RETARDED GROWTH OF LOWLAND RICE IN SALINE SOIL INOCULATED WITH NITROGEN-FIXER AZOTOBACTER

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ABSTRACT

Low-land rice (*Oryza sativa* L.) cultivation in saline soils face some constraints include nitrogen availability. Saline-resistant nitrogen fixing bacteria Azotobacter are expected to increase supply nitrogen in saline soils. The objectives of the study were to determine the effect of liquid inoculant concentration of two Azotobacter isolates on early vegetative growth of lowland rice grown in potted saline soil. The greenhouse trial design was a randomized block design with seven treatments and four replications. The treatments were combination of isolates and Azotobacter liquid inoculant concentrations in single and mixed inoculation. The results showed that all plants experienced chlorosis and stunt due to high Electrical Conductivity. Inoculation of different isolates and concentrations did not influence the growth of lowland rice in soil with high EC at the end of experiment. Therefore, neither isolates nor concentration of Azotobacter could improve retarded-growth of lowland rice in saline soil.

Keywords: Azotobacter, Saline soil, Electrical conductivity, Lowland rice

INTRODUCTION

Limited arable land leads lowland rice cultivation in saline soils with low nitrogen availability. Climate changes and irrigation practices also increase the distribution of saline soil. In general saline soil located in coastal area affected by sea tidal and salt water intrusion. The main features of saline soil are sodium content between 8-15%, pH <8.5, EC > 4 dS/m (equivalent to 4 mmhos/cm) and sodium adsorption ratio >15% (Yan et al., 2015). Saline soil is one of the most destructive environmental conditions that causes major considerable decreased in plant growth, yield and crops quality (Shahbaz and Ashraf, 2013).

Plants grow in saline agroecosystem are not able to adsorb water as much as they need. Limited water absorption in saline and sodic condition lead to water scarcity (Assouline et al., 2015) and then low uptake of plant nutrient. Nitrogen (N) uptake restriction is always evidence in saline soil resulted in retarded early vegetative growth and plant stunting (Hoorn et al., 2001; Assouline et al., 2015). Rice is not resistance to saline soil but some cultivars are more tolerant compared to the others. Researcher reported that irrigation water with EC >2 dS/m cause yield loss of rice up to 1 ton/ha (Asch and Wopereis, 2001). High level of salt in paddy soil influenced early vegetative growth include seed germination, seedling growth, leaf size, shoot growth, shoot and root length, shoot dry weight, shoot fresh weight, and number of tillers per plant (Reddy et al., 2017).

Rhizobacteria might play a prominent role in saline soil since they have certain biological characteristics such as tolerance to saline conditions, plant nutrients provision, plant growth promoting hormones synthesis, develop a specific interaction with crop plants (Shrivastava and Kumar, 2015). Nitrogen fixing bacteria (NFB) are natural rhizosphere inhabitants involved in soil nitrogen cycle and hence N availability for root uptake. In tropics, most of plant cultivations were limited by N and phosphorus (P) since tropical soil naturally contain low available N and P. In conventional agriculture low N availability might be overcome by chemical fertilizer but nowadays NFB inoculation play an important role to supply N for plants.

Azotobacter is prominent and accepted NFB in food crop cultivation since the bacteria enable to produce phytohormone auxin, cytokinins and gibberellines as well as exopolysaccharides in addition to fix dinitrogen (Jnawali et al., 2015). Phytohormones plays an important role in many biochemical processes in plants. Research verified that bacterial exopolysaccharides increased root-associated soil, improve soil porosity and enhance nutrient uptake (Gauri et al., 2012).

The resistance of Azotobacter to saline environment has been reported. Azotobacter sp. enables to proliferate and fixed N in broth contained sodium chloride up to 1.5 M (Sangeeta et al. 2014). Azotobacter K4, S2 and S1 isolated from saline soil were resistance to 1.7%-3.4 % sodium chloride in laboratory test, and the K4 and S2 increased shoot height, leaves number and root's dry weight of four weeks old tomato (Hindersah et al., 2019). The objective of this pot experiment was to verify the resistance of rice cv *Ciherang* on saline condition; and the effect of isolates and concentration of Azotobacter liquid inoculant on early vegetative growth of rice grown in saline soil.

