Changes in some characters of soybean leaves inoculated with mycorrhiza in salinity stress

N Rahmawati1*, Rosmayati1, Delvian2 and M Basyuni2

1Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia
2Faculty of Forestry, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia

E-mail: *nini@usu.ac.id

Abstract. Increasing soybean production can be done by expanding the planting area on suboptimal land such as saline soils. Leaves which are an important part of plants where the photosynthesis takes place also experience disturbances due to salinity stress. Efforts can be made to overcome salinity stress, among others by planting salinity tolerant soybean genotypes and mycorrhiza inoculation. This study aims to analyse changes in leaf morphology characters in two soybean genotypes inoculated with mycorrhiza in salinity stress. The study used split-plot design with the main plot of soybean genotype (Grobogan variety and Grobogan soybeans selected for salinity tolerance) and subplots arbuscular mycorrhizal fungi isolates (control, Glomus sp. 1, Glomus sp. 2, Glomus sp. 3, Glomus sp. 4, Glomus sp. 5, and isolates the mixture of all isolates) in the experimental field of Paluh Merbau Village Deli Serdang with 1.5 m above sea level and salinity level of 5-6 dS/m in February-May 2013. The results showed the number of leaves, leaf area, chlorophyll a, and chlorophyll b were significantly affected by the interaction of soybean genotype and mycorrhizal inoculation. While the treatment of soybean genotypes and mycorrhizal inoculation showed significant differences in the number of stomata and thickness of cuticles.

1. Introduction

Soybeans are an important plant that is widely used to meet the needs of human food, animal feed and biodiesel due to the high content of protein and oil. Soybean is also a raw material that is widely used for human health and industrial products. Therefore, soybean needs continue to increase throughout the world. Growth, development and production of soybeans is the result of the interaction of genetic potential with its environment. [1]. Efforts to increase soybean production continue to be carried out, among others through efforts to expand planting areas on marginal lands such as land with high salinity.

Soybean germplasm shows salt tolerance spectrum from high to low range. The ability of salt tolerance in soybeans (characterized by the level of leaf necrosis) among various varieties depending on the stage of growth. In general, salt-tolerant varieties show the ability to grow and produce better than sensitive varieties [1-2].

Efforts that can be made to reduce the impact of salinity stress are arbuscular vesicular mycorrhizal inoculation. Although the positive effects of mycorrhizae to reduce salinity stress have been reported, a
beneficial role depends on the character and efficiency of fungi. Mycorrhiza species that are salt tolerant can increase plant growth under salinity pressure through increasing carbohydrate concentration and increasing leaf respiration and transpiration, increasing nutrient uptake (N and P) of host plants [3-4]. Therefore, choosing an efficient mycorrhizal species and evaluating the function of mycorrhiza is important in order to successfully obtain the benefits of mycorrhizae in salinity stress [5].

Leaves are the most important and vital organs of physiological activities such as photosynthesis, respiration, transpiration, photoreception and the synthesis and supply of signaling compounds, including growth regulators [6]. Salinity stress causes reduced leaf cell expansion due to low turgor pressure which is controlled by processes associated with water uptake and cell wall extension which results in decreased leaf area and weight. The increase in salt concentration in the canopy is due to increasing the transpiration rate and greatly affecting the leaves. Soy responses to salinity stress depend on both genotypes and environmental conditions [6-8].

This study aims to analyze changes in leaf morphology characters in two soybean genotypes inoculated with mycorrhizae in salinity stress.

2. Materials and Methods
This research was carried out in the experimental area of Paluh Merbau Village, Percut Sei Tuan Subdistrict, Deli Serdang Regency with an altitude of 1.5 m above sea level and a salinity level of 5-6 dS/m in February to May 2013. Research using split plot design with the main plot soybean genotypes (Grobogan variety and Grobogan salinity tolerant genotype) and subplots of indigenous arbuscular mycorrhizal fungi isolates (without inoculation of mycorrhizal inoculum (control), Glomus sp. 1, Glomus sp. 2, Glomus sp. 3, Glomus sp. 4, Glomus sp. 5, and mix of all inoculum). The research stages include land preparation, planting, plant maintenance and parameter observation activities. Parameter observation is done when the plant enters the R1 phase (beginning bloom phase).

