Colonization of Arbuscular mycorrhizal fungi improve salinity tolerance of Eucalyptus (Eucalyptus camaldulensis Dehn.) seedlings

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Abstract

Background: Soil salinity is an important problem for agriculture and effecting in the inability to use soil for cultivation. High salt levels reduce plant performance. Arbuscular mycorrhizal fungi (AMF) have been reported to enhance the tolerance of plants under salinity stress. For promote cultivation of economic plant in salt stress area that univariable to use to produce raw material for pulp industry. We determined the effects of AMF on the growth and nutrient status of eucalyptus (*Eucalyptus camaldulensis* Dehn.) seedlings under salinity stress condition.

Results: Three different clones of Eucalyptus seedlings were pre-inoculated with three salt-tolerant AMF species, namely *Glomus* sp.2, *Gigaspora albida* and *Gigaspora decipiens* or without pre-inoculated. The seedlings were grown in a greenhouse for 45 days. They were then transplanted into individual pots, filled with field soil and subsequently treated with NaCl solution until the electro-conductivity (EC) reached 10, 15 and 20 dS m^{-1}. They were watered for 90 days under nursery conditions. Increasing salinity levels reduced plant performance, fractional root colonization and the number of spores. Increasing salinity also resulted in a lower K/Na ratio. At the same time, performance of the pre-inoculated plants was significantly higher than that of plant that relied on field inoculum only. AMF also significantly increased chlorophyll and leaf proline concentrations and improved the K/Na balance.

Conclusion: The results indicate that pre-inoculation with AMF before out planting improves plant performance under salinity stress due to AMF can improve the negative impacts of salinity on the studied physiological, nutrients uptake and biochemical parameters.

Background

Saline soil and water in 21st currently are increasing [1]. Increased salinization of arable land may have a large negative global effects, predicted to result in a 30% loss of land within the next 25 years, and up to 50% by the middle of the 21st century [2]. Soil salinity is a serious problem for agriculture and is steadily increasing in many parts of the world, particularly in arid and semi-arid regions [3]. Salinity stress limits crop productivity, growth and plant metabolism. In salt stress conditions, the three main problems for plant growth include water potential (physiological drought), the toxic effect of ions, notable sodium (Na) ions and nutrient imbalance, notably K^+:Na^+ balance [4].

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of many plant species. AMF are found naturally in saline environments [5]. AMF can benefit the host plant by enhancing growth, regulating substances, improving soil properties, and increasing the photosynthetic rate and resistance to plant pathogens and environmental stress [6]. Although AMF fungi alleviate growth reduction caused by salinity stress, the mechanisms involved remain partly unresolved. Studies on salinity stress tolerance in mycorrhizal plants have suggested that AMF plants grow better than non-AMF plants due to increased nutrient uptake and photosynthesis, water use efficiency, the production of osmoregulator, higher K /Na ratios and compartmentalization of Na within certain plant tissues reduction caused by salinity [7].

Although AMF can increase host plant tolerance to salinity stress, the capabilities depend on the behavior of each fungal species and strain [8].

Eucalyptus (Eucalyptus camaldulensis Dehn.) or river red gum is a fast-growing plant native to Australia. The species can grow in a wide range of soil properties, from very poor to rich soils. Eucalyptus is one of the most important economic plants in Thailand and is used as a raw material in the production of pulp, oil, furniture and housing [9]. Three Eucalyptus species; E. alba, E. microtheca, and E. camaldulensis have been investigated for their salt tolerance [10]. Eucalyptus camaldulensis Dehn. is a first choice for many growers in Thailand as it can adapt to the saline soil of the northeastern region of Thailand such as in Khon Kaen and Kalasin province. The species is also tolerant to various climate conditions. Cultivation of this species is therefore one option for producing wood in areas with saline soil. An effective way of expanding saline-land usage in Thailand could be to use saline-tolerant strains of AMF for the production of pre-colonized roots of eucalyptus seedlings before planting of cuttings in order to increase the viability of plants after transplanting into saline soil. This study therefore aimed to investigate the potential interaction between saline-tolerant strains of AMF and eucalyptus cultivation under salt stress conditions. We hypothesized that : (1) Increasing salinity levels would reduce plant and mycorrhizal fungal performance; (2) Pre-inoculated plants would be more tolerant to salinity stress than plants that have not been pre-inoculated; (3) Conferral of salt tolerance would be fungal species-specific and eucalypt clone dependent.

Results

Results of the analysis of variance are provided in Table 1. In almost all cases salinity and mycorrhiza were significant sources of variation. Interactions between AMF and clone were almost always significant sources of variation, indicating species-specific AMF responses on different eucalyptus clones. Eucalyptus clone and the other interactions were occasionally significant sources of variation as well.

