrated in rice crops. Various saline-resistant cultivars combined with various halotolerant N-fixing bacteria have been shown to increase the growth and yield of rice plants (Shultana et al. 2020). Phytohormone-producing bacteria may offer the additional potential to combine increased tolerance to salt stress with other beneficial and growth-promoting effects (Ortíz-Castro et al. 2008). An understanding of the relationship between various soil and plant factors under saline environmental conditions can serve as the basis for a cost-effective as well as an environmentally friendly microbial-based strategy (Parnell et al. 2016). Growth-promoting halotolerant microbes can reduce salt stress by increasing the stress resistance of crop plants, potentially in combination with additional beneficial effects, as a tool for sustainable agriculture (Bhattacharyya et al. 2016).

The role of beneficial N-fixing bacteria and their correlation with various factors affecting rice yields in saline soils is still unclear. Therefore, it is necessary to study the correlation and quantity of the contribution of various variables as factors that affect rice yield in saline soils. The quantity of rice yields in saline soils is influenced by various factors or variables, including plant nutrient uptake, beneficial microbial populations, growth components and yield components of rice plants. The study of the role and contribution of N-fixing bacteria to increase rice yields is useful for evaluating the use of N-fixing bacteria biofertilisers in saline soils as environmentally friendly, sustainable microbial-based alleviate plant salinity stress strategy.

## MATERIAL AND METHODS

**Research methodology.** The first experiment was conducted at the Biology Laboratory, Department of Soil and Land Resources, Padjadjaran University, from September 2019 to November 2020. Composite of soil samples from rice rhizosphere of saline soils in Karawang District 6°11'27.7''S, 107°36'50.0''E, Indonesia was taken for isolation of halotolerant nitrogen fixer phytohormone producing rhizobacteria (HNF-PPR) by using modified salinised NFB nitrogen-free medium (Baldani et al. 2014). A simple experiment was done to observe the ability of isolated isolates to increase plant height, root length, and dry weight of rice biomass, produce growth substance IAA (indole acetic acid) and measure its nitrogenase activity.

The second experiment was set up in Karawang District, about 1.0 m a.s.l., with high salinity (ECe = 6.6 mS/cm), soil organic carbon: 1.36%, $N_{tot}$ 0.1%, $P_{tot}$ 36.0 mg/100 g, $K_{tot}$ 30.13 mg/100 g. Soil texture is clay with the composition of sand (37%), silt (14%), and clay (49%).

The weather was rainy season, temperature range of 27.45 °C to 30.93 °C, and the pot experiment for all treatments was set up on 20 kg soil/pot. The grain yield per area in Karawang district ranged between 4.0 to 5.1 t/ha. Design of experiment was arranged in a randomised block design consisting of 8 treatments (control and 20 g inoculant/kg of seed as seed treatments) combined with soil application of inoculant (0, 500, 1 000 and 1 500 g/ha or 0, 2.5, 5.0, 7.5 mg/pot) and provided with 3 repetitions using saline-tolerant rice cv. Inpari 34. The nutrients in the form of inorganic fertilisers given per pot were urea 1.5 g/pot, superphosphate (SP-36) 1.0 g/pot, and potassium chloride (KCl) 0.75 g/pot, which was equivalent to 300, 200, and 150 kg/ha, respectively. Rice seeds planted in each pot were two plants. Setting the water in the pot is regulated by adding water starting at the time of planting rice. They were gradually watering the soil 2–5 cm until the plants were 10 days old. Then the soil is flooded with water as high as 5 cm. From the flowering phase until 10 days before harvest, the soil is continuously irrigated to a depth of 5 cm. When the rice grains are yellow and ripe, the water is reduced until water is dry. The rice panicles that had been cut, collected and dried in the sun for 3 days were then weighed. The observed variables were population NFB, N-uptake, the growth character, rice grain yield and related traits.

