Increasing plant tolerance grown on saline soil: The role of tripartite symbiosis

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Annals of Biology **36** (2) : 346-353, 2020

Increasing Plant Tolerance Grown on Saline Soil : The Role of Tripartite Symbiosis

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ABSTRACT

One abiotic barrier that can inhibit the growth of soybean plants is salt stress. This study was conducted to describe the role of tripartite symbiosis between *Glosum mosseae* and *Rhizobium japonicum* on plant tolerance grown under salt stress conditions using soybean as a plant tested. There were two conditions of salinity (0 mM NaCl and 200 mM NaCl) with four treatments, namely, 1. non-inoculant plants, 2. plants inoculated with rhizobium, 3. plants inoculated with mycorrhizae and 4. plants inoculated with rhizobium and mycorrhizae. The results showed that plant tolerance to salt stress increased in inoculation with mycorrhizae and rhizobium compared to non-inoculant plants. The tolerance increased along with the increase in the absorption of P and N which had an impact on increasing plant biomass. The tripartite symbiotic between soybeans, *G. mosseae* and *R. japonicum* revealed positive effects on the parameters of mycorrhizal colonization, root nodules, plant biomass, microbial dependency, stress tolerance index and nutrient uptake compared to uninoculated plants or inoculated plants with *Glomus mosseae* or inoculated plants with *R. japonicum* only. It reflected that tripartite symbiosis was more effective than single inoculation in salt stress conditions.

Key words : Plant tolerance, tripartite symbiosis, saline soil

INTRODUCTION

Indonesia's population is increasing every year. Based on data from the central statistics agency, Indonesia's population reaches 265 million with a population growth rate of 1.3% (Badan Pusat Statistika, 2018). In addition, Indonesia is the fourth country with the largest population in the world (Sulaiman *et al.,* 2019). Along with the increase in population, food security is one of the concerns of the government. One of the efforts to increase food production is by utilizing wetland such as coastal land as agricultural land because Indonesia is the country with the second largest coastal area after Canada with the coastal length of 99,093 km (Hazazi *et al.,* 2019). With the use of coastal land as agricultural land, it can become an alternative to increase food production in Indonesia. However, in the utilization of coastal land, it is found that there are obstacles in the salinity level where coastal lands have high salinity that classified as saline soil (Hairmansis *et al.,* 2017).

Saline soils are soils with high salinity levels. In agricultural lands, high salinity has a negative effect on plant growth and crop yields.

Salinity also has an impact on urban structures due to subsidence, corrosion and ground water quality, leading to further soil erosion and land degradation (Abuelgasim and Ammad, 2019). Under high salinity conditions, salt accumulates in the soil and causes a decrease in plant production (Asfawa *et al.,* 2018). High salinity in soils can cause disturbance to plant growth. During salt stress conditions, soluble salts are accumulated in the root zone of plants that causes osmotic and ionic imbalance. Plant growth highly reduces by soil salinity through three different mechanisms like nutrient ion imbalance which reduces K^+, PO_4^-, and NO_3^- uptake with high concentration of sodium chloride and osmotic stress which inhibits water availability (Shehzad *et al.* 2019). Other effects of salinity on plants reduced the net of photosynthetic rate, stomatal conductance and relative water content. Sun *et al.* (2019) reported that salt stress had significant effect on plant growth. In salt stress, plant height and leaf number decreased significantly. Besides chlorophyll content of leaves also decreases in salt stress conditions. This can be caused by the disturbance of nutrient

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absorption due to hypertonic soil conditions. To be able to use saline soil as a planting medium, it is necessary to attempt to reduce levels of salt and reduce the negative effects arising from salt stress. One effort that can be done is to use the symbiosis of mycorrhizae (Bhattacharyya *et al.,* 2018).

Arbuscular Mycorrhizal Fungi (AMF) have a positive effect on plant growth under high salinity. AMF inoculation increases the gas exchange performances in plant under salt stress (Chandrasekaran *et al.,* 2019). AMF can mitigate salt stress in host plants. Under salt stress conditions, AMF can significantly increase plant photosynthesis rate and increase K^+ ion levels. Plants that are symbiotic with AMF have a relatively higher water content compared to plants that are not symbiotic with AMF. Wang *et al.* (2019) reported that mycorrhizae had a positive effect on plant biomass and mineral absorption. Symbiotic plants with AMF have increased plant biomass and nutrient uptake including an increase in the K^+/Na^+ ratio compared to non-AMF plants. AMF establish symbiotic interaction with many of higher plants including soybean (Bahadur *et al.,* 2019).