[Insert Table 1 here]

AMF colonization and spore density

Table 2 shows fractional root colonization and spore abundance of AMF at different salinity levels. Control plants were also colonized, as the field inoculation had not been sterilized, however, colonization levels were much lower than in the pre-inoculated seedlings. Colonization declined with increasing salinity levels. Next to mycorrhiza and salinity as significant sources of variation, variety (clone) was also a significant source of variation. Fractional root colonization on H8 was higher than that on H4 and P6. The significant interaction mycorrhiza × variety was due to the fact that *Glomus* sp.2 reached highest colonization on P6, whereas *G. albida* achieved highest colonization on H8 and H4. Spore densities were much higher in the pre-inoculated clones that in the clones that did not receive pre-inoculation. Spore densities salinity levels. Variety and the interaction mycorrhiza × variety were also significant sources of variation (Table 1). Like fractional root colonization spore densities with H4 and H8 were highest with *G.*

albida, while spore densities with P6 were highest with *Glomus* sp.2. Mycorrhizal colonization and spore abundance were very significantly correlated ($r = 0.64$; $n = 36$; $P < 0.001$).

[Insert Table 2 here]

Plant performance

Shoot dry weight was significantly affected by mycorrhiza and salinity. The interaction mycorrhiza × variety was also a significant source of variation (Table 1). Shoot dry weight was negatively influenced by increasing salinity levels, both for pre-inoculated and control plants. The symptoms of salt toxicity were observed, with leaves becoming sapless and showing signs of chlorosis. The damage to plants was more severe in control plants than in pre-inoculated plants at the same salinity level. At all salinity levels, plant pre-inoculated with *G. albida* usually showed much higher shoot dry weight than plants pre-inoculated with the other AMF or without pre-inoculation. However, at the intermediate salinity levels P6 plants, when pre-inoculated with *Glomus* sp.2, were significantly heavier than when pre-inoculated with the other AMF or when not pre-inoculated. Under those conditions, plants pre-inoculated with *G. albida* had lowest shoot dry weight (Table 3). Plant height followed more or less the same pattern as shoot dry weight, with a negative effect of salinity and a positive effect of pre-inoculation by AMF. Plant height of clone H4 was lower than that of the other two clones. Shoot dry weight and plant height were very significantly positively correlated ($r = 0.83$. $n = 36$; $P < 0.001$).

Leaf relative water content was also significantly affected by salinity, mycorrhiza, variety, and the interaction between mycorrhiza × variety and salinity × variety (Table 1). Salinity reduced and mycorrhiza increased LRWC. Again, the combination of clone H8 with *G. albida* and the combination P6 with *Glomus* sp.2, resulted in positive interactions. Clone H8 exhibited somewhat lower LRWC than the two other clones (Table 5).

For root dry weight, all three main factors were significant sources as variation. The mycorrhiza × variety interaction was also a significant source of variation (Table 1). Increasing salinity levels reduced root dry weight. Mycorrhizal plants, especially cutting pre-inoculated with *G. albida*, increased root dry weight. Root dry weight of clone H4 was significantly higher than that of the other two clones, especially at the two higher salinity levels (Table 3). Root dry weight was very significantly correlated with shoot dry weight ($r = 0.85$, $n = 36$; $P <0.001$).

[Insert Table 3 here]

Root length and root surface area were both significantly affected by salinity level, mycorrhiza and the interaction mycorrhiza × variety. In the case of root surface area, the interaction salinity × variety was also a significant source of variation (Table 1). Root length had high significantly positive correlation with leaf relative water content (LRWC) but root diameter had significantly negative with root length, specific root length and root tissue density (Table 4). Salinity reduced, and pre-inoculation with mycorrhiza increased

root length and root surface area. Clone H4 and H8 showed the strongest effect in interaction with G. albida, whereas clone P6 showed the strongest interaction with Glomus sp.2 (Table 5)

[Insert Tables 4 & 5 here]

Plant nutrient concentration

AMF, salinity and variety of eucalyptus had significantly with all of nutrients concentration in plant tissue. Especially, the interaction of AMF and salinity had high significantly in concentration of N, Na and K/Na ratio (Table 1). Concentrations of N, P and K in the plant shoots decreased with higher NaCl concentrations, whereas Na increased with higher NaCl concentrations (Table 6**).** In this study, AMF inoculation was found to enhance nutrient uptake more than the non-AMF plants across all salinity levels. Eucalyptus H4 and H8 strains inoculated with *Gi. albida* had significantly higher total N, K, and lower Na absorption than the control. In the case of P, the results reveal that H4 and H8 had significantly higher concentrations than the control as a result of the fungus at all salinity levels, with the exception of P at 20 dS m^{-1}. The eucalyptus P6 strain inoculated with *Glomus* sp.2 showed significantly higher N, P and K, and lower Na absorption than the control (Table 6**).**

[Insert Table 6 here]

Leaf chlorophyll concentration

Leaf chlorophyll concentration, an important physiological indicator for plant photosynthetic capacity, was significantly affected by all three main factors (salinity, mycorrhiza, variety) and by all two-way and three-way interactions (Table 1). All of chlorophyll had high significant positive correlation but negative correlation with proline concentration (Table7). Mycorrhiza significantly increased leaf chlorophyll concentration, whereas increasing salinity reduced it. In some combinations of variety and AMF species, there was a major effect of increasing salinity levels from 10-15 dS m^{-1}, whereas in other combinations a major decline was observed only when salinity was increased from 15 to 20 dS m^{-1}. Due to the fact that two-way and three-way interactions were significant, other patterns were difficult to explain. Eucalyptus clones H4 and H8 inoculated with *G. albida* had higher chlorophyll concentration compared to other mycorrhizal treatments, while eucalyptus clone P6 inoculated with Glomus sp.2 had higher leaf chlorophyll concentration (Fig. 1-3) than the other mycorrhizal treatments.

