Glomus etunicatum improved salt tolerance in Dalbergia latifolia Roxb. through physiological adjustment

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Abstract. Many studies have reported that salinity has a negative impact on mycorrhiza but mycorrhizae can increase plant growth under salinity stress. This study was conducted to determine the growth and level of dependence of Dalbergia latifolia on mycorrhiza under saline conditions. Dalbergia latifolia is inoculated with Glomus etunicatum and grown on media that has been given a different concentration of sodium chloride solution. The results showed that an increase in salinity led to reduced root colonization of G. etunicatum in D. latifolia but on the other hand, the presence of G. etunicatum increased the growth and biomass of D. latifolia in all salinity levels. Phosphorus uptake of plants colonized by G. etunicatum also increased. This result confirms the degree of dependence of D. latifolia on G. etunicatum under salinity stress. Improved growth of D. latifolia in saline soils reflects the importance of G. etunicatum which can be used to improve the productivity of saline soils.

1. Introduction
Soil salinity issue impacts all aspects of plant physiology and biochemistry with significant decrease of plant productivity. Almost half of the irrigated agricultural land or around 20% of the world's agricultural area experienced salinity issues. Salinity is one of the most important abiotic factors and becoming a limiting factor for plant growth and yield [1]. Under saline condition, several important processes in plant physiology are significantly altered, especially regarding the photosynthesis. The reduced rate of photosynthesis under salinity stress is not only related to the inhibition of stomata opening resulting in reduced CO₂ concentrations, but also other physiological factors, such as chlorophyll and carotene content [2–4].

Many studies have shown that arbuscular mycorrhizal fungi (AMF) may increase plant tolerance to salinity and improve plant growth compared to plants without AMF inoculation. AMF improve the plant growth through several mechanisms, including increasing plant nutrient uptake [5], stimulating growth hormone production and improving rhizosphere conditions [6], increasing water use efficiency and photosynthesis rate [7], increasing the accumulation of solutes [8], especially the anti-oxidant enzymes [9].

Some other researchers have also shown that under stress conditions salinity of the mycorrhizal plants is able to grow better and produce greater biomass than the plants without mycorrhizae [8–10]. The increase in growth and production of plant biomass under conditions of salinity stress is associated with an increase in nutrient uptake by mycorrhizal plants. The presence of mycorrhizae in plant roots that experience salinity stress will affect the morphological characteristics of the roots. The
apical meristematic activity of the roots changes and more lateral root formation. Changes in root morphology will affect nutrient uptake and efficient use of host plant water. In addition, the mutualistic relationship between mycorrhiza and salinity stress plants can affect various morphological parameters of plant growth, including plant height, leaf area index, plant biomass, and root system [11–13]. This study then evaluates the effect of inoculation of *Glomus etunicatum*, a mycorrhizal species to the growth performance of *Dalbergia latifolia*, an important timber species from Indonesia.

2. Materials and methods

2.1. Experimental design

The experiment was designed as a completely randomized factorial design with a factor of 4 salinity levels (0, 100, 100, 300 ppm NaCl) and two factors of AMF inoculum (+/-) with 5 replications. Salinity treatments were adjusted with 50 ml NaCl solution for each treatment once a week during the trial period. Control plants were irrigated with distilled water. *Glomus etunicatum* was chosen as mycorrhizal species priorly propagated in pot culture using *Pueraria javanica* as host. The inoculum used was a mixture of *G. etunicatum* spores (80 spores/10 g media) and root pieces colonized by mycorrhiza (75% colonization). Inoculation was carried out with a layering system and control plants were inoculated with growth media and root pieces without mycorrhizal colonization.

The soil used for the experiment was analyzed for chemical physical properties and the results are presented in table 1. Soil pH was determined by a pH meter, while soil salinity was measured by measuring electrical conductivity (Model LF 539, Germany). Measurement of soil organic carbon used Walkley and Black method [14], N content using the Jackson method [15], P content using the Olsen method [16] and K content using the ammonium acetate method [17].

| Soil pH | EC (mS/cm) | Organic-C (%) | N (%) | Available K (mg/Kg) | P (mg/Kg) |
|---------|------------|---------------|-------|---------------------|-----------|
| 7.1     | 0.13       | 1.1           | 0.56  | 61                  | 47        |

2.2. Observation of root colonization

Observation on *D. latifolia* root colonization followed the procedure by Giovannetti and Mosse [18]. Root colonization was calculated using the colonized root length method as described by Kormanik and Mc Graw [19].

2.3. Analysis on the plant growth and physiology

Measurement of *Dalbegia latifolian* Roxb. growth was monitored at 60 days after planting. The shoot and root biomass were weighed after dried in an oven at 60°C for 48 hr. In addition, the phosphorus absorption by shoot and root tissue was also measured by the ammonium paramolybdate-vanadate method [20] and the proline content in the leaves by the colometry method [21]. Mycorrhizal dependency was calculated based on the formula of Plenchette et al. [22]. The parameter illustrated the extent of plant species colonized by mycorrhizae to retain its growth under salinity stress.