Soybean (*Glycine max* L.) is one of the plants commonly grown in agriculture considering that soybeans have a high protein content. Soybean is the third highest national food needs, after paddy and maize (Adriansyah *et al.,* 2020). Salt resistance in soybeans is commonly characterized by rejection of chloride particles from foliar tissues (Nisar *et al.,* 2019). The inhibitors of soybean were osmotic potential and matric potential, that were decreased due to salt stress and water shortage (Khan *et al.,* 2016). Soybean is a plant that is classified in legume plants. Legume plants are able to have a mutualistic symbiosis with rhizobium and mycorrhizae groups. Takacs *et al.* (2018) conducted research to compare the effectiveness of dual symbiosis with tripartite symbiosis. Based on these studies, the results obtained that tripartite symbiosis was more effective than dual symbiosis where symbiosis between soybeans, rhizobium and AMF increased plant production, photosynthesis rate and root activity. This study was aimed at describing the role of tripartite symbiosis between *Glosum mosseae* and *Rhizobium japonicum* on plant tolerance grown under salt stress conditions using soybean as a plant tested. In addition, the influence of saline stress on shift of positive-negative microbial dependence will be performed to illustrate the microbial functions using the microbial dependency and saline resistance index.

MATERIALS AND METHODS

The ultisol soil was collected from the biology department greenhouse of Universitas Negeri Surabaya. The soil was sterilized using the 200 ml 2% formaldehyde each polybag, for five days before the application of basic fertilizer (for each 5 kg soil in polybag with 20 mg urea, 20 mg TSP and 20 mg KCl). Then, watering the media until the soil became moist (300 ml water). Watering with a concentration of 0 mM NaCl or 200 mM NaCl was carried out every morning to prevent drought, especially if the soil conditions dried quickly in the dry season.

Microbial inoculation AM fungus *G. mosseae* and *R. japonicum* were provided by SEAMEO BIOTROP Bogor-Indonesia collection. Mycorrhizal inoculum consisting of spores (50 spores per gram), hyphae and infected root fragments were applied at a 200 g inoculum to 5 kg soil as growth medium. The inoculum was added to the top 5 cm of the growth medium (i. e. the mycorrhizal inoculum layer) at sowing time just below the seeds for mycorrhizal treatments. Non-inoculated treatment received the same amount of sterilized inoculum to provide a similar microbial population that was free from AM propagules. The strain of *R. japonicum* was added close to tap roots (10 ml containing 121 cells per pot) one week after sowing to establish the bacterial treatment. Non-inoculated treatment received 10 ml autoclaved (121°C, 45 min) microbial suspension.

Seeds of *Glycine max* L. were provided by Department of Agriculture, East Java, Indonesia. The seeds were immersed in H_2O_2 (5%) for 10 min to sterilize the seed surface. The selected seeds were germinated on filter paper soaked with distilled water in Petri dishes at 27°C in the dark for five days in a growth chamber.

Before saline treatment, all the pots were maintained under well-watered conditions. At 14 weeks after sowing, the saline treatment was unified for two groups, namely, control (0 mM NaCl = deionized water) and saline stress (200 mM NaCl). The deionized water or saline solution was daily supplemented to maintain the desired moisture content. The plants were harvested after eight weeks. All the plants were in vegetative growth during the whole experimental period. The experiment was set up in a three-factor randomized complete block design. Experimental treatments included the following : (1) non-AM fungus/rhizobium inoculation as control; (2) inoculated with AM fungus; (3) inoculated with rhizobium and (4) co-inoculated with AM fungus and rhizobium. All the treatments were cultivated under normal and saline stress conditions. There were eight treatments (full combinations of inoculation status and saline conditions) with four replicates, giving a total of 32 pots. The experiment was carried out under greenhouse conditions (day min./max. temperatures of 28/34°C; max light intensity of approximately 900 lux).

Roots and shoots were harvested eight weeks after sowing to determine the root colonization by AM fungus, root nodulation, to measure the plant biomass, and to analyze the plant nutrient uptake (P and N concentrations and contents). Parameter measurements from plant roots and shoots were separately harvested. Roots were carefully washed with deionized water, and the nodules were separated. Fresh weight of shoots, roots and total nodules was recorded. The number of nodules was estimated by directly counting. Sub-samples of fresh roots (0.5 g) were stored in a 4°C refrigerator for measuring mycorrhizal colonization. The rest of the roots, shoots, and nodules were dried at 80°C for 48 h for dry biomass and nutrient content analyses. Plant nutrient contents refer to total P or N contained within the shoot or the root. Spectrophotometer was used to measure P concentrations. Percentage of root length colonized by AM fungi was determined on roots stained in tryphan blue using the gridline-intersect method (Koske and Gemma, 1989).