[Insert Table 7 here]

[Insert Figures 1 - 3 here]

Leaf proline concentration

Like chlorophyll, leaf proline concentrations were significantly affected by all main factors (mycorrhiza, salinity, variety) and all two-way and three-way interactions (Table 1). Proline concentrations increased with increasing salinity and were lower for plants that were pre-inoculated than for control plants, At the

lowest salinity level there were significant differences between varieties, with H8 showing lowest proline concentration and H4 showing highest concentrations. With increasing salinity levels, the differences between the varieties attenuated. Eucalyptus H4 and H8 inoculated with *G. albida* and P6 inoculated with *Glomus* sp.2 had significantly lower proline concentrations across all salinity levels (Fig. 4).

[Insert Figure 4 here]

Discussion

In this research, salinity stress caused by increasing levels of NaCl significantly reduced plant growth and AMF root colonization, as confirmed by [N Aliasgharzadeh, NS Rastin, H Towfighi and A Alizadeh [11]] for glycophytes (Allium cepa L., Medicago sativa L., Triticum aestivum L. and Hordeum vulgare L.), A Campanelli, C Ruta, G De Mastro and I Morone-Fortunato [12] for Medicago sativa L. var. icon, and NB Talaat and BT Shawky [13] for Triticum aestivum L. Colonization rates declined with increasing NaCl levels, indicating that salinity suppressed the growth of AMF [14], inhibited spore germination, inhibited the growth of hyphae after initial infection had occurred [15], and reduced the number of arbuscule [16]. Our results show that increasing levels of salinity resulted in corresponding reductions of plant growth. However, the AMF colonized plants had better results than the non-AMF plant, which agrees with QS Wu and YN Zou [17] for citrus. AAH Abdel Latef and H Chaoxing [18] found that salt stress reduced root, stem and leaf dry matter, but AMF colonization had the effect of improving the dry matter of tomato and increased the growth of Jutropha curcas L. [19]. AMF spores g^{-1} dry soil was supported by H Evelin, R Kapoor and B Giri [20] review, who found that many researchers reported that in saline soil where low or zero spores population were found in high soil salinity level. The present study found that AMF spore and root colonization in the non-AMF plants (control) treatment were observed, which was caused by the experiment using non-sterile soil. Natural AMF in soil may therefore grow and infect host plants.

Leaf relative water content (LRWC) decreased with rising levels of soil salinity. In the present study, AMF plant increased LRWC in salinity level when compared with the non-AMF plant (control) which was supported by QS Wu, RX Xia and YN Zou [21], A Campanelli, C Ruta, G De Mastro and I Morone-Fortunato [12], A Kumar, S Sharma and S Mishra [22]. There were a several reasons supported why the AMF plants have a higher LRWC, consisting of: (1) AMF roots had higher hydraulic conductivity at low water potential [23]; (2) AMF induced alterations to the root system [24]; (3) mycorrhizal plants had higher stomatal conductance which increased transpiration[25] ; (4) AMF accumulated solutes and improved plant osmotic adjustment [26]. Meanwhile, RM Auge [27] reported that this may have been the result of an improved water relation by AMF hyphae.

An interaction between the genotype of eucalyptus and the AMF species was found in this study. Plant strains H4 and H8 had the highest growth promotion from Gi albida, while the P6 strain had the best growth parameters when inoculated with Glomus sp.2. These findings are supported by the report of AMF's ability to protect plants from the detrimental effects of salt stress may be dependent on the behavior of each species [28], while the plant growth parameters are dependent on the AMF inoculant

species when examined against the same host species [29].Therefore, arbuscular mycorrhizal colonization was dependent upon different plant species and cultivars [30].