Parameters observed were number of leaves, leaf area, chlorophyll a and b, number of stomata and thickness of cuticles. Statistical data analysis using F test and further test using Duncan's Multiple Distance Test at the level of α = 5%

3. Results and Discussion
Salinity stress negatively affects soybean growth, including leaves as an important organ supporting plant growth and production. The results in Table 1 showed the interaction of soybean genotype and mycorrhizal inoculation significantly increased the number of leaves. The number of leaves on soybean plants which were inoculated with mycorrhiza was significantly different compared to soybean which was not inoculated with mycorrhiza.

| Arbuscular vesicular mycorrhiza | Soybean genotype | Mean of arbuscular vesicular mycorrhiza |
|--------------------------------|-----------------|----------------------------------------|
|                                | Grobogan variety | Grobogan tolerant salinity              |
| Without mycorrhiza             | 10.20 k         | 11.67 gh                                | 10.94 |
| Glomus sp.1                    | 10.57 j         | 12.37 de                                | 11.47 |
| Glomus sp.2                    | 10.83 j         | 12.53 cd                                | 11.68 |
| Glomus sp.3                    | 11.20 i         | 12.70 bc                                | 11.95 |
| Glomus sp.4                    | 11.63 h         | 12.77 bc                                | 12.20 |
| Glomus sp.5                    | 11.97 fg        | 12.93 b                                 | 12.45 |
| Mix of all inoculum            | 12.13 ef        | 13.23 a                                 | 12.68 |
| Mean of soybean genotype       | 11.22           | 12.60                                   |

Table 1. Number of leaves of two soybean genotypes inoculated with arbuscular vesicular mycorrhiza
The highest number of leaves was in Grobogan salinity tolerant genotype which was inoculated with a mixture of all inoculums, the least of which was in Grobogan varieties which were not inoculated with mycorrhiza (Table 1). The difference in the number of soybean leaves in the two combinations reached 29.71%. Wu et al. [9] research on citrus plants also showed the same results, where the application of mycorrhiza in plants that were caught in salinity could increase the number of leaves.

Table 2. Leaf area of two soybean genotypes inoculated with arbuscular vesicular mycorrhiza

| Arbuscular vesicular mycorrhiza | Soybean genotype | Mean of arbuscular vesicular mycorrhiza |
|-------------------------------|-----------------|---------------------------------------|
|                               | Grobogan variety | Grobogan tolerant salinity              |
| Without mycorrhiza            | 392.70 k        | 563.04 e                              |
| Glomus sp.1                   | 405.97 jk       | 575.85 e                              |
| Glomus sp.2                   | 419.35 j        | 598.42 d                              |
| Glomus sp.3                   | 473.68 g        | 617.97 c                              |
| Glomus sp.4                   | 441.11 i        | 627.63 c                              |
| Glomus sp.5                   | 461.23 h        | 659.58 b                              |
| Mix of all inoculum           | 495.46 f        | 689.29 a                              |
| Mean of soybean genotype      | 441.36          | 618.83                                |

The results in Table 2 showed that the interaction of soybean genotypes and arbuscular vesicular mycorrhiza inoculation significantly affected leaf area of soybean in salt stress. The highest leaf area was in the interaction of Grobogan salinity tolerant genotypes and mixed inoculated from all types of mycorrhiza which were significantly different from all other treatment combinations, namely an increase of 29.71% compared to the Grobogan which was not inoculated with mycorrhizae. Grobogan salinity tolerant also has a higher leaf area of 12.30% compared to Grobogan variety. Ghassemi-Golezani et al. [8] stated that soybean response to salinity stress depends on genotype and environment. The results of Khan et al. [6] study also showed that soybean varieties that are tolerant to salinity reduction are less leaf area than sensitive soybeans. Munns [10] states that excessive salt absorption can cause leaf death and reduce leaf area. As a result, there is a decrease in photosynthate supply in plants, affecting the carbon balance needed for growth. Furthermore, Lutts et al. [11] also stated that the reduction in leaf area is a common phenomenon in glycophytes plants that grow in salt stress conditions.

