CMA-Salinity-Chia

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EFFECT OF *Glomus manihotis* INOCULATION AND SALT STRESS ON PHYSIOLOGICAL PROPERTIES OF CHIA (*Salvia hispanica* L.)

ABSTRACT

Plant productivity is often constrained by the presence of abiotic stress in the form of high salt levels. However, a symbiosis between plant and arbuscular mycorrhizal fungi can reduce the intensity of the effect of salinity stress on cultivated plants. The aim of this study was to determine the impact of salt stress on the physiology of chia (*Salvia hispanica* L.) plants that had been inoculated with the fungus *Glomus manihotis*. The experimental design used was a factorial randomized block design with seven replications. Inoculation of the fungus *G. manihotis* as the first factor and the concentration of NaCl (0, 50, 100 and 200 mM) as the second factor. Several parameters in chia plant were measured at the end of the observation including: root infection, levels of phosphorus, chlorophyll and carotenoids, several antioxidant enzyme activities (superoxide dismutase and catalase) and malondialdehyde levels. The results showed that high salt levels was able to reduce all measured parameters except malondialdehyde levels. However, inoculation of the fungus *G. manihotis* can reduce the negative impact of NaCl treatment. This study can be concluded that the symbiosis between chia plant and *G. manihotis* is able to decrease the adverse effects of high salinity conditions.

Keywords: antioxidant; chia; mycorrhizae; salt stress

1. INTRODUCTION

Cultivation in high salinity soil disrupts agricultural production (Rivero et al., 2018). The presence of high salinity land is exacerbated by population growth and global warming (Okur & Örçen, 2020). In fact, the ability of plants to survive in high salinity conditions is associated with mechanisms that maintain ion homeostasis and chloroplast function (Li et al., 2020). More specifically, the plant's strategy to tolerate high salt levels is the production of antioxidant enzymes that against to free radicals attack (Cen et al., 2020).

Currently, biological methods are getting greater attention in an effort to reduce stress of high salt levels that can affect agricultural production (Noreen et al., 2021). According to Heydari & Pirzad (2020) more than 90% of the plants that live on the earth's surface have a symbiotic relationship with fungi, either wholly (endophytes) or partially (mycorrhizae) in plant cells or tissues. This symbiosis allows plants to adapt well to biotic and abiotic factors such as salt stress. In addition, the nature of symbiosis is beneficial for the survival of the plant itself and its fungi in the face of stressful habitat conditions (Diao et al., 2021).

Glomus manihotis is a mycorrhizal fungus of the order Glomales and belongs to the arbuscular mycorrhizal type. In this type of mycorrhiza, plants exchange photosynthate with minerals and water taken up by mycorrhizal fungi from the soil (Borde et al., 2017). Furthermore, there is no clear evidence whether the ability of plants to tolerate high salt levels is directly by the plant itself or mediated by the presence of mycorrhizal symbiosis.

The plant's response to various environmental factors such as high salt level is thought to be influenced by these fungal symbionts. Research data (Ait-El-Mokhtar et al., 2020) explained that inoculation with arbuscular mycorrhizal fungi can enhance plant growth and nutrient absorption in high-salt soil conditions and reduce crop loss. In addition, the ability of arbuscular mycorrhizas to damage reactive oxygen species (ROS) is believed to be a mechanism that may increase the effectiveness of host plant defenses (Abdelaziz et al., 2019). However, the mechanism of arbuscular mycorrhiza that increases the resistance of host plants to high salt stress is not yet understood.

Understanding the symbiosis of mycorrhizal fungi and their host plants enables strategies to address disorders caused by abiotic environmental factors, including high salinity stress (Kong et al., 2019). The presence of this fungal symbiosis with host plants that form mycorrhizal structures is believed to be an inexpensive and viable way to reduce the effects of global warming on plants and plant communities. Currently, with the global warming, the land affected by salt is increasing, so it is necessary to understand the mechanism of plants that can withstand this high salt stress immediately, and ultimately it will not affect agricultural productivity. (Malhi et al., 2021).

Chia (*Salvia hispanica* L.) plants are horticultural crops that grow at medium levels that are sensitive to high light intensity (Aguilar-Toalá et al., 2020). The role of mycorrhiza in the mechanism of tolerance of chia plants to high salt stress is not yet well understood (Mlinarić et al., 2020). The hypothesis of this study is that *Glomus manihotis* can increase the tolerance of chia plants grown in high salt medium. On the other hand, the effects of mycorrhiza on antioxidant enzyme activity and lipid peroxidation during salt stress are not yet known. This article describes parameters such as phosphorus content, antioxidant enzyme activity, photosynthetic pigments, and lipid peroxidation that indicate the high salt tolerance of chia plants inoculated with the mycorrhizal fungus *Glomus manihotis*.