Salinity significantly reduced leaf chlorophyll concentration. Increasing salinity levels causes reduced chlorophyll content [25] due to repression of specific enzymes for the photosynthesis system [31] and reductions of nutrient uptake such as Magnesium (Mg) and Nitrogen (N) for chlorophyll biosynthesis. This is because NaCl has an antagonistic effect on N absorption which is essential for the molecular structure of chlorophyll [32]. In this study, all of chlorophyll concentration had positive correlation and AMF plants had significantly higher chlorophyll a, chlorophyll b and total chlorophyll than the non-AMF plant in all of salinity level. This suggests that mycorrhizal inoculation could enhance phosphorus (P) and magnesium (Mg) to reduce sodium concentrations in plant, resulting in increased chlorophyll content and overall capability [33]. The accumulation of free amino acid, proline reported modifications induced by water and salt stress [34] and an exogenous application of proline could play an important role in enhancing plant stress tolerance [3]. In saline conditions, numerous plants accumulate proline as a protective osmolyte to maintain an osmotic balance under low water potentials [35]. This study found significantly higher proline concentrations with increased salinity levels. However, proline concentrations were significantly lower in the AMF plant compared to the control. Proline accumulation in plants may be responsive in less salt-tolerance species or to salinity and not necessarily to AMF, because many authors have reported that proline concentration increased in AMF plants compared to non-AMF plants [36]. Conversely, other authors have reported greater proline accumulations in non-AMF plants than AMF plants for example, in Ocimum basilicum L. [37] and Arachis hypogaea L. [38]. From the results, AMF could enhance eucalyptus nutrients uptake; high concentrations of nitrogen (N), Phosphorus (P) and Potassium (K) and low concentrations of Sodium (Na) were found in Gi. albida with eucalyptus strains H4 and H8, and in Glomus sp.2 with eucalyptus strain P6. Although salinity stress decreased nutrient uptake, many studies have reported increasing salinity levels lowered N and K concentrations, for example in pepper, olive, peanut and chili [39–42]. High concentrations of K can be helpful to maintain K/Na ratio, cell osmotic potential from plays control of water relation and effects to increase photosynthetic rate. The results from this study showed that salt tolerance of AMF plant having highest of K shoot concentration, similar the report which explain that enhancing K uptake by inoculated AMF plants under salinity stress [43]. AMF interaction with eucalyptus that resulted in an increased growth response was observed, which may be from the specific mechanism and different characteristics such as hyphae distribution, the length of external mycelium and/or nutrient translocation, which supported by report that the relationship between the length of root that had been colonized and the ability to alleviate nutrient limitations caused by soil salinity [44] .

Conclusion

Salinity altered the growth of Eucalyptus camaldulensis Dehn. due to effects on the physiological and some biochemical parameters. Salinity reduced the uptake of important mineral elements and nutrients. From the study, AMF can improve the negative impacts of salinity on the studied physiological and biochemical parameters. AMF alleviated salt stress by preventing the uptake of Na and enhancing uptake

of major plant nutrients, causing the AMF plants to have better growth in saline soil. Thus, the use of AMF provides a sustainable and environmentally safe treatment to improve the salinity tolerance of plants. Future field trials in areas with saline soils are required as the next step.

Methods

AMF inoculum and plant preparation

Three AMF species, which are most frequent in saline soil areas in Khon Kaen province, viz., *Glomus* sp.2 (KKU-BH-001), *Gigaspora albida* (KKU-BP-001) and *Gigaspora decipiens* (KKU-BP-002), were isolated from the rhizosphere of eucalyptus from planting sites on saline soil in Ban Phai (6.92 dS m^{-1}), Ban Haeat (5.35 dS m^{-1}). These AMF strains were selected after screening their soil salinity tolerance by growing them in soils supplemented with a solution (NaCl) at a maximum salinity of 20 dS m^{-1}, which is considered very strongly saline [45], using the technique described by CM Hepper [46] with minor modifications. The AMF species were propagated in maize (*Zea mays* L.) that has short life and a lot of root system by the pot culture technique in a sterilized sandy loam, and then placed in a greenhouse under natural lighting conditions for three months. Colonized root fragments (fractional root colonization 70-90 %) and spores (24 spores g^{-1} dry soil) were used as inoculum. Forty-five day old eucalyptus cuttings from three different salt tolerance clones containing commercial clone H4 can growth in sand, P6 which can growth in loam and non-commercial clone H8 which can growth in sandy loam which developed and cutting by using the patent of SCG packaging public company limited, Phoenix Pulp & Paper Public Co. Ltd. and Siam Forestry Co., Ltd, Thailand. All of cuttings were grown in sterilized coconut dust were inoculated with 40 g inoculum for mycorrhizal treatment, and 40 g sterilized inoculum as a non-mycorrhizal treatment.

Experimental design

The eucalyptus cuttings were transplanted into individual pots that filled with field soils, pH 4.87, EC 5.72 dS m^{-1}, 0.350 % of organic matter, total N 195.30 ppm, total P 50.05 ppm and total 5,948.98 mg kg^{-1}, exchangeable Ca 100 mg kg^{-1} and Na 464.26 mg kg^{-1} . The experiment was a 3x3x4 complete factorial in a randomized complete block design (RCBD) with three salinity levels (10, 15 and 20 dS m^{-1}), three eucalyptus clones (H4, H8 and P6) and four AMF treatments (*Glomus* sp.2 KKU-BH-001, *Gigaspora albida* KKU-BP-001, *Gigaspora decipiens* KKU-BP-002 and a treatment without pre-inoculation). Each treatment had three replicates. After fourteen days, 5% of NaCl solution was added to the soil every seven days to increase the initial EC from 0.24 (0% NaCl) to 10, 15 and 20 dS m^{-1}, respectively. All Eucalyptus cuttings were watered with 1,000 mL every three days. Assessment of plant and fungal performance parameters was conducted at 90 days.