**Biochemical analysis and molecular identification of isolate.** The activity of the nitrogenase enzyme was measured by the ARA method (acetylene reduction assay). This assay is based on the $N_2$ase-catalysed reduction of $C_2H_2$ to $C_2H_4$, gas chromatographic isolation of $C_2H_2$ and $C_2H_4$, and quantitative measurement with an $H_2$-flame analyser (Hardy et al. 1968). Indole acetic acid production from pure strains was measured by high-performance liquid chromatography (HPLC) (Sadaf et al. 2009) and, afterwards, identified by partial sequencing of the 16S rRNA

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Table 1. Effect of halotolerant nitrogen fixer-phytohormone producing rhizobacteria isolates on the growth of rice seedlings at 3 weeks after planting

| Isolate | Shoot height (cm) | Roots length (cm) | Dry weight (mg) shoot | Dry weight (mg) root | Dry weight (mg) total | IAA (µg/mL) | ARA (µmol/mL/h) | Identification result | Similarity (%) |
|---|---|---|---|---|---|---|---|---|---|
| Control (without isolate) | 2.87 ± 0.67 | 6.90 ± 0.46 | 6.72 ± 2.89 | 1.533 ± 0.47 | 8.25 ± 0.20 | 0 | 0 | – | – |
| As-2 | 9.13 ± 7.07 | 12.07 ± 1.27 | 13.67 ± 9.61 | 1.967 ± 0.46 | 15.64 ± 0.81 | 0.593 | 0.114 | *Klebsiella pneumonia* | 99.63 |
| As-3 | 6.13 ± 2.61 | 12.17 ± 0.70 | 7.33 ± 3.79 | 2.000 ± 0.62 | 9.33 ± 0.31 | 0.648 | 0.078 | *Pseudomonas stutzeri* | 98.88 |

Numbers ± standard deviation; IAA – indole acetic acid; ARA – acetylene reduction assay

gene (Janda and Abbott 2007). The sequenced strains were the two best isolates that increased the growth of rice plants compared to those without isolates in the first experiment. A fragment of the 16S rRNA gene from the total genomic DNA was amplified by polymerase chain reaction (PCR) using universal primer, P1 (5'-GAGTTTGATCCTGCTCAG-3') and P6 (5'GTTACCTTGTTACGACTT-3').

HNF-PPR inoculant used is two isolates of NFB in an organic powder carrier (30% paddy straw compost, 40% peat, 20% biochar, 10% Ashby's and Okon broth as additive). A bacterial density of about $10^8$ CFU (colony forming unit)/g in HNF-PPR inoculant was achieved by incorporating one-third (*v*/*w*) of NFB starter liquid containing $10^9$ CFU/mL into a powder carrier. The control treatment was also dosed with the organic carrier without NFB.

**Statistical analyses.** Observed data were analysed using IBM SPSS version 26 (New York, USA). Analysis of varians (ANOVA) was used to determine the significant effects of tested treatments. If there was a significant effect of the tested treatments, then continued with Duncan's multiple range test (DMRT) at $P < 0.05$ to find out the mean difference between the treatments (Gomez and Gomez 1984). The path analysis was used to interpret equations by finding the determination coefficient of multiple regression. The correlation and regression analysis were performed using IBM SPSS version 26 and continued with path analysis/structural equation modelling (SEM) using IBM SPSS Amos version 26.

## RESULTS AND DISCUSSION

**Halotolerant nitrogen fixer phytohormone producing rhizobacteria characteristics.** Inoculating two potential isolates into growing media showed a different performance on rice seedlings. The isolate of As-2 and As-3 were able to increase shoot height, roots length and dry weight and were compared to those without isolate (control). As-2 and As-3 isolates as a pure culture on N-free slant agar (JNfb medium) were also able to fix nitrogen compared control slant agar without bacteria. Those isolates also produce the growth substances of IAA (Table 1). Furthermore, these isolates were identified bio-molecularly as *Klebsiella pneumonia* and *Pseudomonas stutzeri*, as shown in Figure 1.

