
EFFECT OF AZOTOBACTER AND ANTIBIOTIC ON SOIL NITROGEN AND RICE SEEDLING GROWTH IN SALINE SOIL

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ABSTRACT

Saline soil in general has low nitrogen (N) availability and restricted N uptake. Application of manure and nitrogen fixing bacteria (NFB) in addition to inorganic fertilizer are needed but manure amendment on soil will contribute to increase antibiotic residue in soil. A pot experiment has been conducted to verify the effect of antibiotic-resistance NFB *Azotobacter* on nitrogen availability and growth of rice seedling in saline soil contaminated with tetracycline and ampicillin. Green house experiment was arranged in factorial Randomized Block Design to examine four concentrations of *Azotobacter* sp. liquid culture and two kind of generic antibiotic. The first factor was without and with 106, 107, and 108 CFU g⁻¹ of *Azotobacter* inoculation, and the second one were antibiotic treatment consisted of tetracycline and ampicillin applied at 1 mg/kg. The results showed that neither *Azotobacter* nor antibiotic affected plant height, soil pH and EC, as well as *Azotobacter* population in rice rhizosphere. The interaction effect on both treatment was significant on total nitrogen in soil. This research suggested that *Azotobacter* maintained soil N when inoculated to soil up to 106 CFU/g and 108 CFU/g irrespective of antibiotic contamination but the bacteria had not yet affect growth of rice seedling in high sodium soil.

Keywords: Nitrogen, *Azotobacter*, Sodium, Rice Seedling, Soil Electrical conductivity

1. INTRODUCTION

To increase rice production, suboptimal land area of saline soil in Indonesia is put in use. Saline soil contains excessive soluble salts mainly sodium in soil solution and cause high osmotic pressure [1]. Salinity influence nitrogen transformation in soil resulting in reduced nitrogen availability for roots uptake. Soil nitrification at EC of 20 dS/m was inhibited up to 75% and 83% when ammonium sulfate and urea were applied respectively [2]. Medium to high salinity promote nitrite accumulate due to suppression on second step of nitrification, leaving more ammonium accumulation in soil [3].

High osmotic pressure and low available nitrogen in saline soil are unsuitable for plant growth. The limitation of N in saline soils could be overcome, among others, by application of organic matter and nitrogen fixing bacteria. Amendment of 20 t/ha of manure on saline soil with EC 4.1

dS/m significantly increased growth and dry matter weight of *Sesbania grandiflora* [4]. Organic matter widely used in rice production in Indonesia is chicken manure which contain some antibiotic residue [5]. and contributed to the antibiotic resistance bacteria in soil [6, 7]. Widely used antibiotic in poultry farming in Indonesia was tetracycline and ampicillin.

Biofertilizers are alternative to chemical fertilizers for nutrient source in food crops production. *Azotobacter* sp. is a prominent nitrogen fixing bacteria which widely used as biofertilizer. The bacteria fix nitrogen and produce phytohormones indole acetic acid (IAA) and gibberellins which contribute to plant growth [8, 9]. *Azotobacter* inoculation combined with 25%-100% urea improved rice growth [10]. *Azotobacter* is heterotrophic bacteria that utilize organic matter as carbon and energy source. Regarding the presence of antibiotics in manure, the utilization of *Azotobacter* as biofertilizer for rice production in saline soils requires *Azotobacter* which is resistant to antibiotics and at the same time to high salinity. Salt-tolerant *Azotobacter* was reported elsewhere. *Azotobacter salinestris* fixed nitrogen in the presence of 1.0 to 1.5% Sodium Chloride (NaCl) and the cells grew well in marine broth alone [11]. Certain isolates of *Azotobacter* were reported showed a tolerance for NaCl up to 10% [12, 13]. In the presence of 0.3 M of salt, *Azotobacter* H12 enhanced nitrogen fixation, and some isolates increased IAA production in media with 0.3% salt although their growth was restricted [14].