Plant growth measurement and biochemical analysis

The plant fresh and dry weight (g), plant height (cm), and plant symptoms were measured. The Eucalyptus roots were scanned by an Epson scanner V700 PHOTO and analyzed by WINRHIZO Pro2004a (REGENT Instruments Inc., Qc, Canada). Root colonization was calculated from roots stained with trypan blue [47] according to the method described by A Trouvelot, JL Kough and V Gianinazzi-Pearson [48]. Spore abundance (number of spores g^{-1} dry soil) was observed after sucrose centrifugation [49]. Plant N concentration was determined by the Kjeldahl method [50], while P and K concentrations were determined by the wet oxidation method [51] and Na concentration were determined by BS Martinez, AP de Oliveira, FGG Pedro, JC de Oliveira and RD Villa [52] .

Leaf relative water content (LRWC)

Leaf samples from the top of the plants at the end of the 90 days were used to determine the tolerance of the mycorrhizal and non-mycorrhizal plants at each salinity level, according to . We calculated LRWC using the following equation [53]:

LRWC (%) = (FW – DW / TW- DW) x 100

Where FW is leaf fresh weight, DW is leaf dry weight after 24 h of drying at 70 $^{\circ}$C, and TW is leaf turgid weight after being soaked in distilled water for 24 h.

Leaf chlorophyll concentration

Leaf chlorophyll concentration (chlorophyll a, chlorophyll b, and total chlorophyll) was determined by the method described by DI Arnon [54]. 0.5g of fresh leaf was ground with 20 mL of 80% acetone. The homogenate was then centrifuged at 4,000 rpm for 15 min. The supernatant was read using a spectrophotometer (Thermo Scientific GENESYS 10S UV/Vis Spectrophotometer, model EW-02654-22) at 645 (Chlorophyll a) and 663 nm (Chlorophyll b). The chlorophyll content was calculated using the following formulae:

Chlorophyll a (mg/L) = (12.7 x OD663) – (2.69 x OD645)

Chlorophyll b (mg/L) = (22.9 x OD645) – (4.68 x OD663)

Total chlorophyll (mg/L) = (8.02 x OD663) + (20.2 x OD 645)

Chlorophyll concentrations are given in mg g^{-1} fresh leaf weight.

Proline concentration

Proline determination was performed according to the method described by LS Bates, RP Waldren and ID Teare [55]. 0.5 g of fresh leaf material was homogenized in 10 mL of 3% sulfosalicylic acid and then sieved through Whatman's No. 1 filter paper. Then 2 mL filtrate solution were mixed with 2 mL of acid-ninhydrin and glacial acetic acid in a test tube, respectively. The test tubes were placed in a water bath at 100 ^{o}C for 1 h and then placed in ice to stop the reaction. The mixture was extracted by 4 mL toluene and

the chromophore containing the toluene was separated to measure absorbance of 520 nm using a Thermo Scientific GENESYS 10S UV/Vis Spectrophotometer (model EW-02654-22). The calculated proline concentration was then compared with the proline standard.

Statistical analysis

The treatment effects were tested by three-way analysis of variance (ANOVA) using the Statistix program version 8.0. All data complied with the ANOVA assumptions of homoscedasticity and normality. Means were compared between treatments using the Tukey's Honestly Significant Difference (HSD) at a 0.05 probability level

Abbreviations

AMF:arbuscular mycorrhizal fungi; EC:electro-conductivity; S:salinity; V:variety; SFW:shoot fresh weight; SDW:shoot dry weight; RFW:root fresh weight; RDW:root dry weight; PH:plant height; RL:root length; RS:root surface; LRWC:leaf relative water content; RC:AMF root colonization; TS:total spore; Chl. a:chlorophyll a; Chl. b:chlorophyll b; T Chl.:total chlorophyll; N:nitrogen; P:phosphorus; K:potassium; Mg:magnesium; NaCl:sodium chloride; HSD:honestly significant difference; C:control; ns:non-significantly; FW:fresh weight; DW:dry weight; TW:leaf turgid weight

Declarations

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Authors' contributions

C.K. and S.B. designed, conducted the experiments, interpreted data and wrote the manuscript. T.W.K. and S.L. revised manuscript. All of authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, all table files are only available for download from the Supplementary Files section.