2. MATERIAL AND METHODS

2.1. Plant material and mycorrhizal fungi preparation

It In this study, chia seeds were purchased from an agricultural shop in Tanjungsari sub-district, Sumedang district, West Java. Meanwhile, the mycorrhizal culture of *G. manihotis* was propagated in the Integrated Garden of Saintek UIN Bandung. *G. manihotis* inoculum 100 grams (containing more than 1000 spores) was applied to a depth of 3 cm in a 5 kg polybags already contained sterile zeolite medium. Seedlings of 7-day-old chia plants were planted in zeolite medium inoculated with mycorrhiza. A chia plant not inoculated with mycorrhiza was prepared as a control. All chia plants were watered with aquaDM every 2 days and with a half-concentrated nutrient solution of the Hoagland formula every 7 days.

2.2. Experimental design

The experimental design used a randomized block design with a factorial pattern and seven replications. The first factor was inoculation and non-inoculation of *G. manihotis*. The second factor is treatment with several concentrations of NaCl (0, 50, 100, 200 mM). The observation was conducted in a greenhouse, an integrated garden in Saintek UIN Bandung, for three months from September to November 2021. The main experiments were conducted for 45 days with an ambient temperature coefficient of 27/21 ° C (day / night), a relative humidity of 70%, and a light intensity of 6500 lux.

2.3. Infection percentage

Root samples from 45-day-old chia plants (at the end of the experiment) were cleaned in running water to remove the medium used during the study. Furthermore, the roots can be stored in 5% KOH solution, while for observations the roots are soaked using trypan blue for 6 hours. Calculation of root infection by mycorrhizal fungi using the grid-line intersection method observed under a surgical microscope (Parvin et al., 2020). The percentage of infected roots is calculated using the following formula:

% infection = 100 x (number of mycorrhizal roots / total observed roots).....(1)

2.4. Phosphorus estimation

The third leaf from the chia plant shoots tip that aged 45 days was dried at 70 °C for 48 hours. After drying, it is incinerated in a furnace at 550 ° C for 24 hours. Phosphorus concentration was measured using a spectrophotometer at a wavelength of 882 nm (Qiu et al., 2020).

2.5. Chlorophyll & Carotenoid Estimation

Leaf sample chia plants was weighed as much as 1 g for maceration and extracted with 10 ml of 80% acetone. Furthermore, the supernatant was filtered using Whatman filter paper no. 1 and the filtrate was added to 50 ml in a volumetric flask by adding solvent. The solution was measured by spectrophotometer at wavelengths of 440.5, 646 and 663 nm. To obtain the concentrations of chlorophyll and carotenoids, the data obtained were entered into the following formula:

Chlorophyll a = 12,21(A663) – 2,81(A646).....(2)

Chlorophyll b = 20,13(A646) – 5,03(A663).....(3)

Carotenoid = 4,69(A440,5) – 0,268(Chl a + Chl b).....(4)

Chlorophyll concentration is expressed in units of mg/g fresh weight and carotenoid concentration is expressed in units of g/g fresh weight (Kazemi et al., 2019).

2.6. Superoxide dismutase estimation

Superoxide dismutase was measured according to the method explained by Santander et al. (2020). Next, the ready-to-use enzyme sample was mixed with 2.9 ml of 30% hydrogen peroxide. The prepared mixture was measured for absorbance at a wavelength of 240 nm using a UV-Vis spectrophotometer. The level of this enzyme indicates its activity which is expressed in mmol/min/mg protein.

2.7. Catalase estimation

The catalase enzyme was identified using the method showed by (Wang et al., 2020) The ready-to-use enzyme sample was then mixed with 2.9 ml of 30% hydrogen peroxide. The absorbance of the prepared mixture was measured at a wavelength of 240 nm using a UVVis spectrophotometer. The concentration of this enzyme indicates its activity, represented by the mmol / min / mg protein.

2.8. Malondialdehyde estimation

Lipid peroxidation was tested from the formation of malondialdehyde using the thiobarbituric acid method described by (Kabir et al., 2020). The third leaf sample from the shoots tip as much as 0.2 g was homogenized by adding 1 mL of 5% trichloroacetic acid solution. Then the homogenate was centrifuged at 12,000 rpm for 12 min at room temperature. The supernatant was analyses on a spectrophotometer with a wavelength of 532 nm.

2.9. Statistical analysis

The data obtained was analyzed using one-way ANOVA (analysist of variance). If there was a significant difference ($p < 0.05$) between the processes, further testing was identified using Duncan's multi-range test. Data is managed by SPSS software version 20.