Table 3. Chlorophyll a content of two soybean genotypes inoculated with arbuscular vesicular mycorrhiza

| Arbuscular vesicular mycorrhiza | Soybean genotype | Mean of arbuscular vesicular mycorrhiza |
|--------------------------------|-----------------|---------------------------------------|
|                               | Grobogan variety | Grobogan tolerant salinity              |
| Without mycorrhiza            | 1.07 i          | 1.61 d                                |
| Glomus sp.1                   | 1.27 h          | 1.61 d                                |
| Glomus sp.2                   | 1.34 g          | 1.65 cd                               |
| Glomus sp.3                   | 1.42 f          | 1.69 bc                               |
| Glomus sp.4                   | 1.47 ef         | 1.72 b                                |
| Glomus sp.5                   | 1.50 e          | 1.74 ab                               |
| Mix of all inoculum           | 1.60 d          | 1.78 a                                |
| Mean of soybean genotype      | 1.38            | 1.69                                  |
Chlorophyll content is an important parameter to determine the tolerance level of plants to salinity stress. The results of the study in Table 3 and Table 4 show the chlorophyll a and chlorophyll b content in salinity tolerant genotype of soybean inoculated with a mixture of all mycorrhizal inoculants higher than other treatments. Al-Khaliel’s [12] study also showed that peanut plants inoculated with mycorrhiza had higher chlorophyll content than those not inoculated with mycorrhizae. The higher chlorophyll content may indicate the higher rate of photosynthesis needed to support the amount of carbon needed by mycorrhiza [13]. The increase in photosynthesis of plants which are inoculated with mycorrhiza is also caused by increased absorption of phosphate nutrients to increase plant growth.

### Table 4. Chlorophyll b content of two soybean genotypes inoculated with arbuscular vesicular mycorrhiza

| Arbuscular vesicular mycorrhiza | Soybean genotype | Mean of arbuscular vesicular mycorrhiza |
|---------------------------------|-----------------|----------------------------------------|
|                                 | Grobogan variety | Grobogan tolerant salinity              |
| Without mycorrhiza              | 0.59 g          | 0.72 e                                  |
| *Glomus* sp.1                   | 0.61 g          | 0.81 d                                  |
| *Glomus* sp.2                   | 0.65 fg         | 0.84 cd                                 |
| *Glomus* sp.3                   | 0.63 g          | 0.80 bc                                 |
| *Glomus* sp.4                   | 0.66 efg        | 0.92 b                                  |
| *Glomus* sp.5                   | 0.70 ef         | 0.94 b                                  |
| Mix of all inoculum             | 0.72 e          | 1.11 a                                  |
| Mean of soybean genotype        | 0.65            | 0.88                                    |

The decrease in chlorophyll content in salinity stress is associated with an increase in chlorophyllase activity which causes degradation of the pigment causing a decrease in photosynthesis rate and plant growth. High salinity also limits de novo protein synthesis and the function of pigment-protein complexes [14-16]. Mycorrhizal inoculation is an effort that can be done to increase the chlorophyll content of plants that experience salinity stress, as the results obtained in this study. Chlorophyll content increased by 12.12% and chlorophyll b increased by 39.39% in soybean plants inoculated with a mixture of all mycorrhizal inoculums compared to treatment without inoculation.