Microbial biodiversity and abundance of rhizomicrobiome, which are caused by the application of HNF-PPR inoculant (*P. stutzeri* and *K. pneumonia*)

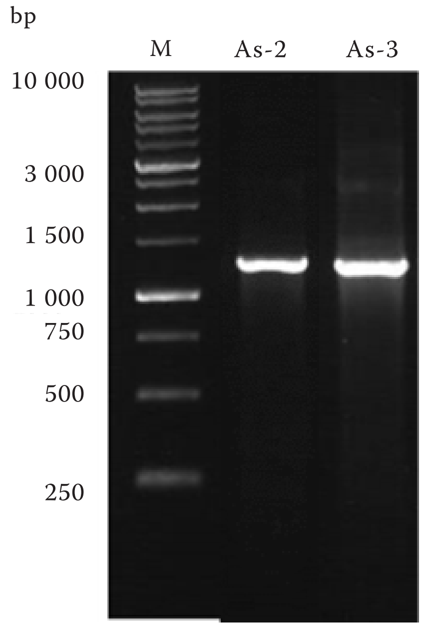


Figure 1. DNA fingerprint of *Klebsiella pneumonia* (As-2) and *Pseudomonas stutzeri* (As-3)

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and increasing the population of beneficial HNF-PPR indigenous, plays an important role in managing the availability of nutrients and growth factors for rice plant development and growth (Nosheen et al. 2020). The plant health, improvement of rice growth, and enhancement of rice productivity in saline soils are contributed by the presence and domination of beneficial microbes in rhizomicrobiome (Yao et al. 2010). The PPR application combined with ameliorant (organic fertilisers, compost, or dolomite) resulted in biofertilisers increasing their effectiveness (Shilev 2020).

**NFB population, N-uptake, and rice growth characters.** The NFB population and growth of plant traits (plant height and the number of tillers) at 40 days after the plant (DAP) were significantly influenced by the seed treatment and soil application (Table 2). The abundance of NFB was increased significantly by 20 g inoculant/kg seed treatment combined with 1 500 g inoculant/ha of soil application. The increasement was more than 150% higher than the control. Application of NFB at 500 g SoA (soil application, g inoculant/ha) did not increase its abundance in contrast to when applied together with 20 g SeT (seed treatments, g inoculant/kg seed). Application of 20 g SeT combined with 500 g SoA can increase the population of NFB compared to without NFB application through seed treatment in saline soil.

The increase in N-uptake due to the application of inoculants was only slightly compared to the control, so the increase was not significantly different. Plant height and tiller of rice plant were increased significantly by inoculant addition of 20 g/kg to the seed treatment combined with 1 500 g/ha to the soil application. The application of inoculants through soil treatment starting at 500 g has increased plant height as well as when combined with 20 g of seed treatment. However, rice plant height did not increase if only 20 g of inoculant was given through seed treatment. Meanwhile, the tiller can be increased with inoculant doses starting from 1 000 g through soil application, and the inoculant dose used will be lower when combined with a seed treatment. Grain straw ratio control was higher than the treatment given the NFB inoculant over 500 g through soil application or its start with 500 g soil application combination with seed treatments.

Seed and soil application as inoculant treatment methods could increase the population of NFB compared to without inoculant application in saline soil. These results exhibit that the incorporated inoculant was able to adapt and reproduce in the rhizomicrobiome. These microbes are well known as N-fixer bacteria, and PGPR (plant growth promoting rhizobacteria) is bacteria which contributes to plant growth, plant health, nutrient availability, and reducing salinity stress (Benaissa et al. 2019, Setiawati et al. 2020).

The plant height of rice plants inoculated with HNF-PPR was higher than those without inoculants. Meanwhile, the tiller number increased when the inoculant was given through a soil application of 1 000 g/ha. With the application of seed treatment combined with soil application, a lower dose of soil application was obtained to produce a larger num-