MATERIALS AND METHOD

A pot experiment was conducted in the green house of Faculty of Agriculture, Padjadjaran University at Jatinangor Campus in Sumedang Regency, West Java. The experimental site was located in tropic at the altitude of 732 m above sea level. Saline soil was taken up from the top soil of Subang Regency of West Java. The soil was clay in texture and has pH 6.75, organic-C 0.65% (low), total N 0.21% (medium), C/N 3 (very low), total P_2O_5 9.67 mg/100g (low), total K_2O 7.2 mg/100g (low). The soil was low in Cation Exchange Capacity but high in electrical conductivity (EC 8.6 dS/m).

Azotobacter sp. isolate S1 and S2 were the collection of Soil Biology Laboratory, Faculty of Agriculture and isolated from rice rhizosphere grown in saline soils. Both isolates enabled to proliferate in saline broth with 1.7% sodium chloride. Bacteria were maintained in N-free Ashby's mannitol slant and retransferred to the Ashby's slant 3 days before used.

Greenhouse experiment was setup in randomized block design with seven treatments and four replications. The treatments were the combination of Azotobacter isolates and concentration of liquid inoculant of Azotobacter:

A: Control, without Azotobacter
B: *Azotobacter* sp. S1; 0.5%
C: *Azotobacter* sp. S1; 1.0%
D: *Azotobacter* sp. S2; 0.5%
E: *Azotobacter* sp. S2; 1.0%
F: Mixed of *Azotobacter* sp. S1 and S2; 0.5%
G: Mixed of *Azotobacter* sp. S1 and S2; 1.0%

Soil was collected from the top soil by using auger from several points in paddy field of Subang Regency. The soil was air dried in the shade, ground, homogenized and then filtered with a 5 mm filter. A total of

5 kg of soil was mixed with 50 g of cow manure and 1.5 L of ground water in a 6 L black plastic pot. The soil then was saturated with ground water and left for a week before rice cv. *Ciherang* transplanting.

The liquid inoculant was prepared by pouring 1% of Azotobacer mother culture into 200 ml of sterilized free-N Ashby's mannitol broth and incubated for 72 hours at room temperature on the shaker at a speed of 115 rpm. At the end of incubation, liquid inoculant contained 10^8 colony forming unit (CFU)/ml of Azotobacter counted by hemocytometer. Azotobacter liquid inoculant was diluted by ground water to get final bacterial population of 10^7 CFU/ml. A total of 20 ml of said inoculant was mixed with soil at the same time of soil saturation, 7 days before planting.

A single 14-day old rice seedlings were planted in each pot on 7 days after soil saturation and placed in the green house for three weeks with 20 cm distance between pots. A 50% of recommded dose of chemical fertilizers were applied at the planting time. Fertilizer doses recommended by Indonesia Rice Research Institute for Sumedang area are 300 kg/ha of urea, 125 kg/ha of SP-36 and 75 kg/ha of KCl.

Plant height, number of stems (tillers) and number of expanded leaves per pot were observed at 7, 14 and 21 days after planting. Electrical conductivity and soil acidity has been measured at the end of experiment. Soil samples for EC and pH analysis were taken up from the soil near the roots. All data were subjected to analysis of variance (F-test; $p<0.05$), if the effect of experimental treatment were significant, then Duncan Multiple Range Test ($p<0.05$) were performed.

RESULTS AND DISCUSSION

The results showed that plant growth and performance was very poor due to salinity (Fig 1). Decreased of plant growth was evidence irrespective of Azotobacter treatments. Control plant that received no inoculation also suffered from salinity stress because of high EC of soil. All rice transplant was stunt and has yellow leaves that was not fully expanded. The severe toxicity was shown even at 3 days after planting (Fig 1).