Research of antibiotic-resistance *Azotobacter* on rice grown in saline soil have not been done intensively in Indonesia. Rice is a major staple food in Indonesia and their tolerance to high salt concentration will be important when rice production carried out in the coastal area dominated by saline soil. Salt-tolerant characteristics was depend on rice variety although it was reported that rice was susceptible on salt [15]. The objective of this research was to verify the effect of antibiotic-resistance NFB *Azotobacter* on nitrogen availability and growth of rice seedling in saline soil contaminated with tetracycline and ampicillin.

2. MATERIAL AND METHOD

Green house experiment was carried out in April to Mei 2018 at Faculty of Agriculture Universitas Padjadjaran, Jatinangor Campus, West Java located in tropic at the altitude of 625 above sea level. *Azotobacter* sp. K4 which was resistance to 50 mg/L tetracycline and ampicillin belongs to Laboratory of Soil Biology Faculty of Agriculture. Liquid inoculants was prepared di N-free Ashby medium; a total of 5% *Azotobacter* pure culture inoculated to sterilized liquid media and incubated on 115 rpm shaker for 72 hours at room temperature (24-27 °C).

The soil was saline clay soil collected from Karawang District, West Java; soil characters were slightly acid (pH 6.05); low in C-organic (1.24%), total nitrogen (0.19%) and ratio C to N (6.5); very low in P₂O₅ and K₂O (0.14 mg/100 g and 9.41 mg/100 g respectively). with very high exchangeable sodium (2.39 cmol/kg). Electrical conductivity (EC) of soil was high (1.8 dS/m); Clay soil with EC 1 dS/m–2 dS/m determined by the 1:5 dilution method is classified as a high salinity soil [16]. Experiment was carried out by used of salinity stress resistant rice variety namely Inpari-34 produced by Food Crops Development and Research Centre of Indonesian Ministry of Agriculture.

The experimental design was Factorial Completely Randomized Block Design to test *Azotobacter* and antibiotic treatment factor. *Azotobacter* treatment consisted of *Azotobacter* inoculation to soil up to 10^6 , 10^7 and 10^8 Colony Forming Unit (CFU)/g before rice seedling transplanting; the antibiotic treatments were kind of generic antibiotic tetracycline and ampicillin applied in soil at 1 g/kg. Control treatments received neither *Azotobacter* nor antibiotic. All treatments were replicated three times.

A total of 1 kg soil mixed with 50 g chicken manure was put on 1.5 L black pot; all soil in pot was saturated with ground water; treated with antibiotic and placed 3 days in the green house. Two weeks-old day rice seedlings were grown on soil saline soil and transferred to potted soil as much as 4 seedlings each. Bacterial inoculation and 50% of recommended dose nitrogen-phosphorus-potassium compound fertilizer were applied at the planting time. Liquid-culture's *Azotobacter* as much as 10 mL was diluted by ground water and poured equally surrounding rice seedling; the amount of water was depended on expected final population of exogenous *Azotobacter* in soil. Inorganic fertilizer was placed 5 cm below mud soil in two holes. Rice were growing in green house for 9 days.

Soil total nitrogen, *Azotobacter* population, acidity and EC were measured at 9 days after transplanting. Soil total nitrogen was determined by Kjeldahl method after acid destruction. Population of *Azotobacter* was count from rice rhizosphere using serial dilution plate method with N-free Ashby's medium. Soil acidity and EC were measured after being diluted with 2.5 part and 5 part of distilled water respectively. All set of data were subjected to analyze of variance with 95% significance level; and 95% Duncan's Multiple Range Test whenever the effect of treatment on parameters was significant.

3. RESULTS AND DISCUSSION

3.1 Soil Total Nitrogen and *Azotobacter* Population

Analysis of variance showed that the interaction between the *Azotobacter* and antibiotic treatments on total nitrogen in soil was significant (Table 1). There was no change in N content when soil was inoculated by 10^6 CFU/g and 10^8 CFU/g of *Azotobacter* but N content in ampicillin-contaminated soil with *Azotobacter* of 10^7 CFU/g was lower than those in soil without antibiotics and in the presence of tetracycline. *Azotobacter* inoculation and antibiotic contamination didn't show significant interaction effect on *Azotobacter* population in rice rhizosphere at 9 days; main effect of both treatments didn't influence *Azotobacter* count (Fig 1) based on variance analysis.