The microbial dependency of inoculation treatment was calculated according to Van der Heijden (2003). If biomass of $\Sigma^n{}_1 a_n$ > bn, then microbial dependency = $(1 - (bn/\Sigma^n{}_i\, a_n) \times 100$. If biomass of $\Sigma^n{}_1 a_n$ > bn, then microbial dependency = $(-1 - (bn/\Sigma^n{}_i\, a_n) \times 100$, where 'a' was the plant dry weight of a treatment inoculated with microbe, n is the number of treatments where plants were inoculated with microbe and 'b' was the plant dry mass of the non-inoculated treatments. These equations ensured that positive and negative values for microbial dependency were comparable and symmetrical. Saline stress index as stress tolerance index (STI) was calculated using the following formula : STI= $(Bc \times Bs)/Mc^2$, where Bc and Bs were the plant biomass under control and stress conditions, respectively, and Mc was the mean biomass over all plants under control condition (Clarke *et al.,* 1992).

Data were shown as the mean±standard error (SE) of independent replicates except for microbial dependency and STI, which were calculated from the mean value of the different treatments. The data were checked for normality and homogeneity of variance before performing the analysis of variance (ANOVA) using SPSS (IBM SPSS Statistics). One-way ANOVA followed by Duncan's multiple-range test was ?rst performed with treatment as the main factor. Then, a three-way ANOVA was performed to examine the significance of treatment effects and their interactions on the observed parameters using SPSS.

RESULTS AND DISCUSSION

There were differences in dry weight, root infection by AMF and root nodules in soybean which were at 0 and 200 mM NaCl (Table 1). In addition, differences also appeared in the four treatments applied in this study, namely, control in the form of non-inoculants (non-mycorrhizae and non-rhizobium), inoculation with mycorrhizae i. e. *G. mosseae*, inoculation with *R. japonicum*, and co-inoculation with *Glomus* sp. and *Rhizobium* sp. Table 1 reveals that tripartite symbiosis between soybeans with mycorrhizae and rhizobium had a dry weight, root infection by AMF, and root nodules that were higher than the symbiosis between plants with rhizobium, and with mycorrhizae and were clearly much higher compared to plants non-inoculant both in soil with 0 mM NaCl or 200 mM NaCl.

Shoot and root dry weight of soybean (*Glycine max* L.) decreased in media with a salinity of 200 mM NaCl (Fig. 1). In general, inoculation of *G. mosseae* and *R. japonicum* triggered the improvement in dry weight of soybean plants (shoot and root dry weight) compared to non-inoculant. It was revealed in the condition of 0 mM NaCl where the highest dry weight was found in symbiosis with rhizobium and

Table 1. Root colonization by AMF and nodulation of plants root inoculated with/without arbuscular mycorrhizal (AM) fungus *G. mosseae* and *R. japonicum* under different concentrations of salinity conditions (mean±SE, n=4)

Salinity (mM NaCl)	Type of soil organism	Dry weight (g)		Root infection by AMF (%)	Root nodule	
		Shoot	Root		Number	Dry weight (g)
0	NM-NR	2.1+0.3cd	1.5+0.2e	6.1+0.4e	6.1+0.7d	0.02+0.00e
	M	4.8+0.5b	3.1+0.3b	51.8+5.6b	8.9+0.5c	0.05+0.01d
	R	3.1+0.4c	2.2+0.2c	16.2+2.3d	26.5+4.2ab	0.16+0.03b
	M-R	6.2+0.5a	4.9+0.6a	78.4+10.9a	32.1+4.9a	0.25+0.07a
200	NM-NR	1.3+0.3d	1.0+0.2f	1.2+0.5f	2.3+0.4e	0.01+0.00f
	M	3.2+0.5c	2.3+0.4cd	38.5+7.6c	5.2+1.1d	0.03+0.01de
	R	2.8+0.3cd	1.9+0.3d	12.1+2.8d	11.3+2.2c	0.09+0.02c
	M-R	4.6+0.8b	3.6+0.7ab	54.4+9.3b	20.1+3.6b	0.16+0.04b

NM-NR represents non-inoculation control, M represents inoculation with *Glomus mosseae*, R represents inoculation with *Rhizobium japonicum* treatments and M-R represents the co-inoculation treatments. Data of columns indexed by the same letter are not significantly different at P< 0.05.

mycorrhizae (*Glomus* sp.). In saline soil with 200 mM NaCl, the dry weight of soybean decreased but plant biomass treated with rhizobium and mycorrhizal co-inoculation remained higher than non-inoculants.