3. RESULTS

Chia plants inoculated with the mycorrhizal fungus *G. manihotis* had a higher phosphorus concentration than chia plants that were not mycorrhizal (P<0.05). The salt treatment reduced the phosphorus levels of chia plant leaves, both mycorrhizal and non-mycorrhizal (Figure 1).

Chia plants that were not inoculated with *G. manihotis* did not show any root infection. Root infection was seen in plants inoculated with *G.manihotis* which decreased the percentage of root infection in line with the increase in the saline treatment given. The percentage of root infection of chia plants at 50 mM NaCl concentration was not significantly different from the control (p>0.05) (Figure 2).

Leaf chlorophyll content of chia plants inoculated with *G. manihotis* was higher than that of non-mycorrhizal chia plants (Table). The chlorophyll levels decreased in line with the increase in the salt levels of both the chia plants inoculated with the *G.manihotis* fungus and those without mycorrhizal. Mycorrhizal plants and did not receive salt treatment had the highest chlorophyll levels of 4.70 mg.g-1. Meanwhile, the levels of carotenoids in chia plants inoculated with *G.manihotis* were higher than those in non-mycorrhizal chia plants. Treatment of salt content has reduced levels of carotenoids in chia plant leaves. Chia plants that were mycorrhizal and treated with 50 mM and 0 mM salt levels (control) had the highest carotenoid levels, namely 8.27 and 8.35 $g.g^{-1}$ fresh weight, respectively. (Table).

The activity of the antioxidant enzyme superoxide dismutase in mycorrhizal chia plants was higher than in chia plants without mycorrhizae. Superoxide dismutase activity reached its peak in mycorrhizal chia plants treated with NaCl 100 and 200 mM. The activity of superoxide dismutase among chia plants without mycorrhizae treated with 50 mM NaCl concentration was the same as that of plants not receiving NaCl treatment (control) (Figure 3).

The activity of the catalase enzyme in chia plants with mycorrhizae was higher than in chia plants without mycorrhizae. Increasing NaCl treatment resulted in decreased catalase activity. Peak catalase activity was achieved in mycorrhizal chia plants treated with 50 mM NaCl and control (without NaCl treatment) (Figure 4).

Malondialdehyde levels in mycorrhizal chia plants were lower than in non-mycorrhizal chia plants (Figure 5). The highest levels of malondialdehyde were achieved in chia plants that were not mycorrhizal and were treated with 200 mM NaCl (Figure 5).

4. DISCUSSION

The presence of mycorrhizal fungi on plant roots will help these plants get more phosphorus nutrients (Diagne et al., 2020). The zeolite used in this study contains very little or no phosphorus. However, the nutrient content of phosphorus in the media was available because Hoagland's solution provided sufficient nutrients, including phosphorus for the growth of chia plants(Figure 1). This is in line with the statement of Wang et al. (2021) that P available in soil under salt stress is an important factor determining the effectiveness of arbuscular mycorrhizae.

The roots of chia plants are normally capable of symbiosis with arbuscular mycorrhizal fungi (Younis et al., 2021). Ebrahim & Saleem (2017) stated that the ability of mycorrhizal fungal infections can be influenced by environmental stresses such as temperature. In line with this study, saline treatment can reduce the colonization ability of *G. manihotis* (Figure 2). Garg & Bhandari (2015)

report that the germination of arbuscular mycorrhizal fungus spores followed by the formation of hyphae would decrease along with increasing salt levels in the surrounding environment.

The beneficial effect of the presence of mycorrhizae on plant roots is the increased chlorophyll levels and high photosynthetic activity (Hashem et al., 2015). Ouzounidou et al. (2015) reported that *Glomus sp* inoculation was able to increase the chlorophyll concentration of chia plants. In line with this study, mycorrhizal chia plants had a high chlorophyll content when compared to non-mycorrhizal chia plants (Table 1). Inhibition of photosynthetic reactions will only occur in conditions of high salt level due to inhibition of the diffusion of chemicals that support photosynthesis in stomata and leaf mesophyll cells (Shin et al., 2020). Likewise, the condition of high salt level in cultivated land affects the accumulation of minerals in the soil resulting in low water potential. The absorption of water carrying minerals will be hampered to enter the plant roots and result in a lack of water supply to the leaf mesophyll tissue. Furthermore, such conditions will affect the closing and opening of stomata which affect the process of plant photosynthesis (Lotfi et al., 2020). In this study, it was seen that the treatment of high salt level could reduce the chlorophyll content of chia plants (Table 1).