### Table 5. Number of stomata and cuticle thickness of two soybean genotypes

| Soybean Genotype       | Stomata (number of stomata/mm) | Cuticle thickness ((µm)) |
|------------------------|--------------------------------|--------------------------|
| Grobogan variety       | 317.62 b                       | 1.32 a                   |
| Grobogan salinity tolerant | 336.67 a                  | 1.27 b                   |

Salinity stress can affect various cellular mechanisms, biochemistry, and plant physiology. At the cellular level salinity results in protoplasmic water loss resulting in increased ion concentration, inhibits metabolic functions, and increases the likelihood of interactions between molecules which can cause protein denaturation and membrane fusion. One mechanism of tolerance in plants as a response to salinity stress is to optimize the role of stomata to prevent loss of water through the leaves. With the osmotic adjustment allows growth to continue and the stomata remain open [17]. The results showed that the Grobogan salinity tolerant genotype had a greater number of stomata and thinner stomata than the selected Grobogan varieties. Whereas the cuticle on the leaf is a layer containing wax which is
produced by the epidermal layer of the leaves, the tip of the stem and the parts that come into contact with the other air. The function of cuticles, among others, is to reduce evaporation of water from the surface of plants and reduce water loss in plants. In salinity-tolerant soybean genotypes, the osmoregulation mechanism and the ability of plants to absorb water are better than intolerant varieties.

### Table 6. Number of stomata and cuticle thickness of soybean genotypes inoculated with arbuscular vesicular mycorrhiza

| Arbuscular vesicular mycorrhiza | Stomata (number of stomata/mm) | Cuticle thickness (µm) |
|---------------------------------|-------------------------------|------------------------|
| Without mycorrhiza              | 306.67 d                      | 1.33 a                 |
| Glomus sp-1                     | 315.00 cd                     | 1.32 ab                |
| Glomus sp-2                     | 321.67 bcd                    | 1.30 b                 |
| Glomus sp-3                     | 326.67 bc                     | 1.30 b                 |
| Glomus sp-4                     | 330.00 ab                     | 1.29 c                 |
| Glomus sp-5                     | 345.00 a                      | 1.27 cd                |
| Mix of all inoculum             | 345.00 a                      | 1.26 d                 |

The addition of stomatal amount and reduction in the thickness of cuticles in soybean leaves which are inoculated with mycorrhiza indicates that the water absorption process takes place better. Plants that can absorb enough water will be able to transpose some of the absorbed water through the leaves without having to do morphological adaptations such as reducing the density of stomata and thickening the cuticles to reduce the rate of transpiration. Plants planted in saline fields often experience the disruption in the process of absorbing water from the rhizosphere into the roots of plants due to osmotic stress which causes plants to experience water shortages that can inhibit plant growth and development. The presence of mycorrhizae in plant roots can help overcome these problems through several mechanisms including the expansion of the absorption area in the presence of external hyphae. Ruiz-Lozano (2003) describes several studies indicating the role of mycorrhizal hyphae in the absorption and transfer of water to host plants. Hyphae with a diameter of 2–5 µm can penetrate the soil pore that cannot be reached by hair roots with a diameter of 10-20 µm so that the absorption of water cannot be carried out by plants that do not have microcorrhiza. Allen [18] estimated the rate of water absorption by external hyphae to plant roots was 0.28 ng/sec per entry point. Faber et al. [19] stated that the rate of water transpiration by hyphae ranged from 375 to 760 nl H2O per hour.

### 4. Conclusions

The results showed the number of leaves, leaf area, chlorophyll a, chlorophyll b was significantly affected by the interaction of soybean genotype and mycorrhizal inoculation. While the treatment of soybean genotypes and mycorrhizal inoculation showed significant differences in the number of stomata and thickness of cuticles. The best leaf character to support the growth and production of soybean in saline area is Grobogan salinity tolerant and inoculation of all mycorrhizal isolates.