Figures

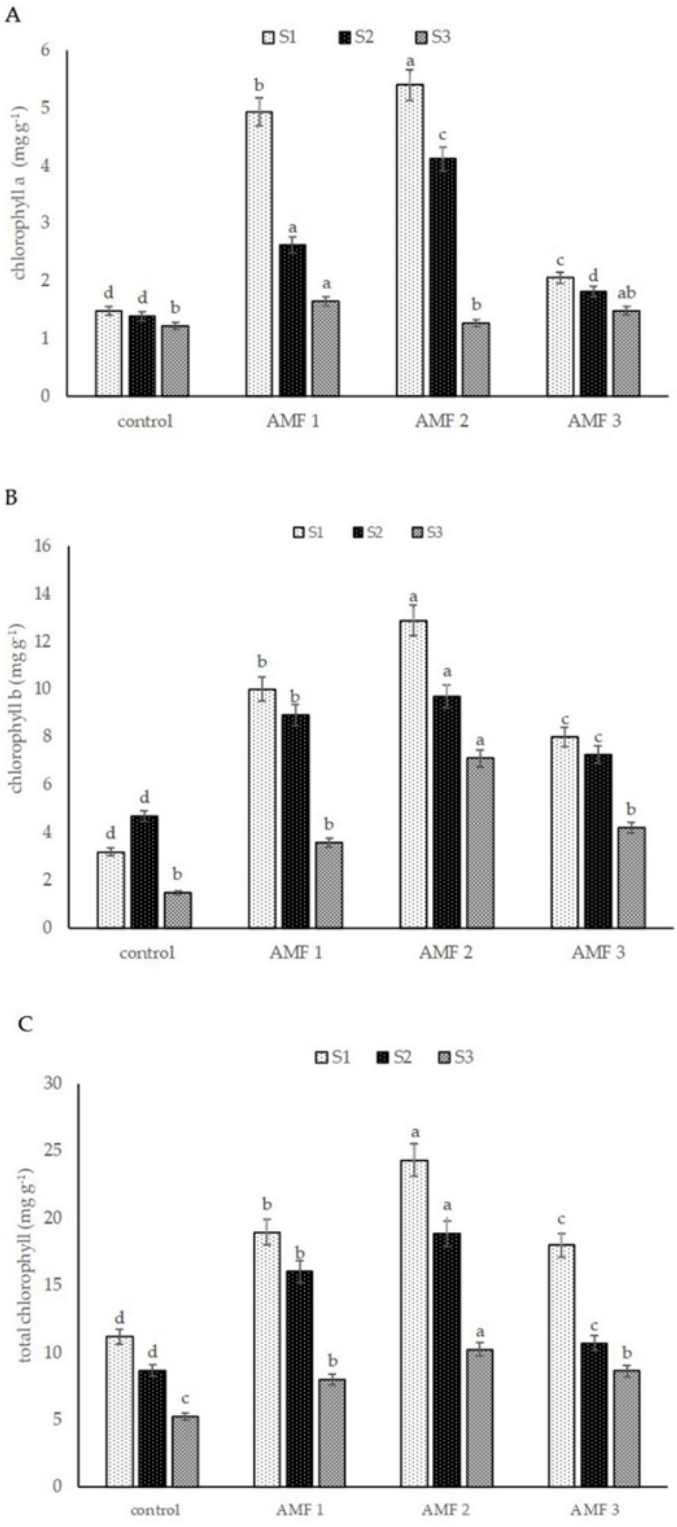

Figure 1

Effect of AMF inoculation and NaCl stress on leaf chlorophyll, A; chlorophyll a, B; chlorophyll b and C; total chlorophyll concentration of eucalyptus H4 after cultivated for 90 days. Mean values shown, in which the same letters above the bars represent no significant difference, according to HSD at $P \leq 0.05$. Abbreviation: AMF1; Glomus sp.2, AMF2; Gigaspora albida, AMF3; Gigaspora decipean, control; non-AMF inoculation, S1; 10 dS m-1, S2; 15 dS m-1, S3; 20 dS m-1