In the research of Shahvali et al. (2020)) reported that the application of arbuscular mycorrhizal fungi can enhance the antioxidant system. Sadak et al. (2019) found a correlation between environmental stress and antioxidant activity such as superoxide dismutase in cucumber plants. Meanwhile, the results of this study showed a relationship between the inoculation of the fungus *G. manihotis* with salt stress and the antioxidant system (Figure 3). However, the antioxidant system of chia plants changed depending on the salt stress treatment. According to Porcel et al. (2016) antioxidant activity such as increased superoxide dismutase plays an important role in reducing free radicals.

The presence of catalase activity occurred at the beginning of the symbiosis of mycorrhizal *G. mossae* with asparagus plant roots (He et al., 2020). Soybean plants inoculated with *G intraradices* in conditions of sufficient water or dry growing media will show high catalase activity. Furthermore (Etesami & Shafiei, 2020) states that gradually increasing salt stress will reduce catalase activity. From the results of this study, it was shown that the catalase activity of mycorrhizal chia plants was higher than that of non-mycorrhizal chia plants (Figure 4). According to (Haque & Matsubara, 2018) the response of antioxidant compounds from the enzyme group will vary depending on mycorrhizae, plant species and stress conditions. In principle, based on the data obtained, the response of chia plants can vary to the presence of the mycorrhizal fungus *G. manihotis* and salt stress conditions.

Malondialdehyde is a compound produced in lipid peroxidation reactions (Álvarez-Robles et al., 2020) So its presence indicates how much the oxidation reaction damages lipids found in plant tissues such as chia plants. Research Hu et al. (2020) reported that mycorrhizal plants had lower levels of malondialdehyde than non-mycorrhizal plants. The presence of mycorrhizae will be able to reduce oxidative damage to lipids during environmental stress conditions such as high salt content. This is in line with the results of this study, that the level of malondialdehyde is low because there are mycorrhizae in chia plants that reduce high salt stress effect (Figure 5). The application of mycorrhizae on chia plants is not only at the acclimatization stage but is continued as long as the chia plants grow until harvest time (Sabouri et al., 2021). Thus, the application of mycorrhizae can be used as a strategy to reduce the damaging effects of land conditions under high salt stress. The right

combination of arbuscular mycorrhizal fungi species and their host plants will be able to overcome the effects of high salt stress.

5. CONCLUSION

From the results of this study it is clear that chia (*Salvia hispanica* L.) plants showed increased survival to high salt levels because it has formed a mycorrhizal association. Even though mycorrhizal chia plants were grown in a high salinity environment, they were able to increase enzymatic antioxidant compounds such as superoxide dismutase and catalase. Moreover, they are able to reduce the intensity of lipid peroxidation by producing a small amount of malondialdehyde compounds. Other parameters measured like chlorophyll and carotenoid level and phosphorus concentration in chia plants grown at high salinity and inoculated with mycorrhizal fungi *Glomus manihotis* were seen to increase significantly compared to those without mycorrhizal fungi inoculation. In short, the productivity of chia plants will not decrease even if they are planted under conditions of high salt stress when inoculated with the mycorrhizal fungus *Glomus manihotis*.

Figure and Table

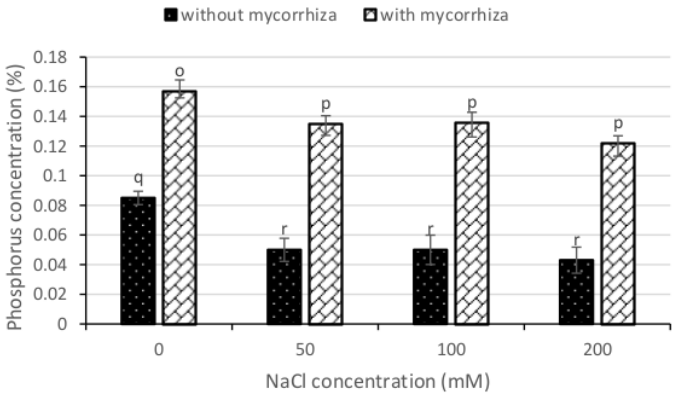

Figure 1. Phosphorus concentration of chia plant with and without mycorrhiza inoculation grown in various NaCl concentration at the end of the observation (day 45). Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the column of the graph to indicate statistical significance ($p<0.05$)