### References

[1] Phang T H, Shao G and Lam H M 2008 Salt Tolerance in Soybean Journal of Integrative Plant Biology 50 10 pp 1196-212
[2] Shao G H, Song J Z and Liu H L 1986 Preliminary Studies on The Evaluation of Salt Tolerance in Soybean Varieties Acta Agronomy Sinica 6 pp 30-5
[3] Tian C Y, Feng G, Li X L and Zhang F S 2004 Different Effects of arbuscular Mycorrhizal Fungal Isolates From Saline or Non-Saline on Salinity Tolerance of Plants Applied Soil Ecology 26 pp 143–8
[4] Miransari M 2010 Contribution of Arbuscular Mycorrhizal Symbiosis to Plant Growth Under Different Types of Soil Stress Plant Biology 12 pp 563–9
[5] Zou Y N and Wu Q S 2011 Efficiencies of Five Arbuscular Mycorrhizal Fungi in Alleviating Salt Stress of Trifoliate Orange *International Journal of Agricultural and Biological* **13** pp 991-5

[6] Khan M S A, Al-Mamun M A, Al-Mahmud A, Bazzaz M M, Hossain A, Alam M S, Shamimuzzaman M and Karim M A 2014 Effects of Salt and Water Stress on Leaf Production, Sodium and Potassium Ion Accumulation in Soybean *Journal of Plant Sciences* **2** 5 pp 209-14

[7] Cramer G R and Bowman D C 1993 *Cell elongation control under stress conditions. In: Handbook of Plant and Crop Stress, M Pessarakli (Ed.)* (New York: Marcel Dekker) pp 303–19

[8] Ghassemi-Golezani K, Taifeh-Noori M, Oustan S and Moghaddam M 2009 Response of Soybean Cultivars to Salinity Stress *Journal of Food, Agriculture & Environment* **7** 2 pp 401-4

[9] Wu Q S, Zou Y N, Liu W, Ye X F, Zai H F and Zhao L J 2010 Alleviation of Salt Stress in Citrus Seedlings Inoculated With Mycorrhiza: Changes in Leaf Antioxidant Defense Systems *Plant Soil Environ.* **56** 10 pp 470–5

[10] Munns R 2002 Comparative Physiology of Salt and Water Stress *Plant Cell Environ.* **25** pp 239-50

[11] Lutts S, Kinet J M and Bouharmont J 1996 Effects of Salt Stress on Growth, Mineral Nutrition and Proline Accumulation Concerning Osmotic Adjustment in Rice (*Oryza sativa L.*) Cultivars Differing in Salinity Tolerance *Plant Growth Regul.* **19** pp 207-18

[12] Al-Khaliel A S 2010 Effect of Salinity Stress on Mycorrhizal Association and Growth Response of Peanut Infected by *Glomus mosseae* *Plant Soil Environ.* **56** 7 pp 318–24

[13] Wright D P, Read D J and Scholes J D 1998 Mycorrhizal Sink Strength Influences Whole Plant Carbon Balance of *Trifolium repens* L. *Plant, Cell and Environment* **21** pp 881–91

[14] Hashema A, Abd-Allah E F, Alqarawi A A, Aldubise A and Egamberdieva D 2015 Arbuscular Mycorrhizal Fungi Enhances Salinity Tolerance of *Panicum tungidum* Forssk by Altering Photosynthetic and Antioxidant Pathways *Journal of Plant Interactions* **10** pp230–42

[15] El-Tayeb M A 2005 Response of Barley Grains to the Interactive Effect of Salinity and Salicylic acid *Plant Growth Regul.* **45** pp 215–24

[16] Azooz M M, Youssef A M and Ahmad P 2011 Evaluation of Salicylic Acid (SA) Application on Growth, Osmotic Solutes and Antioxidant Enzyme Activities on Broad Bean Seedlings Grown Under Diluted Seawater *Int. J. Plant Physiol. Biochem.* **3** 14 pp 253–64

[17] Lestari E G 2006 Hubungan antara kerapatan stomata dengan ketahanan kekeringan pada somaklon padi Gajah Mungkur, Towuti, dan IR 64 [Relation between stomata density and drought resistance in Gajah Mungkur, Towuti, and IR 64 rice somaclons] *Jurnal Biodiversitas* **7** pp 44-8

[18] Allen M F 1991 *The Ecology of Mycorrhizae* (Cambridge: Cambridge University Press)

[19] Faber B A, Zasoski R J and Munns D N 1991 A Method For Measuring Hyphal Nutrient and Water Uptake in Mycorrhizal Plants *Canadian Journal of Botany* **69** pp 87–9