Table 2. Nitrogen-fixing bacteria (NFB) population, growth component affected by halotolerant nitrogen fixer-phytohormone producing rhizobacteria as seed treatments (SeT, g/kg) and soil application (SoA, g/ha) on saline soils

| Application treatment | NFB population ($\times 10^7$ CFU/g) | N-uptake (mg/plant) | Plant height (40 DAP) | Tiller number (tiller/plant) | Grain straw ratio |
|---|---|---|---|---|---|
| $T_0$ = control | $1.97^{a}$ | $8.87^{a}$ | $62.93^{a}$ | $17.58^{a}$ | $1.32^{c}$ |
| $T_1$ = 500 g SoA | $2.17^{a}$ | $10.76^{a}$ | $66.16^{bc}$ | $21.50^{abc}$ | $1.10^{bc}$ |
| $T_2$ = 1 000 g SoA | $2.77^{b}$ | $10.80^{a}$ | $69.11^{de}$ | $25.58^{cd}$ | $0.98^{ab}$ |
| $T_3$ = 1 500 g SoA | $3.47^{c}$ | $10.79^{a}$ | $72.51^{f}$ | $31.42^{f}$ | $0.75^{a}$ |
| $T_4$ = 20 g SeT | $2.00^{a}$ | $9.80^{a}$ | $63.99^{ab}$ | $19.67^{ab}$ | $1.22^{bc}$ |
| $T_5$ = 20 g SeT + 500 g SoA | $2.83^{b}$ | $11.02^{a}$ | $66.86^{cd}$ | $23.42^{bcd}$ | $0.89^{ab}$ |
| $T_6$ = 20 g SeT + 1 000 g SoA | $3.40^{c}$ | $11.12^{a}$ | $69.27^{de}$ | $26.50^{de}$ | $0.88^{ab}$ |
| $T_7$ = 20 g SeT + 1 500 g SoA | $4.93^{d}$ | $11.20^{a}$ | $71.47^{ef}$ | $30.58^{ef}$ | $0.88^{ab}$ |

Different letters in each column indicate significant differences between different application treatments ($P < 0.05$; Duncan's test). CFU – colony forming unit; DAP – day after planting

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ber of tillers. The role of HNF-PPR in increasing the number of tillers was obtained through the two application methods.

Grain straw ratios (GSR) of rice plants without inoculant (control) showed high values and were not different from rice plants that were given 500 g of inoculant through soil application or with 20 g of seed treatment. This result was in contrast with the weight of the rice grain produced. This illustrates that the GSR calculation is only appropriate for unstressed soil conditions. When in stressed conditions, such as the salt content in saline soil, it will cause low straw weight and increased grain weight, but there is a lot of empty grain.

**Rice yield and related traits in saline soils.** The yield component (pithy and empty grain and the number of grains), as well as total grain weight, was significantly influenced by the application of the HNF-PPR inoculant (Table 3). In general, the enlarged dosage of HNF-PPR inoculant increased pithy grain, number of grains, percentage of pithy grain and total grain weight. Even though the empty grains also grew, the percentage of empty grains was lower than those with the control.

The increase in component rice grain yield traits (pithy grain or filled grain and number grain) was strongly influenced by the increase in the inoculant dose administered through soil application. Meanwhile, when soil application inoculants were combined with seed treatment, the combination with the largest soil application dose (1 500 g/ha) resulted in the highest pithy grains. If the inoculant was only given through 20 g/kg seed, it was not able to increase the pithy grain per plant, and the results were the same as those in control. In saline soil that was inoculated with or without NFB inoculants, high empty grains were produced, namely 11.54–18.74 g/plant or 32.50–46.31% of the total grain yield. The high empty grain is related to the difficulty of plants to absorb nutrients in saline soil conditions. Application NFB inoculants, either through soil application or combined with seed treatment, escalated the percentage of pithy grain and reduced empty grain.

The application of inoculant 20 g/kg seed combined with HNF-PPR inoculant 1 500 g/ha on soil obtained the highest rice grain yield (57.65 g/plant or 8.4 t/ha). Compared to the control, the application of inoculant 20 g/kg seed treatment combined with HNF-PPR inoculant 500–1 500 g/ha on soil application contributed to the growth of the rice grain yield by 43.8–130.6%; meanwhile, the increase by 32.8–111.8% was achieved by soil application of inoculant 500–1 500 g/ha. Moreover, the application of 20 g/kg seed of inoculant combined with 1 500 g/ha soil application showed the highest grain yield. But this application did not demonstrate a significant effect on grain yield when compared with 1 500 g/ha soil application. Nevertheless, an inoculant of HNF-PPR could be used to engineer the soil rhizomicrobiome and alleviate plant salinity stress.