3. Results and discussion

The response of *D. latifolia* Roxb. to salinity stress and AMF colonization is presented in figure 1 to 4. The mycorrhizal dependency of *D. latifolia* Roxb to *G. etunicatum* is presented in figure 5. The results showed that *G. etunicatum* was able to colonize *D. latifolia* roots under salinity stress conditions (figure 1). Observation on colonized roots showed the presence of mycorrhizal structures in the form of internal hyphae and vesicles while the structure of the arbuscula was not observed. Presumably, the absence of arbuscular structure may be related to the age of arbuscula and the condition of the roots.
during the observation period [23]. The highest colonization occurred in plants without salinity stress. The presence of dissolved salts pose hazardous effects on plants and mycorrhizae, thus reducing the percentage of mycorrhizal colonization in plant roots. The manifestation of the effect may be observed through hyphal inhibition thereby reducing root spread and colonization [24].

**Figure 1.** Salinity effect to root colonization by *G. etunicatum*. Data are presented as mean ± S.D from five replications. (S0) Control, (S1) 100 ppm, (S2) 200 ppm, (S3) 300 ppm.

Increased NaCl concentrations or salinity levels resulted in growth reduction of *D. latifolia* seedlings and showed a negative correlation between plant growth and salinity. Both shoot and root biomass were reduced by increasing salinity as shown in figure 2 A and B. However, the impact of salinity to shoot and root biomass was greater in uncolonized plants than those colonized by *G. etunicatum*. Under salinity stress, the presence of *G. etunicatum* was still considerably beneficial by increasing the root and shoot biomass despite the lower percentage of root colonization. Salinity has a negative effect on the effectiveness of mycorrhizal fungi but the beneficial effect of mycorrhizal fungi on plants did not seem to be independent of the percentage of root colonization [25].

**Figure 2.** Salinity effect to (A) shoot biomass and (B) root biomass of *D. latifolia*. Data are presented as mean ± S.D from five replications. (S0) Control, (S1) 100 ppm, (S2) 200 ppm, (S3) 300 ppm.
Many studies have shown that plants inoculated with AMF grow better than uninoculated plants under poor environmental condition, one of which in saline soils and lead contaminated soil [9,11,26–29]. The beneficial properties facilitated by AMF are related to the increased nutritional absorption by the plants. Increased phosphorus (P) and water uptake is considered as a major factor contributing to the improved plant growth under salinity stress [9,30]. Figure 3 shows the increased P absorption of shoots and roots of D. latifolia under salinity stress.

**Figure 3.** Salinity effect to (A) shoot P uptake and (B) root P uptake of D. latifolia. Data are presented as mean ± S.D from five replications. (S0) Control, (S1) 100 ppm, (S2) 200 ppm, (S3) 300 ppm.

The symbiosis of mycorrhizal fungi and plants under salinity stress not only provides benefits for plants in the form of increased P uptake of plants and water, but also in terms of crop osmotic pressure adjustment. The physiological change in osmotic pressure must be adjusted to maintain the plant's ability to absorb water and nutrients. One form of osmotic adjustment is the accumulation of low molecular weight and soluble compounds, such as proline in plant leaves. In this study, the proline content of inoculated D. latifolia was lower than control plants under salinity stress (figure 4).

**Figure 4.** Salinity effect to proline accumulation in D. latifolia leaves. Data are presented as mean ± S.D from five replications. (S0) Control, (S1) 100 ppm, (S2) 200 ppm, (S3) 300 ppm.
Proline accumulation is a mechanism for adjusting plant osmotic pressure to increase the plant tolerance towards salinity stress by protecting various enzymes from dehydration [31–33]. The presence of mycorrhizal fungi in plant roots can modify the osmotic adjustment of leaves as mycorrhizal fungi affect carbohydrate composition. Carbohydrates are always associated with active osmotic adjustment wherein carbohydrates are synthesized into low molecular weight compounds, namely proline [26,34].

![Figure 5](image)

**Figure 5.** Mycorrhizal dependency of *D. latifolia* to *G. etunicatum*. Data are presented as mean ± S.D from five replications. (S0) Control, (S1) 100 ppm, (S2) 200 ppm, (S3) 300 ppm.

The beneficial properties provided by mycorrhizal fungi in host plants in the form of absorption of nutrients and water are measured as the level of plant dependence on mycorrhizal presence. Some plant species may be highly dependent on mycorrhizae, while some other species exposed from a moderate level of dependence to no dependent at all. In this study, *sonokeling* or *D. latifolia* was less dependent on *G. etunicatum* colonization in non-saline conditions, but the mycorrhizal dependency increased following the salinity levels (figure 5). This result is in line with Kumar et al. [31] who obtained the increased mycorrhizal dependency in *Jatropha* up to 20.84% under salinity conditions from 0 to 0.4%.

Mycorrhizal dependency may be altered by many factors, including soil properties, plant growth stages, plant species and mycorrhizal fungal species [35,29]. Under conditions of environmental stress, mycorrhizal fungi will first fight the adverse effects of stress for growth and development, after that fungi will stimulate plant growth. Increased plant nutritional requirements under salinity stress are afforded by increasing the colonization of mycorrhizae thereby increasing the mycorrhizal dependency at the end of plant growth [4,8–10]. Mycorrhizal dependency will be reduced in soils with high P content because plants may absorb their own P requirements readily. The availability of certain nutrients may also affect the mycorrhizal dependency depends on the plant species.

4. Conclusions
Salinity stress does not only interfere with the water potential but also the availability of nutrients for plants. The adverse effects of salinity stress on plant growth can be reduced by the symbiosis of plants with mycorrhizal fungi. Colonized *D. latifolia* grew better than control plants under salinity stress. The phenomenon is explained through beneficial properties provided by *G. etunicatum* to increase plant tolerance and maintain nutritional requirements, especially the P and water uptake for plant growth.
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