Figure 1. Retarded rice growth with unexpanded leaves in saline soil at 3 days after planting

Before the trial, the EC and pH of soil were 8.6 and 6.75 respectively. In 3 weeks later soil in pot became more saline and alkaline, indicated by the increase of EC and pH. At the end of experiment EC and pH in soil were around Nonetheless, statistical analysis showed that Azotobacter. The increase in EC was up to 10.29 (Table 1).

Tabel 1. Effect of Azotobacter inoculation on soil acidity and electrical conductivity in saline soil after 3-week rice estabilishment

Azotobacter inoculation	Electrical conductivity (dS/m)	Soil Acidity
A: Control	9.37	8.26
B: isolate S1; 0,1%	10.38	8.42
C: isolate S1; 0,5%	9.25	8.50
D: isolate S2; 0,1%	8.94	8.47
E: isolate S2; 0,5%	10.02	8.30
F: isolate S1 and S2; 0,1%	10.29	8.30
G: isolate S1 and S2; 0,5%	8.02	8.45

The mean square of treatment on growth traits at 1st, 2nd and 3rd week after planting was not significant. Azotobacter inoculation didn’t change plant height, number of leave at any week observation (Fig 1). We observe no fully expanded leave in this pot trial. Either plant height or number of leaves were lower than that those of rice grown in non-saline soil. Plant heigt at 30 days in experimental field was around 40 cm (Donggulo et al., 2017).

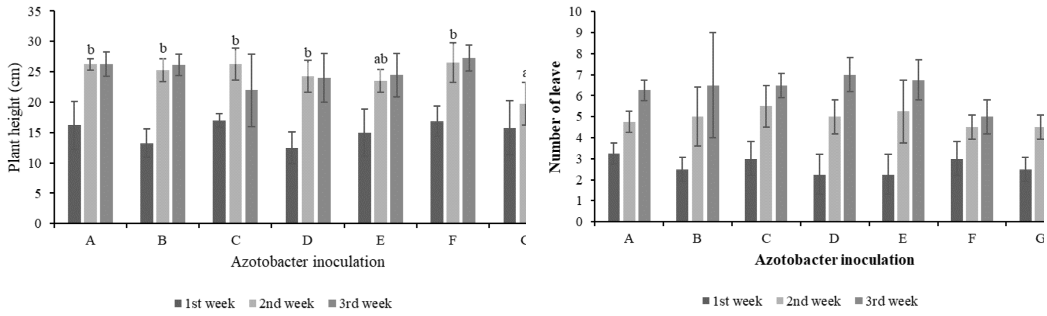

Fig 2. Effect of isolates and concentration of Azotobacter liquid inoculant on plant height and number of leave of rice grown in saline soil at 3 weeks after transplanting. A: control, B: isolate S1, 0.1%; C: isolate S1, 0.5%; D: isolate S2, 0.1%; E: isolate S2, 0.1%; F: isolates S1 and S2; 0.1%; G: isolates S1 and S2; 0.5%.

The result found that Azotobacter inoculation to rice in saline soil didn't affect number of stem (tiller) of individual pot (Fig 2). The increase of tiller in each treatment plants from 1st to 3rd week was evidence but they are thin and weak. The number of tiller at 3rd week was 6.3 in average. Rice grown in normal soil had around 8 tiller at 15 days after planting that increased to around 27 at three weeks after (Jalil et al., 2015).