**Correlation of NFB, growth, and rice yield components on saline soils.** Correlation analysis was performed on NFB, growth, and yield components of rice plants on saline soils to determine the relationship between these variables (Table 4). The

Table 3. The yield of rice plants and related component traits on saline soils due to the application of halotolerant nitrogen fixer-phytohormone producing

| Application treatment | Pithy grain (g/plant) | Empty grain (g/plant) | Number of grains (number/plant) | Pithy grain (%) | Empty grain (%) | Total grain weight (g/plant) |
|---|---|---|---|---|---|---|
| $T_0$ = control | 13.45$^{a}$ | 11.54$^{a}$ | 560.34$^{a}$ | 53.69$^{a}$ | 46.31$^{d}$ | 25.00$^{a}$ |
| $T_1$ = 500 g SoA | 19.07$^{b}$ | 14.14$^{ab}$ | 773.27$^{b}$ | 57.31$^{abc}$ | 42.69$^{cd}$ | 33.20$^{bc}$ |
| $T_2$ = 1 000 g SoA | 25.55$^{c}$ | 15.60$^{ab}$ | 1 001.52$^{c}$ | 62.03$^{cd}$ | 37.97$^{abc}$ | 41.14$^{de}$ |
| $T_3$ = 1 500 g SoA | 35.11$^{d}$ | 17.85$^{b}$ | 1 356.52$^{d}$ | 66.58$^{de}$ | 33.42$^{a}$ | 52.96$^{f}$ |
| $T_4$ = 20 g SeT/kg seed | 15.37$^{a}$ | 12.17$^{a}$ | 628.02$^{a}$ | 55.94$^{ab}$ | 44.06$^{dc}$ | 27.54$^{ab}$ |
| $T_5$ = 20 g SeT + 500 g SoA | 21.17$^{b}$ | 14.77$^{ab}$ | 831.55$^{b}$ | 58.84$^{bc}$ | 41.16$^{bcd}$ | 35.95$^{cd}$ |
| $T_6$ = 20 g SeT + 1 000 g SoA | 29.16$^{c}$ | 16.00$^{ab}$ | 1 123.34$^{c}$ | 64.64$^{de}$ | 35.36$^{ab}$ | 45.16$^{e}$ |
| $T_7$ = 20 g SeT + 1 500 g SoA | 38.92$^{e}$ | 18.74$^{b}$ | 1 412.65$^{d}$ | 67.50$^{e}$ | 32.50$^{a}$ | 57.65$^{f}$ |

Different letters in each column indicate significant differences between different application treatments ($P < 0.05$; Duncan's test). SoA – soil application; SeT – seed treatment

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Table 4. Correlation analysis of N-fixing bacteria population (NFB), rice yield and related traits

| | PG (g) | EG (g) | GN | PGP (%) | EGP (%) | TN | PH (cm) | GSR | NFB (CFU/g) | RGYP (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| PG (g) | 1 | 0.714** | 0.983** | 0.896** | –0.868** | 0.879** | 0.922** | –0.680** | 0.887** | 0.981** |
| EG (g) | | 1 | 0.693** | 0.445* | –0.306 | 0.663** | 0.628** | –0.463* | 0.751** | 0.836** |
| GN | | | 1 | 0.907** | –0.870** | 0.896** | 0.937** | –0.721** | 0.878** | 0.962** |
| PGP (%) | | | | 1 | –0.954** | 0.732** | 0.886** | –0.699** | 0.747** | 0.825** |
| EGP (%) | | | | | 1 | –0.760** | –0.871** | 0.652** | –0.674** | –0.765** |
| TN | | | | | | 1 | 0.901** | –0.640** | 0.782** | 0.872** |
| PH (cm) | | | | | | | 1 | –0.649** | 0.754** | 0.896** |
| GSR | | | | | | | | 1 | –0.564** | –0.661** |
| NFB (CFU/g) | | | | | | | | | 1 | 0.903** |
| RGYP (g) | | | | | | | | | | 1 |