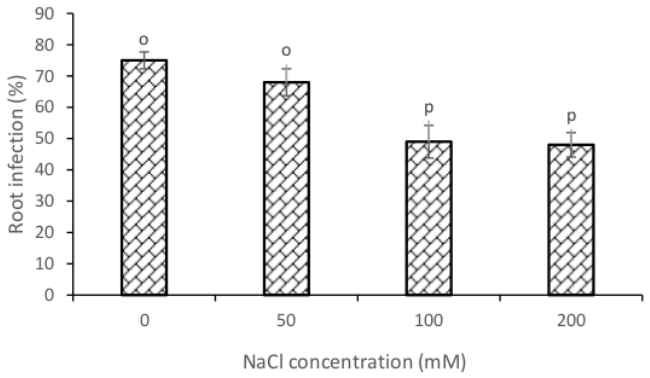

Figure 2. Mycorrhizal colonization of chia plant roots grown in various NaCl concentration at the end of the observation (day 45). Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the column of the graph to indicate statistical significance ($p<0.05$)

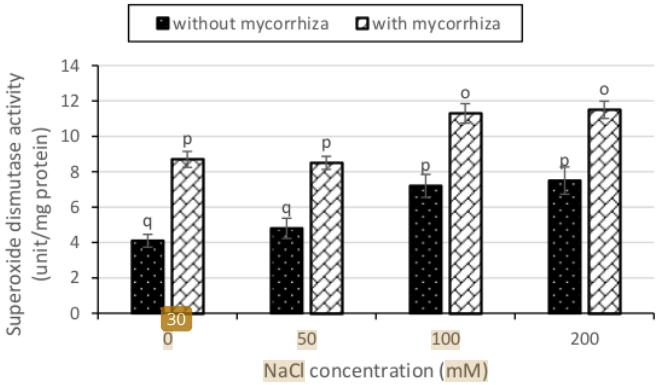

Figure 3. Superoxide dismutase activity in chia plants with and without mycorrhiza inoculation grown in various NaCl concentration at the end of the observation (day 45). Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the column of the graph to indicate statistical significance ($p<0.05$)

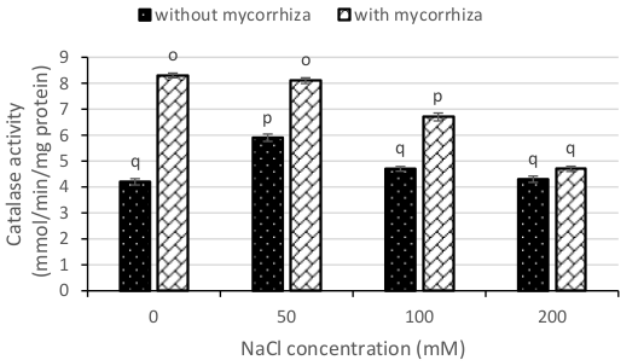

Figure 4. Catalase activity of chia plants with and without mycorrhiza inoculation grown in various NaCl concentrations at the end of the observation (day 45). Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the column of the graph to indicate statistical significance ($p<0.05$)

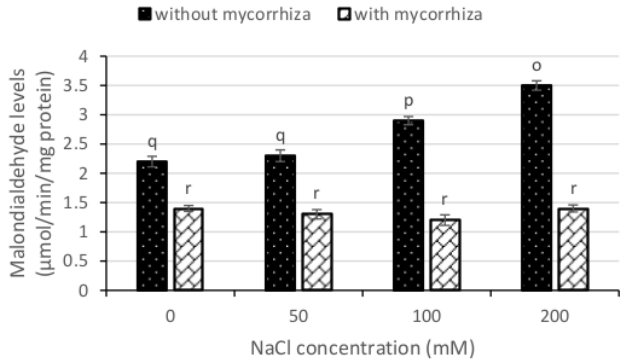

Figure 5. Malondialdehyde levels of chia plant leaves with and without mycorrhiza inoculation grown in various NaCl concentration at the end of the observation (day 45). Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the column of the graph to indicate statistical significance ($p<0.05$)

Table 1. Total chlorophyll and carotenoid levels in chia plant leaves with and without mycorrhiza inoculation grown in various NaCl concentration at the end of the observation (day 45).

Treatment	Salt (mM NaCl)	Total Chlorophill (mg/g fresh weight)	Total Carotenoids (µg/g fresh

			weight)
Without Mycorrhiza	0	3,65(q)	7,42(q)
	50	3,59(q)	7,38(q)
	100	3,32(r)	7,17(r)
	200	3,28(r)	7,13(r)
With Mycorrhiza	0	4,60 (o)	8,25(o)
	50	4,36(p)	8,17(o)
	100	4,39(p)	8,00(p)
	200	4,31(p)	8,05(p)

Note: Data were expressed as the mean + standard error of seven repetitions. ANOVA and Duncan's test were used in data analysis. Different letters in the same column indicate significantly different ($p<0.05$)

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CMA-Salinity-Chia

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