B -27

THE ROLE OF MYCHORHIZAE AND RHIZOBIUMTO INCREASE PLANT TOLERANCE GROWN ON SALINE SOIL

Yuni Sri Rahayu

Biology Department - *The Surabaya State of University – Surabaya*

Abstract

Saline soil has a high salt content with pH between 7,3 – 8,5. These conditions contribute decreased of plant growth, yield quality and metabolic disorders related to salt (Na and/or Cl) toxicity. The series of experimental studies have been conducted to describe the effect of mychorhizae and rhizobium to increase plant tolerance based on the percentage of mychorhizal infection and nodule infection, the absorbtion of Phosphate, Natrium, Clorin, also the plant growth using the soybean as a model plant. Data were analyzed statistically. The *Glomus etunicatum* and *Glomus facsiculatum* had an optimal effect on the plant growth, the absorption of Phosphate and the percentage of mychorhizal infection. When the both of mychorhizae were applied together with Rhizobium, affected positively and significantly on the plant growth, percentage of mychorhizal infection, the N and P absorption. These results revealed that the mychorizae and rhizobium affected positively and significantly on plant tolerance in saline soil. As a consequence, the multi simbiotic soil microorganism can be used as an alternative to increase the plant tolerance grown on high saline soil.

Key words: Mychorizae, Rhizobium, plant tolerance, saline soil

INTRODUCTION

The increased agricultural production in Indonesia is still possible by agricultural extension that utilize marginal land into productive land, such as coastal areas in marine. The coastal areas in East Java, for example, has an area nearly twice the land area or reaches 75.700 km^2 (Salahuddin and Mulyana, 2006). However, saline soils contain high levels of salt, with a pH greater than 7.0. These constraints posedonthe plants due toa highsalt content in soil which inhibit root growth, including cell division or cell elongation. In addition, the availability of essential nutrients is very low. Nutrient deficiency is caused by inhibition of the root uptake due to root growth inhibition. Such conditions will obviously interfere the plant growth (Bernstein, 1975; Marschner, 1994).

Salinityis closely related to the water needs by the plant due to the increasing of viscosity which stimulate the entry of large amounts of NaCl causing cellular stress of Na^+ and Cl^- ions accumulation. NaCl accumulation in the soil result in increased ion Ca^{2+} and K^+ which causes the need for essential nutrient for plants such as phosphorus (P) is inhibited (Maathuis, 2001).

Estan *et al*., (2005) showed that the excessive availability of salts, such as sodium chloride (NaCl), sodium carbonate (NaCO3), sodium sulfate (Na2SO4) or salts of magnesium can interfere the plant growth, than can be controlled by plant it self by three mechanisms. First, remove out the salt directly from the root,generally occurs in the mangrove. Second, to develop a tissue to storage water due to reduce the high osmotic pressure. Third, to a bort the plant organs that contain lots of salt.

Mycorrhiza is a mutualistic symbiotic between the plant root witha particular group of obligate soil fungi (Mosse, 1981; Rao, 1994), because the host plants provide nutrients (photosynthesis result) to the fungus, otherwise the host plants get the nutrient from the fungi (Bethlenvalvay, 1992; Suhardi, 1994). Mycorrhizal associate with nearly all plants, including Leguminosae.

Mycorrhizae can increase the absorption of nutrients, especially Pin plants with magniloid roots (cassava, oranges and onions) or the development of disturbed plant roots (due to soil pH, drought stress) (Sieverding, 1991; Kabirun, 2001). Increased absorption of the other nutrients are also reported as N (Marschner and Dell, 1994), K, Mg (Sieverding, 1991), Zn Kothari et al., 1991; Mosse, 1981) and Cu (Li *et al*., 1991, Mosse, 1981). Mycorrhizal are reported to cope with a variety of conditions such as drought stress (Huang *et al*., 1985), the accumulation of heavy metals in the soil such as Cd, Zn, Pb (Duech *et al*. 1986; Heego *et al*., 1990; Mosse, 1984 ;Guo *et al*., 1996; Rogersand Williams, 1986; Cuenca *et al*., 2001). Including to overcome the stress of high soil salinity (Sylvia and Williams, 1992; Pond *etal*., 1984) and acidic or alkaline pH (Sieverding, 1991; Porter *etal*., 1987).