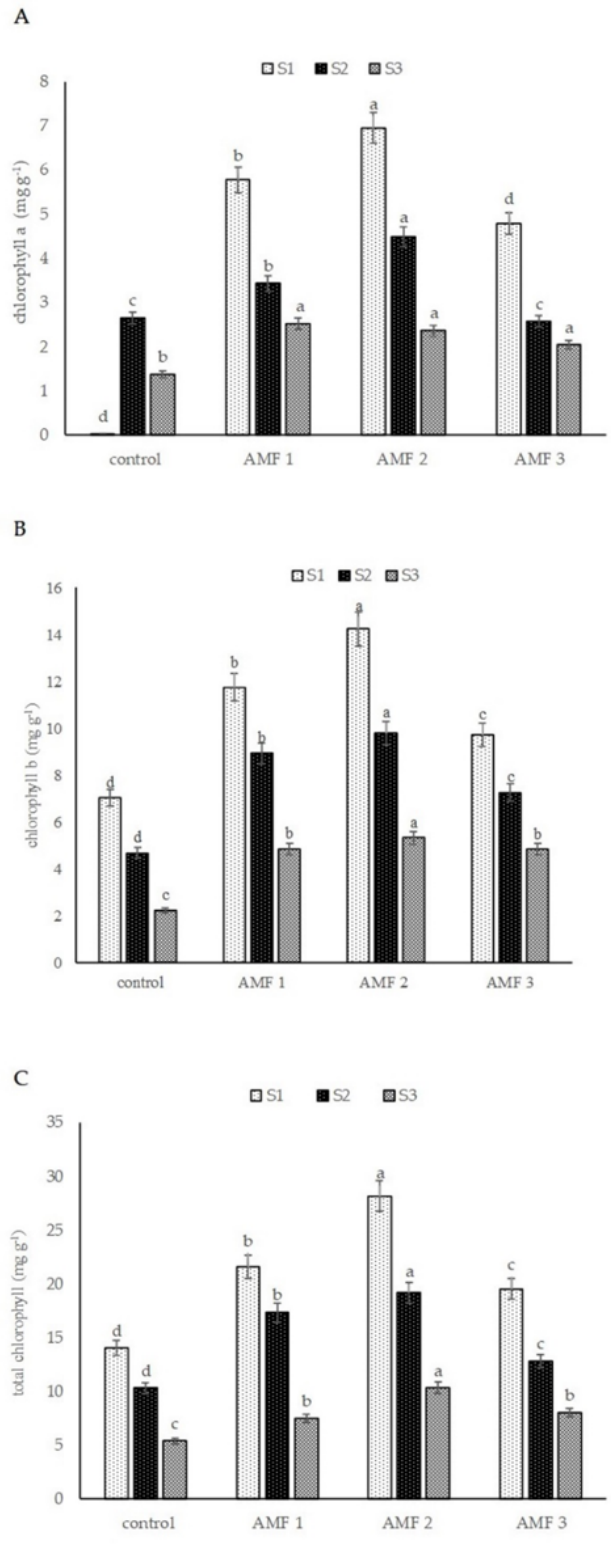

Figure 2

Effect of AMF inoculation and NaCl stress on leaf chlorophyll A; chlorophyll a, B; chlorophyll b and C; total chlorophyll concentration of eucalyptus H8 after cultivated for 90 days. Mean values shown, in which the same letters above the bars represent no significant difference, according to HSD at $P \leq 0.05$.
Abbreviation: AMF1; Glomus sp.2, AMF2; Gigaspora albida, AMF3; Gigaspora decipean, control; non-AMF inoculation, S1; 10 dS m-1, S2; 15 dS m-1, S3; 20 dS m-1.