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). PG – pithy grain; EG – empty grain; GN – grain number of pithy grain; PGP – pithy grain percentage; EGP – empty grain percentage; TN – tiller number; PH – plant height; GSR – grain straw ratio; RGYP – rice grain yield per plant; CFU – colony forming unit

results of correlation analysis showed that there was a strong positive relationship between NFB population and plant height 40 DAP ($r$ = 0.754**) and the number of tillers 40 DAP ($r$ = 0.782**) but there was a negative relationship with grain straw ratio ($r$ = –0.564**). There was a strong positive relationship between plant height and the number of the tiller ($r$ = 0.901**), while with grain straw ratio was a negative relationship ($r$ = –0.649**). In addition, there was a negative relationship between the tiller number and the grain straw ratio ($r$ = –0.640**).

Linear regression analysis was performed to predict how much influence the number of tillers, plant height, grain straw ratio, and NFB population on grain yield per plant on saline soils. The results of the analysis get a multiple linear regression equation with a coefficient of determination ($R^2$) = 0.926 as follows: $y = 0.107x_1 + 1.404x_2 - 3.408x_3 + 5.880 \times 10^{-7}x_4 - 71.850$ where $y$ = grain weight per plant (g), $x_1$ = number of tiller; $x_2$ = plant height (cm); $x_3$ = grain straw ratio, and $x_4$ = NFB population (CFU/g) (Table 5). The effective contribution of each of these variables to the grain weight per plant is calculated using the formula: effective contribution (%) = beta × zero-order correlation × 100 (Lufri 2004, Liu et al. 2014). The relationship between the number of the tiller, plant height, grain straw ratio, and NFB population on grain yield per plant is clearly illustrated through path analysis/structural equation modelling (SEM) diagrams (Figure 2).

The NFB population was positively correlated with plant height; the number of the tiller, rice yield and related traits were very influenced due to the nitrogenase activity of NFB, which contributes

Table 5. Results of linear regression analysis number of the tiller, plant height, grain straw ratio, and N-fixer bacteria (NFB) population on rice grain yield on saline soils

| Model | Unstandardised coefficients | | Standardised coefficients | $t$ | Sig. | Correlations | | |
|---|---|---|---|---|---|---|---|---|
| | B | SE | β | | | zero-order | partial | part |
| Constant | –71.85 | 28.454 | | –2.525 | 0.021 | | | |
| Number of tiller 40 DAP | 0.107 | 0.347 | 0.048 | 0.308 | 0.762 | 0.872 | 0.07 | 0.019 |
| Plant height 40 DAP (cm) | 1.404 | 0.49 | 0.429 | 2.866 | 0.01 | 0.896 | 0.549 | 0.179 |
| Grain straw ratio | –3.408 | 4.192 | –0.068 | –0.813 | 0.426 | –0.661 | –0.183 | –0.051 |
| NFB population (CFU/g) | 5.88E–07 | 0 | 0.503 | 4.909 | 0 | 0.903 | 0.748 | 0.307 |

Dependent variable: grain weight (g/plant); DAP – day after planting; SE – standard error; CFU – colony forming unit