Based on the ability of mycorrhizal fungi to overcome unfavorable environmental conditions (stress), including the ability to increase the absorption of both macro and micronutrientsto theplant, thesemycorrhizalfungi can be used as an alternativetoincrease the resistance of plants grown in saline soils. Infected mycorrhizal roots are able to reduce, and even inhibit the absorption of excessive salt. In addition, it is able to increase the uptake of Ca^{2+} ions that serve as a signal to regulate proline synthesis, which has a function as a trigger for the growth and metabolism of plants. Moreover this plant responses to environmental changes when stress conditions, where high osmotic pressure due to the stress of high NaCl concentration (Tse and Chia, 1999).

Legume is a plant that is capable to obtain N_2 fixation from the air using the root nodule bacteria. Nodule has ared pigment called leghemoglobin which regulates the absorption and reduction of nitrogen, electron carriers specifically on N fixation, oxygen regulator and oxygen carrier (Rao, 1994). Insaline conditions, many species of bacteria can adapt to the intracellular accumulation of low molecular weight organic solution called osmolit (Csonka and Hansonin Zahran, 1999). Osmolit accumulation depends on the level of osmotic stress, growth phase, carbon source, and the percentage of osmolit in the growth medium. Rhizobium can accumulate osmolit such as glycine,betaine, exotin, trehalose and others (Zahran, 1999).

In the symbiosis between legumes, root nodule bacteria and mycorrhizal fungi (tripartite symbiosis), each component symbionts have different roles. Plants played a role in to provide the photosynthate to both Rhizobium and mycorrhizal fungi. Rhizobium provides N to plants throughN_2 fixation and mycorrhizal fungi provides Ptoplants and Rhizobium. The pattern of these interactions provide an information the mechanism of the symbiotic relationship in legume plants so that the plantcan survive on environmental conditions. This study will examine the effect of mycorrhizal and Rhizobium on improving plant tolerance on high saline soil.

RESEARCH METHOD

This research was conducted in the Biology Department green house Unesa. The study consisted of two series of studies, both using a complete randomized block design. The first study used the types of mycorrhizae as a manipulated variable with 5 replications. The response variables were mychorhizal infection, root nodule, P concentration and plant biomass. The second study used concentration mycorrhizae as a manipulated variable with 5 replications. The response variables were mychorhizal infection, N concentration, P concentration, and plant biomass.

The plant media used the soil consist of combination of regosol soil, sand and compost with a ratio of 2 : 1 : 1. It was sterilized by 200 ml of 2 % formaldehyde for each pot (5 kg) for 5 days. The basic fertilizer was applied in 40 mg for urea, TSP and KCl respectively. 300 mL concentrate of salt water (NaCl solution) each pot was every morning to prevent the occurrence of drought, especially when in the dry season. Furthermore, mycorrhizal spore was put at planting area, by making the hole as a deep of 3 cm in a each pot. Mycorrhizal spore then was placed in the planting hole based on the treatments (20 grams of inoculum per polybag for the first study and varies in the second study). Soybean seed planted in these prepared pots.

Data were obtained namely the percentage (%) of mycorrhizal infection in plant roots (Kormanik and Mc-Graw, 1982), the P concentration (AAS), N concentration (spectrophotometer) and plant biomass. Data were analyzed using ANOVA followed by a LSD test.

RESULT AND DISCUSSION

The effect of different types of mycorrhizal (*G.etunicatum*, *G.fasciculatum*and both of *G.etunicatum* and *G.fasciculatum*) on the percentage of mycorrhizal infection, the number of root nodules, P uptake and plant growth can be seen in Table1.