In the beginning of experiment, exogenous *Azotobacter* in soils were 10^6 , 10^7 , and 10^8 CFU/g. *Azotobacter* population in soil before inoculation was 10^5 CFU/g; those in rice rhizosphere at the end of experiment was 10^6 CFU/g. The increase of bacterial population in rhizosphere compared to the count before experiment indicated that exogenous *Azotobacter* colonized the rhizosphere in the presence of high exchangeable sodium (2.39 cmol/kg) and high EC (1.8 dS/m) in soil. Exudates released by seedling root induced microbial proliferation in the rhizosphere due to organic substances [17,18]. Ability of *Azotobacter* to withstand to salinity was reported elsewhere [12,13,14]; in our experiment, *Azotobacter* K4 was isolated from soil with EC of 8 dS/m and can survive in paddy soil which has EC of only 1.8 dS/m. *Azotobacter* population in

control treatments, either without *Azotobacter* or Antibiotic, were statistically similar to those in treated soil which is suggested that the decline of *Azotobacter* count in soil with initial population of 10^7 and 10^8 CFU/g rhizosphere was not caused by the treatments.

Table 1. Interaction effect between *Azotobacter* soil inoculation and antibiotic on total nitrogen in soil at 9 days after transplanting in saline soil

<i>Azotobacter</i> Inoculation (CFU/g)	Soil Total Nitrogen (%)		
	Control	Tetracycline	Ampicillin
Control (without <i>Azotobacter</i>)	0.33a A	0.34a A	0.28b A
10^6 (Liquid inoculant)	0.33a A	0.27a A	0.33b A
10^7 (Liquid inoculant)	0.32a B	0.31a B	0.19a A
10^8 (Liquid inoculant)	0.24a A	0.30a A	0.28b A

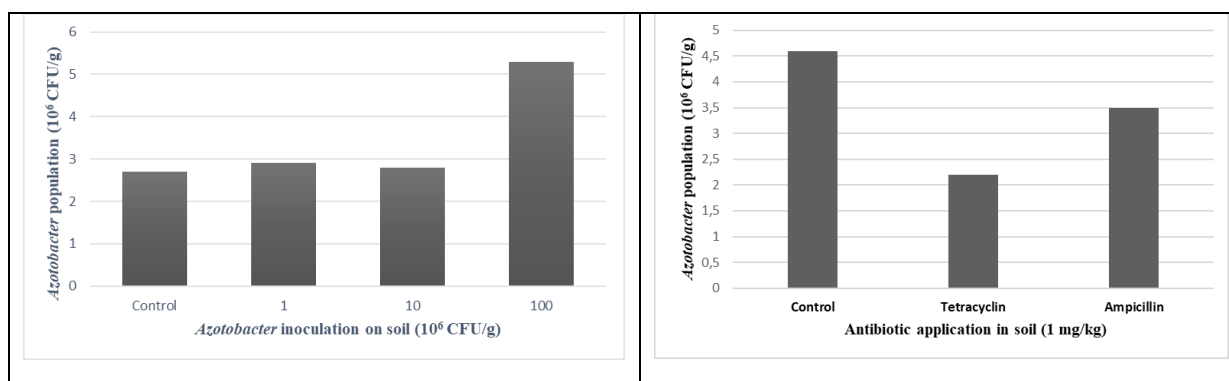


Fig 1. Effect of *Azotobacter* inoculation (left) and antibiotic (rights) on *Azotobacter* population in rice seedling rhizosphere in saline soil at 9 days after transplanting

Before experiment, soil total N was as low as 0.19% but at the end of experiment, N content in soil was generally increased up to around 0.3% (Table 1). Increased in total N did not due to *Azotobacter* inoculation but it came from organic matter amendment before planting and urea application since total N in control treatment did not significantly differ. Free-living N fixing *Azotobacter* is a heterotrophic Plant Growth Promoting Rhizobacteria that depend on organic matter for proliferation. Bacteria living in rhizosphere are more functional in transforming nutrients compared to bacteria in bulk soil [19]; root exudation provide organic matter as carbon and nitrogen source as well as terminal electron for their aerobic metabolism.