Based on plant shoot and root dry weight, microbial dependency was calculated to evaluate contribution of mycorrhizal and rhizobium inoculations to plant growth. There had been an increase of microbial dependencies in plants associated with co-inoculation of *R. japonicum* and *G. mosseae* compared with a single inoculation (Fig. 2). Microbial dependencies based on shoot dry weight increased on 200 mM NaCl with inoculation of rhizobium and mychorrizae compared to 0 mM NaCl. But that was different based on the dry weight of the root, where microbial dependencies based on root dry weight were decresed on 200 mM NaCl even with inoculation with rhizobium and mychorizae still had lower microbial dependency than at 0 mM NaCl.

Soybean (*Glycine max* L.) inoculated with *G. mosseae* alone or inoculated with *R. japonicum*

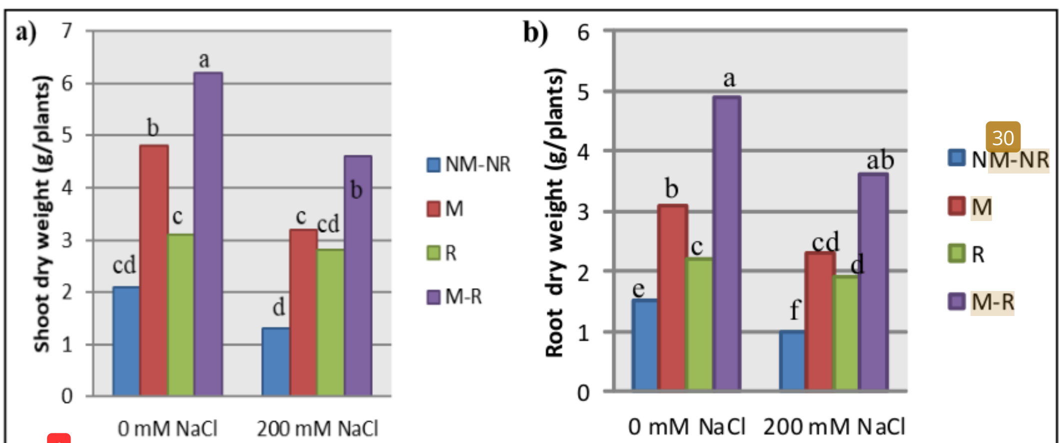

Fig. 1. Shoot (a) and root (b) dry weights of plants inoculated with/without arbuscular mycorrhizal (AM) fungus *G. mosseae* and *R. japonicum* under different concentration of salinity conditions (mean±SE, n=4). NM-NR represents non-inoculation control, M represents inoculation with *G. mosseae*, R represents inoculation with *R. japonicum* treatments and M-R represents the co-inoculation treatments. The same letter above the error bar indicates no significant difference at P<0.05.

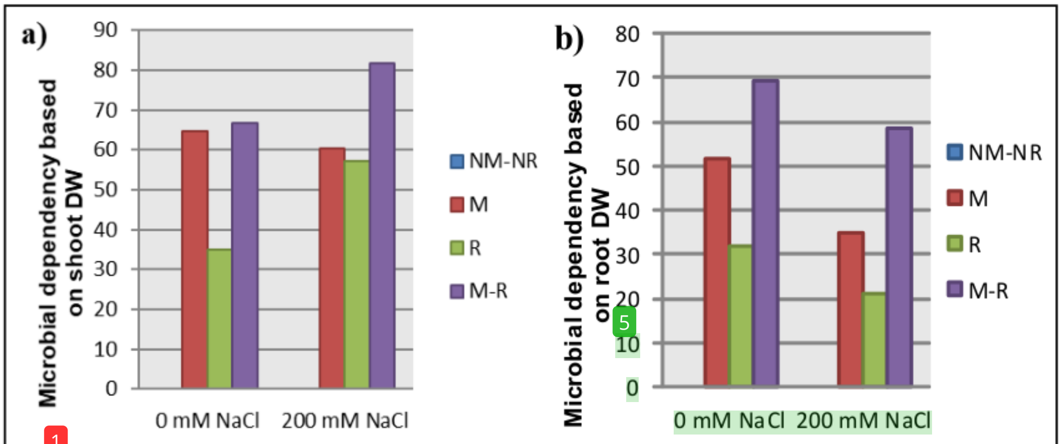

Fig. 2. The microbial dependency calculated according to van der Heijden (2003) based on shoot (a) and root (b) dry weights. NM-NR represents non-inoculation control, M represents inoculation with *G. mosseae*, R represents inoculation with *R. japonicum* treatments and M-R represents the co-inoculation treatments.