Table 1. The Effect of Mycorrhizae Type on Mychorhizal Infection, Root Nodule, P concentration and Plant Biomass

Treatments	Mychorhizal Infection (%)	Root Nodule (%)	P concentration (%)	Plant Biomass (g)
G. etunicatum	66.9 $\pm$ 5.30 ^{a}	43.9 $\pm$ 2.82 ^{a}	0.20 $\pm$ 0.07 ^{a}	5.4 $\pm$ 2.6 ^{a}
G. fasciculatum	72.5 $\pm$ 8.02 ^{a}	45.6 $\pm$ 2.99 ^{a}	0.25 $\pm$ 0.05 ^{ab}	6.0 $\pm$ 1.6 ^{a}
G. etunicatum & G. fasciculatum	81.3 $\pm$ 8.35 ^{b}	41.4 $\pm$ 2.71 ^{a}	0.29 $\pm$ 0.05 ^{b}	10.4 $\pm$ 4.6 ^{b}

Note:Number followedbythe same letterin acolumnare notsignificantly differentatthe 0.05levelaccording toLSDtest.

Table 1 shows that the type of mycorrhizal affect on mycorrhizal infection, P uptake and biomass plants, but did not affect significantly the formation of root nodules. This suggests that the mycorrhizae can adapt well to its environment. Mycorrhizal infection is essential for the plant, which means that the formation of the internal and external hyphae and mycorrhizal vesicles in plant root cells. Theis hyphae is able to increase the capacity of absorption of various nutrients such as that previously reported in terms of increased N uptake (Marschner and Dell, 1994), K, Mg (Sieverding , 1991), Zn (Kothari *et al*., 1991; Mosse, 1981) and Cu (Li *et al*., 1991, Mosse, 1981). In the infected root system, mycorrhizal hyphae would appear in the outside and inside the plant root especially the root cortex cells, including hyphae that spread around the root zone which ultimately serves as a tool of nutrients absorbver. These hyphae can help expand the area of nutrient uptake by plant roots, the higher the level of mycorrhizal infection that occurs in the roots, allowing external network formed hyphae getting longer and make the roots absorb phosphorus faster and more (Hardiatmi, 2008).

In saline soils, P content is very low that it will stimulate the process of mycorrhizal infection in plants faster, than the P content in the plant can be improved due to the ability of the plant to increase the production of phosphatase enzymes and organic acids that increase the P plant availability (Harrison , 1999; Mosse, 1981; Mosse, 1984; Sieverding, 1991; Kabirun, 2001). Mycorrhizae can increase the production of auxin in plant. Auxin delay the root aging so the roots as absorbing nutrients and water will last longer. In addition, mycorrhizal external mycelium can help the formation of soil aggregates better (Siradz and Kabirun, 2003). The high concentration of P in plants is supported by the availability of the auxin. It make grown on saline soils can be improved, even though the concentration of N in plants has not been significantly affected by the formation of root nodules.

Table1 also shows that the mixture of *G.fasciculatum* and *G.etunicatum* able to have the greatest influence on mycorrhizal infection, P uptake and plant biomass compared the effect of *G. fasciculatum* or *G.etunicatum*s olely. It is indicating that the mixture inoculum is more effective than the single inoculum.

Because the mycorrhiza ltypes have no effect on the formation of root nodules, then further research was conducted by manipulating the concentration of mycorrhizal (the mixture of *G. fasciculatum* and *G.etunicatum*, namely 10 g, 20 g and 30 g). In addition, the media also was added by Rhizobiumin the hope of initiating the formation of root nodules that result in increased uptake of N in the plant. Effect of mycorrhizal and Rhizobium on various parameters following such as Mychorhizal Infection, N concentration, P concentration, and plant biomass can be observed in Table2.

Tabel 2. The Effect of Mychorrhiza and Rhizobium on Mychorhizal Infection, N concentration, P concentration, and Plant Biomass

Treatments	Mychorhizal Infection (%)	N concentration (%)	P concentration (%)	Plant Biomass (g)
Rhizobium +10 g G. etunicatum & G. fasciculatum	52 ± 1.17 [a]	2.08 ± 0.15 [a]	0.08 ± 0.01 [a]	1.91 ± 1.39 [a]
Rhizobium +20 g G. etunicatum & G. fasciculatum	74 ± 8.97 [b]	2.28 ± 0.20 [ab]	0.10 ± 0.01 [a]	4.68 ± 2.30 [b]
Rhizobium +30 g G. etunicatum & G. fasciculatum	85 ± 6.48 [c]	2.60 ± 0.14 [b]	0.17 ± 0.03 [b]	8.16 ± 2.92 [c]

Note: Number followedbythe same letterin acolumnare notsignificantly differentatthe 0.05levelaccording toLSDtest.