3.2 Soil Acidity, Electrical Conductivity and Shoot Heights

The experiment showed that interaction between *Azotobacter* and antibiotic treatment did not significantly affect soil acidity, EC as well as shoot heights. Neither *Azotobacter* nor antibiotic influenced the traits measured (Table 2). Comparing to the soil before experiment, soil acidity did not change but soil EC was increased irrespective of the treatments (Table 2). Soil EC increased from 1.7 dS/m before experiment to 3.1-3.4 after rice grows for only 12 days (Table 2). Rice is sensitive to salt-accumulated soil; they did not survive when soil EC is 2-3 dS/m [20, 21].

Electrical conductivity of clay soil in this experiment was 1.8 dS/m which measured after fivefold dilution by distilled water; classified soil with EC 1-2 dS/m as high salinity soil [16]. Toxic effect of sodium on plant growth was clearly demonstrated. Plant growth was inhibited and plant leaves showed salt stress symptoms; leaves desiccation, pale green in color, rolling inward and chlorosis were observed in the oldest leaves. At 12 days after transplanting, all rice plants in 11 pot were not survived. We decided to terminate the experiment at 12 days after planting when soil acidity and EC were analyzed. Growth response of upland rice cultivar Inpari-34 was in accordance with growth of some rice genotype in solution at 8 dS/m at 7 days when their fresh and dry weight as well as shoot heights were reduced [22].

Table 2. The effect of *Azotobacter* inoculation and antibiotic contamination on shoot height at 9 days after transplanting in saline soil, and soil acidity and electrical conductivity at 12 days

<i>Azotobacter</i> inoculation (CFU/g)	Soil Acidity*	EC (dS/m)*	Shoot height (cm)*
Control (Without <i>Azotobacter</i>)	6.27	3.3	17.5
10 ⁶ (Liquid inoculant)	6.26	3.1	17.1
10 ⁷ (Liquid inoculant)	6.24	3.4	17.3
10 ⁸ (Liquid inoculant)	6.26	3.1	16.8
Antibiotic (1 mg/kg)			
Control	6.17	3.4	17.2
Tetracycline	6.29	3.2	17.3
Ampicillin	6.31	3.2	17.0

*Data from three replicates

Damage of rice plant in saline soil in controlled conditions caused by osmotic imbalance and accumulation of chloride ion was reported ([22]. Plant growth inhibition due to excessive sodium (Na²⁺) uptake; causes Na ions to be absorbed enormously by plants [23]. In saline soils, roots were inhibited to absorb nutrients such as Ca²⁺, K⁺, N, and P in soil [24]. Chlorosis in older rice

leaves was due to low N uptake and consequently N from older leaves was mobilized to the young one. Pale color in rice leaves was caused by reduced chlorophyll content since accumulation various salt inhibited biosynthesis of certain chlorophyll fraction [25].

4. CONCLUSION

The effect of *Azotobacter* K4 concentration inoculated to rice seedling in saline soil on total nitrogen content was depend on type of antibiotic contaminated to the soil. Application of salt-resistant *Azotobacter* up to 10^6 and 10^8 CFU/g of maintained nitrogen content in ampicillin-contaminated soil but those of 10^7 CFU/g lowered total nitrogen. Irrespective of *Azotobater* and antibiotic treatments, *Azotobacter* population in the rhizosphere of rice seedling grown in saline soil showed higher bacterial count than those in bulk soil before experiment which is indicated that *Azotobacter* K4 was tolerant to high sodium as well as EC in soil. *Azotobacter* inoculation at any level and type of antibiotics didn't affect soil pH, EC and plant growth; although *Azotobacter* was survive in the rice seedling rhizosphere, their activity to perform PGPR effect was not evidence due to high sodium and EC in soil. This reseach suggest that in saline soil, the contribution of antibiotic-resistant *Azotobacter* on rice seedling growth could not be explained properly since high sodium and EC suppressed plant growth.

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