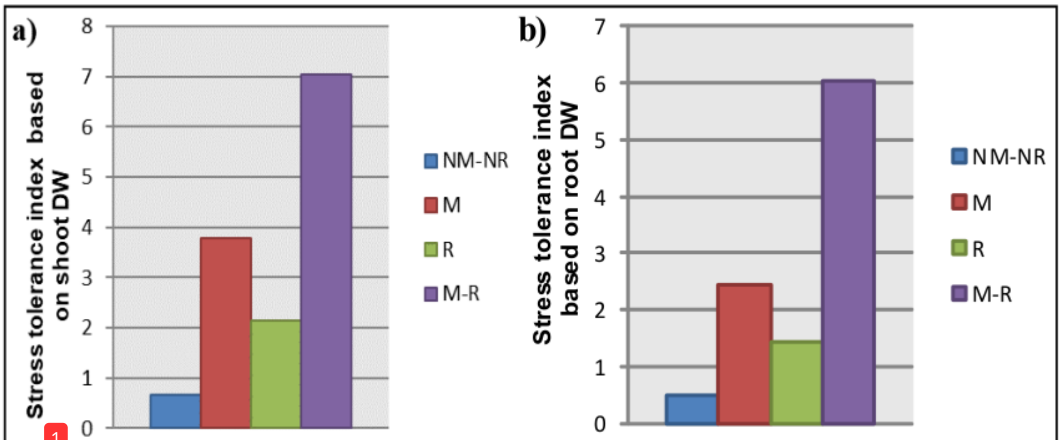

Fig. 3. Stress tolerance index (STI) of plants calculated based on plant shoot (a) and root (b) dry weights. NM-NR represents non-inoculation control, M represents inoculation with *G. mosseae,* R represents inoculation with *R. japonicum* treatments and M-R represents the co-inoculation treatments.

alone had a higher stress tolerance index (STI) compared with non-inoculant (control) (Fig. 3). Plants that were inoculated with *G. mosseae* and *R. japonicum* showed the higher STI compared to other treatment with 11.82 fold STI than non-inoculant. Baik berdasarkan shoot dry weight maupun root dry weight urutan STI dari yang tertinggi sampai terendah yaitu (1) co inoculation with *G. mosseae* and *R. japonicum*, (2) inoculation with *Glomus* sp. (3) inoculation with *R. japonicum* and last (4) non-inoculation (non-*G. mosseae* and non-*R. japonicum*).

There are four main data in the observation of plant nutrient extraction, namely, P concentration, P content, N concentration and N content (Table 2). In general, inoculation with *G. mosseae* and *R. japonicum* had a higher P concentration, P content, N concentration and N content compared with other treatment both in shoot or in the root. P concentration on plant that grown on 200 mM NaCl was higher than 0 mM NaCl with co-inoculation of *G. mosseae* and *R. japonicum* both on shoot and root. While shoot P concent in 200 mM NaCl was almost the same with shoot P content in 0 mM NaCl when plant was inoculated with *G. mosseae* and *R. japonicum* N concentration and

Table 2. Plant nutrient uptake of plants inoculated with/without arbuscular mycorrhizal (AM) fungus *G. mosseae* and *R. japonicum* under different concentration of salinity conditions (mean±SE, n=4)