Table 2 shows that thedifferent concentrations of mycorrhizae in medium affect significantly on mychorhiza linfection, N concentration, P concentration and plant biomass. The best treatment based on the four parameters used are shown in Rhizobium treatment with the addition of 30 g mycorrhizal concentration. In addition, the addition of *Rhizobium sp*. in media reveals that Mycorrhizae can adapt to saline soil conditions. This adaptability is the beginning of the success of the plant to survive in the environmental conditions. This is consistent with the statement of Lozano *etal* (*in* Subiksa, 2002) that mycorrhizal *Glomus spp* types. Are able to live and survive in saline conditions because this mycorrhizae is able to increase the P levels of P to offset the ion salinity, in this case NaCl. The P high availability in the plant is able to improve the N_2 fixationso that the N concentration in the plant is also high. It is known that N_2 fixation requires more energy supply, including for root nodules formation. 16 ATP is required for each N_2 fixation (Luttge, 1997).

The increase in P uptake due to the availability of P have a positive impact on plant growth. P plays a role in the structural constituent of DNA/RNA, plays a role in the cell metabolic reactions and used in the inhibition process of Na and Cl in excess in saline soils, as well asN_2 fixation process that it can improve plant growth. While N plays a role in the building blocks of protein and aminoacids as well as structural constituent molecules such as DNA/RNA. The high concentrations of P and N support plant growth andplant resistance to high saline conditions (Hopkins, 1999; Rao, 1994).

According Sipayung (2003), the mechanism ofplant tolerance to salt adaptation can be seen from the morphological and physiological adaptations. In morphological adaptation, salinity causes structural changes that improve plant water balance, so that the water potential in the plant can maintain turgor and the entire biochemical process for normal growth and activity. Structural changes include a smaller leaf size, smaller stomata, the thickening of the cuticle and wax layer on the surface of the leaf, and root lignification earlier. Smaller leaf size important to maintain turgor. The root lignification is needed for osmosis adjustment which is very important to maintain turgor required for normal plant growth and plant activity.

The form of adaptation using the physiological mechanisms, namely: (1) salt stress induces the accumulation of specific organic compounds in the cell cytosol that can works as osmoregulator. These organic compounds can lower the cell osmotic potential and increase the turgor pressure that is necessary for plant growth. These organic compounds can also protect enzymes against inhibition or deactivation at low water potential (Sipayung, 2003); (2) Plants can also prevent the accumulation of Na and Cl in the cytoplasm through the exclusion of Na and Cl to the external environment (growing medium) (Marschner, 1995); (3) The compartementation into the vacuole or Na and Cl translocation into other tissues. Some plants develop a structure called the salt glands in leaves and stems. The toxic ions are accumulated in these vesicles to osmotic adjustment purposes without inhibiting the metabolism, so the plant cells become more tolerant on the larger amount of salt (Sipayung, 2003); (4) Integration of cell membrane. The system semi- permeable membrane that encloses the cell, organelle, and structural compartments are the most important to regulate ion cell levels. The outermost layer of the cell membrane or cytoplasm separates plasmalema and metabolic components of saline soil solution which is chemically not fit. Semipermeable membrane hinders the free diffusion of salt into plant cells , while giving the opportunity for active absorption of essential nutrients (Harjadi and Yahya *in* Sipayung, 2003).

CONCLUSION AND SUGGESTION

The *G. etunicatum* and *G. facsiculatum* had an optimal effect on the plant growth, the absorption of Phosphate and the percentage of mychorhizal infection. When the both of mychorhizae were applied together with Rhizobium, affected positively and significantly on the plant growth, percentage of mychorhizal infection, the N and P absorption. These results revealed that the mychorizae and rhizobium affected positively and significantly on plant tolerance in saline soil. As a consequence, the multisimbiotic soil microorganism can be used as an alternative to increase the plant tolerance grown on high saline soil

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