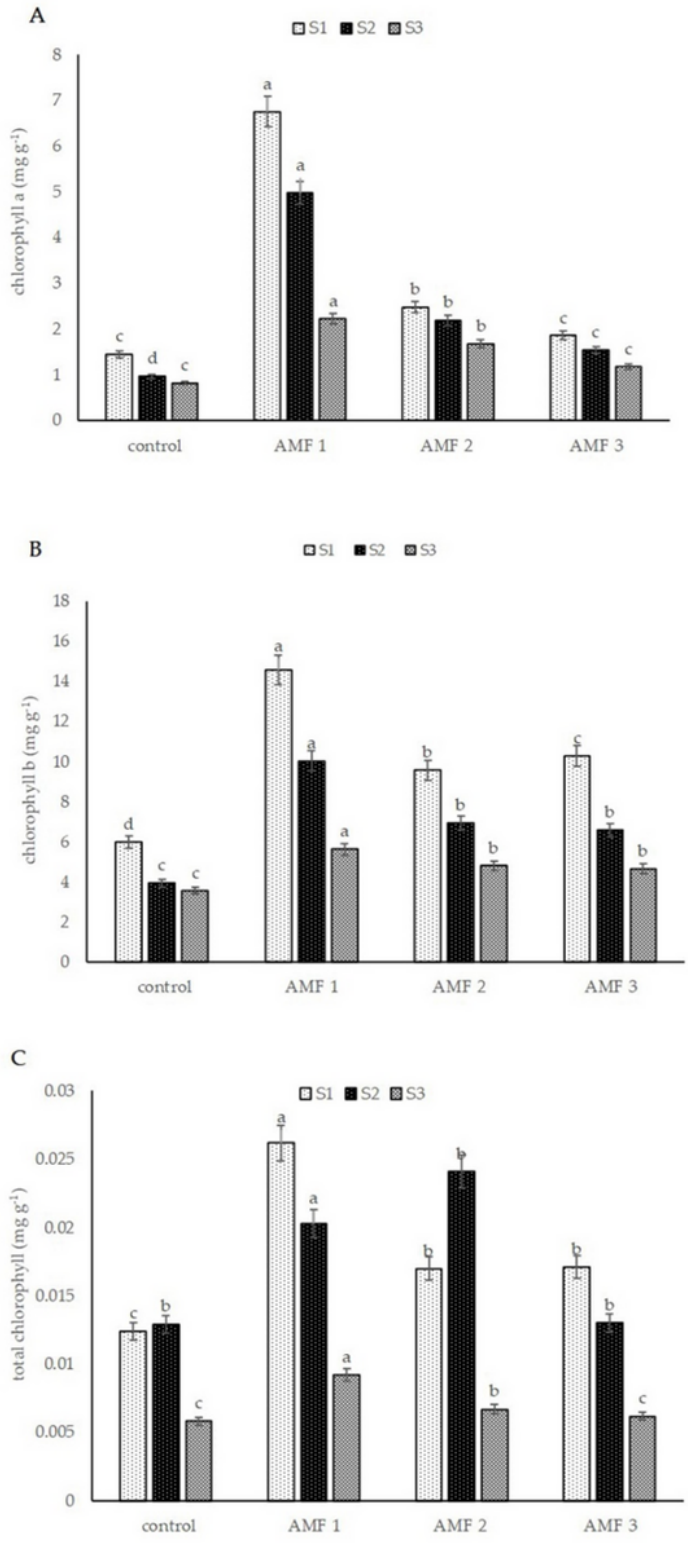

Figure 3

Effect of AMF inoculation and NaCl stress on leaf chlorophyll A; chlorophyll a, B; chlorophyll b and C; total chlorophyll concentration of eucalyptus P6 after cultivated for 90 days. Mean values shown, in which the same letters above the bars represent no significant difference, according to HSD at $P \leq 0.05$.
Abbreviation: AMF1; Glomus sp.2, AMF2; Gigaspora albida, AMF3; Gigaspora decipean, control; non-AMF inoculation, S1; 10 dS m-1, S2; 15 dS m-1, S3; 20 dS m-1.

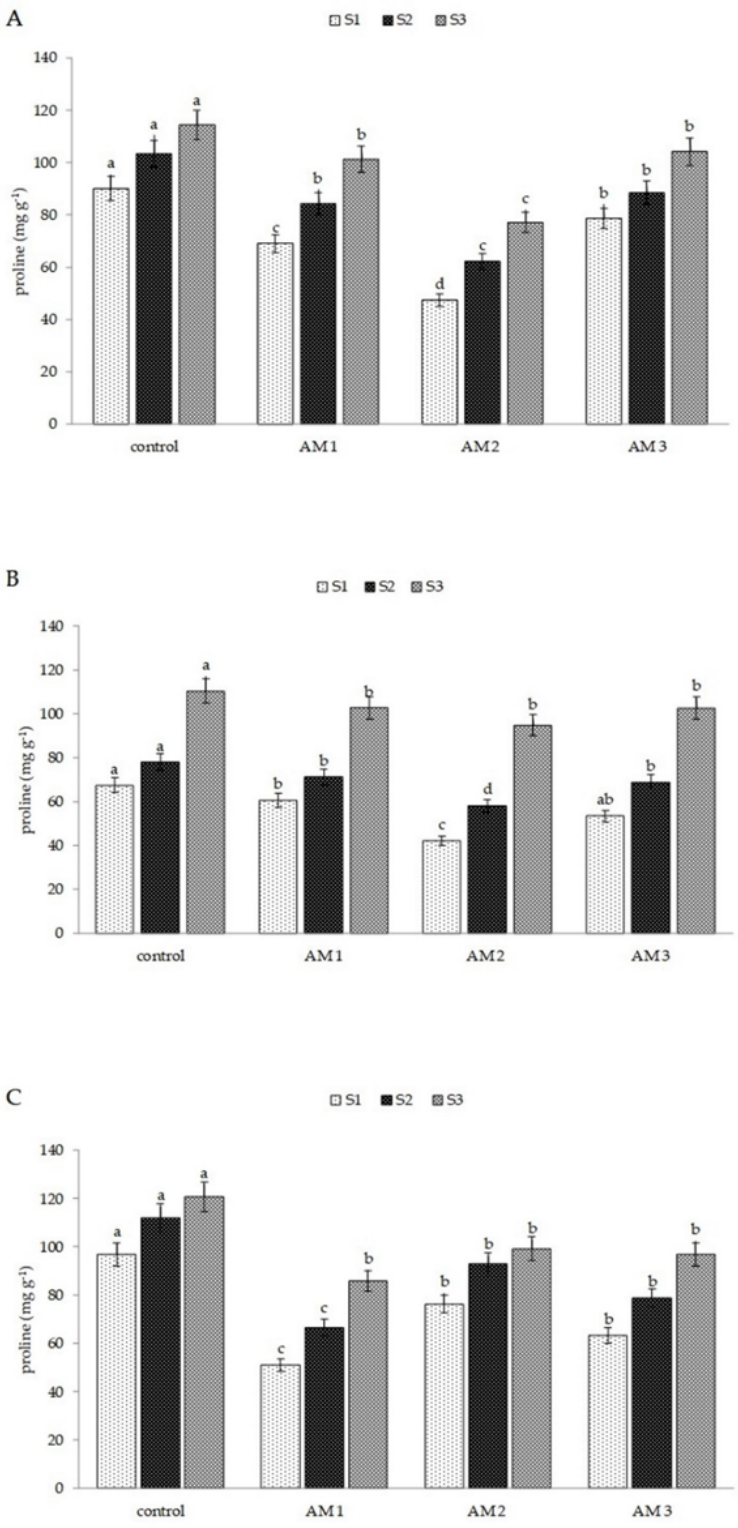

Figure 4

Effect of salinity level and AMF inoculation on leaf proline concentration of eucalyptus clone H4 (A), H8 (B) and P6 (C) 90 days after planting. Mean values shown, in which the same letters above the bars represent no significant difference, according to HSD at P≤0.05. Abbreviation: AMF1; Glomus sp.2, AMF2; Gigaspora albida, AMF3; Gigaspora decipean, control; non-AMF inoculation, S1; 10 dS m-1, S2; 15 dS m-1, S3; 20 dS m-1.

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