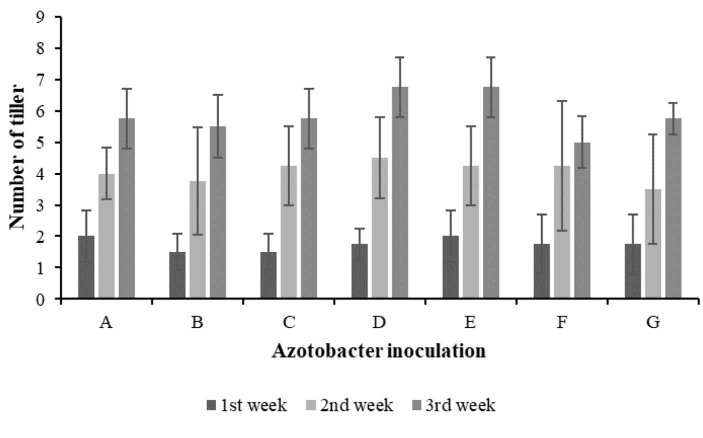

Fig 3. Effect of isolates and concentration of Azotobacter liquid inoculant on number of expanded leaves of rice grown in saline soil at 3 weeks after transplanting. A: control, B: isolate S1, 0.1%; C: isolate S1, 0.5%; D: isolate S2, 0.1%; E: isolate S2, 0.1%; F: isolates S1 and S2; 0.1%; G: isolates S1 and S2; 0.5%.

Higher EC after experiment in all pots was a results of transpiration by which salt accumulation was occured surrounding the roots. The increase in EC was also influenced by low organic matter (Carmo et al., 2006) since the soil contain only low organic carbon (0.65%). Application of 50 g cow manure to 5 kg soil was too low to reduce soil EC and maintain Azotobacter proliferation and their enzymatic activities. In all treatments, increased in pH might be caused by the degradation of organic matter by heterotrophic that use organic matter as a carbon source. Since decades, decomposition of organic matter was verified to increase OH^- concentration and hence the pH (Hopkins et al., 1990).

Decrease of early vegetative growth in saline soil of this pot experiment was clear since rice is a grain crops sensitive to saline soil mainly in the early stage of growth (Makihara et al., 1999; Reddy et al., 2017). Our results was in line with the retarded growth of rice irrigated by floodwater salinity of 2-8 dS/m at germination or 2 weeks after planting (Asch and Wopereis, 2001). The process of photosynthesis is disrupted due to the accumulation of salt in mesophyll tissue and increased CO_2 concentration between cells which can reduce the opening of stomata (Silva et al, 2008). High salt solubility inhibit the absorption of water and nutrients, resulting in low uptake of N (Hoorn et al., 2001).

External input of N should be added to saline soil. Nonetheless the experiment showed that replacing some N fertilizer with Azotobacter inoculation in any isolates and rates were not yet effective to imporove plant performace because of too high salinity of soil decrease the said bacteria involvement in N fixation. High salinity revealed adverse effect on N transformation. Population of diazotrophic bacteria and their N fixing capacity were gradually decline with the increase of sodium chloride in the culture media (Barua et al., 2011).

Salinity stress affected the enzymatic available N production in the soil through N fixation and organic N mineralization (Hoorn et al., 2001). Adverse effect if salinity stress leaving ammonium accumulation that cannot be uptake by roots, and losing ammonia due to volatilization from 14 days to 42 days after salinity stress (Akhtar et al., 2012). Physiological characteristics of microbes also change under saline conditions. Bacteria osmoregulation by synthesizing osmolytes such as glutamine, proline and glycine betaine that accumulate Na^+, K^+ and Mg^{2+} was recorded to protect plant from saline severity (Zahran, 1997; Hmidi et al., 2018).

The results confirmed that high soil salinity lead to growth suppression of rice cv. *Ciherang*, the better growth of rice might be achieved when salt-tolerant rice variety grown in said soil. Both Azotobacter isolates were isolated from paddy field with EC around 8 dS/cm but they didn't show plant growth promoted effect on rice.

CONCLUSION

The research conclude that rice cv. *Ciherang* was failed to perform normal growth in saline stressed soil in the pot trail. Retarded growth of rice also demonstrated during 3 weeks of crop establishment. The EC and pH in soil were increased at the end of experiment but all Azotobacter treatment has not influenced on both traits. We found that the soil become more saline compared with the soil before experiment. Neither isolates nor population of Azotobacter in the liquid inoculants increased plant height, number of tillers and number of expanded leaves of 3-week old rice grown in saline soil compared to the control. The experiment suggested that the activity of saline–resistance Azotobacter as well as plant metabolisms was strongly restricted by salinity stressed soil.

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