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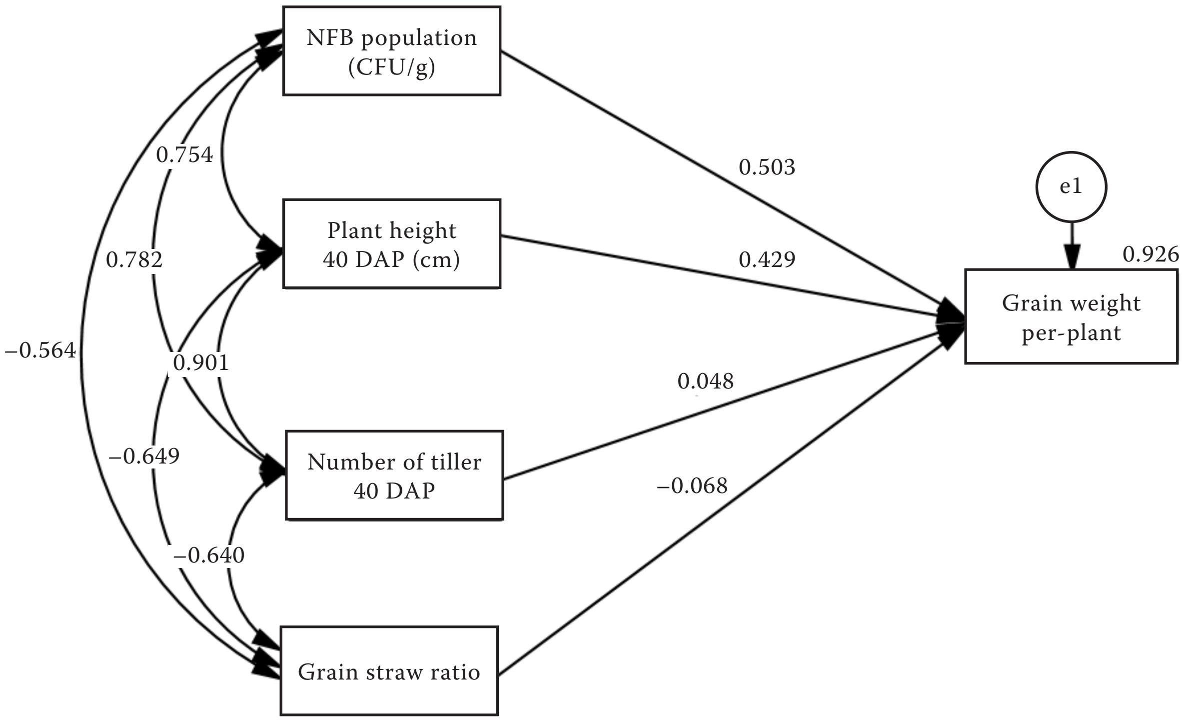


Figure 2. Path analysis model of the number of tillers, plant height, grain straw ratio, and nitrogen-fixing bacteria (NFB) population to the grain yield per plant on saline soils. CFU – colony forming unit; DAP – day after planting

provides N to rice plants in saline soils of 0.114 and 0.078 μmol/mL/h. Besides that, another role of NFB in this experiment was to produce growth hormone IAA of 0.593 and 0.648 μg/mL, which affected root division and elongation. As a result, the extension of the roots of rice plants can expand the volume of roots to absorb nutrients in the soil. IAA plant hormones extend root reinforcement by enhancing the length and surface area of the root, which causes increased nutrient uptake and hence increases plant fertility in a saline toxicity environment (Jha et al. 2013).

The linear regression equation produced a coefficient of determination ($R^2$) = 0.926, which indicated that 92.6% grain weight per plant was influenced by the number of tillers, plant height, grain straw ratio, and NFB population with the effective contribution of each variable were 4.18, 38.45, 4.59, and 45.45%, respectively. NFB population had the greatest influence on grain weight per plant. This is understandable since NFB provide N nutrients and growth hormones so that it affects the grain formation of rice plants.

A new approach through this research has been developed to reduce salt stress in rice plants by inoculating seeds and plant seedlings with identified HNF-PPR. This study has proven that the role of HNF-PPR significantly determines rice yield in saline soils; therefore, the role of HNF-PPR through seed treatment can be improved in several ways. Alternative applications of HNF-PPR on rice seed can be attempted through techniques, namely soaking seeds or immersing the root seedlings in the suspension of HNF-PPR inoculants. Hence, there is a great opportunity for the successful application of HNF-PPR in saline soil management. HNF-PPR inoculation and its application technology are essential for developing commercially effective formulations to alleviate salt stress circumstances.

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