Salinity (mM NaCl)	Type of soil organism	P concentration (mg/g)		P Content (mg/plant)		N concentration (mg/g)		N content (mg/plant)	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	NM-NR	0.5+0.1d	0.3+0.2e	1.05+0.3e	0.5+0.2e	15.2+1.9c	16.7+1.2c	31.9+3.9e	25.1+3.0e
	M	3.0+0.5b	2.7+0.4c	14.4+0.6b	8.4+0.6c	20.1+2.5b	21.3+3.3b	96.5+4.7c	66.0+9.3c
	R	1.2+0.3c	1.0+0.3d	3.7+0.4c	2.2+0.5d	19.9+2.6b	18.9+1.5b	61.7+5.1d	41.6+4.5d
	M-R	2.8+0.6b	2.7+0.6c	17.4+0.6a	13.2+0.7b	36.4+3.1a	29.1+1.9a	225.7+15.3a	142.6+12.3a
200	NM-NR	0.7+0.3d	0.6+0.2e	0.9+0.3e	0.6+0.2e	14.3+3.5c	11.4+1.4d	18.6+3.8f	11.4+3.1f
	M	3.3+0.4ab	3.9+0.3b	10.6+0.5c	9.0+0.4c	20.9+2.9b	18.1+1.6b	66.9+5.7d	41.6+8.9d
	R	0.9+0.2cd	1.1+0.2d	2.5+0.4d	2.1+0.3d	26.6+3.1b	20.5+2.4b	74.5+10.0d	39.0+7.3d
	M-R	3.8+0.5a	4.7+0.5a	17.5+0.7a	16.9+0.7a	35.1+4.2a	29.3+1.3a	161.5+22.1b	105.5+9.2b

NM-NR represents non-inoculation control, M represents inoculation with *G. mosseae*, R represents inoculation with *R. japonicum* treatments and M-R represents the co-inoculation treatments. Data of columns indexed by the same letter are not significantly different at P<0.05.

N content decreased in salt stress (200 mM NaCl) compared with 0 mM NaCl.

Legume plants such as soybeans can form symbiosis with rhizobium and mycorrhizae. Table 1 indicates that the inoculation with tripartite symbiotic between soybeans, *G. mosseae* and *R. japonicum* showed the results of mycorrhizal colonization, root nodules, plant biomass (dry weight), microbial dependence, stress tolerance and nutrient uptake that were higher compared to plants that were only symbiotic with *G. mosseae* or symbiosis with *R. japonicum* only. These results are in accordance with previous studies which reported that symbiotic tripartite between legume, mycorrhizal and rhizobium plants had a greater impact compared to dual symbiosis i. e. only between plants with *Rhizobium* or plants with mycorrhizae only (Takacs *et al.*, 2018).

In addition, research data also revealed that plant tolerance to salt stress increased in inoculation with mycorrhizae and rhizobium compared to non-inoculants (Table 1, Figs. 1 and 2). The tolerance increased along with the increase in the absorption of nutrients P and N which had an impact on increasing plant biomass (Table 2). Garcia *et al.* (2019) reported that inoculation with rhizobium and AMF increased the absorption of plant nutrients under salt stress conditions because rhizobium and AMF increase the relative water content of the soil so that the osmotic pressure in the soil increased and triggered the absorption of water accompanied by nutrient absorption. Microbial inoculants, such as AMF and rhizobium, had been previously studied as they were ubiquitous in soil and provided improving nutrient uptake by plant and improved the availability of nutrient in the soil under adverse stress environmental conditions (Hack *et al.*, 2019).

This study also performed the obtained data that symbiotic tripartite with mycorhizae and rhizobium had higher root nodulation and root infection by AMF compared to other treatments. This was caused by the symbiosis between mycorhizae and plant roots by causing expansion of the area of absorption of plant nutrients and maintenance of ionic homeostasis wherein this study indicated by an increase in both P and N levels in shoot and root. Increased levels of P by mycorrhiza caused an increase in the activity of the nitrogenase enzyme which resulted in an increase in the rate of nitrogen fixation by rhizobium and caused the development of root nodules and the better development of mycorrhizae (Ren *et al.*, 2016). The stress tolerance index (STI) was performed (Fig. 3). STI is used to determine the level of tolerance of plants to abiotic stress. In this study, plant that inoculated with *G. mosseae* and *R. japonicum* resulted in synergistic effects on plant salts tolerance, thereby indicating that the tripartite symbiosis adapted to a wide range of environmental stresses based on STI.

CONCLUSION

It was concluded that tripartite simbiotic between legume plants, mychorrizae and rhizobium were more effective to increase plant tolerance on salts stress condition compared to non-inoculant and dual symbiotic. The inoculation with tripartite symbiotic between soybeans, *G. mosseae* and *R. japonicum* showed the results of mycorrhizal colonization, root nodules, plant biomass (dry weight), microbial dependence, stress tolerance, and nutrient uptake that were higher compared to plants that were only symbiotic with *G. mosseae* or symbiosis with *R. japonicum*. These results also indicated that for better production system using the agriculture land in saline soil, the interactive effects of AM fungal, rhizobium, and the crop above, as a tripartite symbiotic, should be performed well in detail so that the optimized combinations of microorganisms can be implemented as effective soil inoculants for plant growth promotion.

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