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Response of growth and salinity tolerance of *Nauclea orientalis* L. seedlings to arbuscular mycorrhizal fungi

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ABSTRACT. The purpose of this study was to determine the effectiveness of AMF types on the increasing growth of Lonkida (*Nauclea orientalis* L.) plants under salinity stress conditions. This study was carried out in the plastic home of the Indonesian Mycorrhizal Association Southeast Sulawesi branch, Kendari City and Forestry Laboratory, for five months, march - July 2019. This study used a Factorial Completely Randomized Design consisting of 3 replications and three plant units. The first factor included treatment without AMF, *Acaulospora* sp1. and *Clorideglomus etunicatum*. The second factor includes Salinity 0 mM, 50 mM, 100 mM, 150 mM and 200 mM. The results showed that the interaction of AMF and salinity was not effective in increasing plant growth. Inoculation of AMF type *C. etunicatum* can increase height, plant dry weight, root shoot ratio, seed quality index, and root colonization. *N. orientalis* has a high dependence on arbuscular mycorrhizal fungi. Giving 0 mM salinity increases height, diameter, number of leaves, plant dry weight, and seed quality index

Keywords: *Clorideglomus etunicatum, Lonkida, Salinity, Indonesia*

INTRODUCTION

Environmental stress is a limiting factor in the process of plant growth. In biological terms, stress means deviations in physiological processes, development, and function of plants that can be harmful and can cause irreversible damage to plant systems (Sopandie, 2013). One form of environmental stress is salinity. Salinity is when the soil experiences excess salt accumulation, especially on the surface. High salt content (saline soil) results from the formation of dissolved salt minerals, salt accumulation from irrigation that carries salt, intrusion of seawater, rivers, or lakes (Mindari, 2009). Salt stress (saline soil) can cause abnormal plant growth by disrupting physiological mechanisms such as decreased photosynthetic efficiency, gas exchange, membrane disturbances, water status, and others (Evelin *et al*., 2009).

An alternative to overcome problems related to salinity stress for plants is utilizing microorganisms such as mycorrhizae. Arbuscular mycorrhizal fungi (AMF) are roots symbionts in symbiosis with most higher plants and are generally found in terrestrial ecosystems (Smith and Read, 2008). AMF plays a role in increasing the ability of plants to cope with environmental stresses that generally occur in degraded ecosystems (Giri *et al*., 2003). AMF can reduce the detrimental effects of salinity (Feng *et al*., 2002) increase plant productivity by about 25-50%, which includes plant health, yield quality, tolerance to water stress, fertilization efficiency and can suppress the development of pathogenic microbes in the soil (Ansiga *et al*., 2017).

Research conducted by Husna *et al*. (2015) showed that the AMF treatment had a very significant effect on the variables of height, diameter, number of leaves, and number of plant root nodules. Local AMF inoculation increased the average growth in height and diameter of seedlings with an increase of 139% and 37%, respectively, against the control. In addition, local AMF inoculation of Glomus sp. and *Acaulospora tuberculata* are also known to increase plant growth under stress conditions such as serpentine soil media and heavy metal uptake (Tuheteru *et al*., 2017). According to Sundari *et al*. (2011), *Glomus* AMF has the most expansive distribution area and is the most tolerant of stress conditions such as soil salinity.

Lonkida (*Naucleaorientalis* L.) is a tropical tree species that generally live in wetlands and grows naturally in Indonesia. Lonkida plants thrive on three soil types, namely inceptisols, alfisols, and oxisols (Tuheteru *et al.,* 2014). The selection of lonkida plant species in this study was based on the ability of the plant to adapt to various conditions such as drought stress and inundation so that experiments could be carried out under salinity stress conditions. Many studies related to the growth of lonkida in inundation conditions have been carried out but are still limited to salinity stress conditions.

MATERIAL AND METHODS

Location and time of research. This research was carried out at the Mycorrhizal Association Plastic House, Southeast Sulawesi Branch, Kendari City, Southeast Sulawesi Province, and the Laboratory of the Faculty of Forestry and Environmental Sciences, Halu Oleo University, which lasted for five months, from March to July 2019.

Research design. The research design used in this study was a completely randomized design (CRD) consisting of 2 factors, namely AMF inoculation (Control, *Acaulospora* sp1, and *Clarodeoglomusetunicatum)* and salinity (0, 50, 100, 150 mM NaCl). Each treatment consisted of 3 replications, and each replication consisted of 3 plant units, so the number of treatment units was 135 experimental units.

Research procedure

Preparation of growing media for seeds and seedlings. River sand and soil are cleaned and then sterilized by heating the media at a specific temperature using a sterilizer. Meanwhile, rice husks are first burned to become husk charcoal.

Lonkida seed preparation and germination. The seeds of *N. orientalis* were obtained directly from the bottom of the stand. Before planting, the seeds were extracted in running water to separate the seeds from the fruit pulp, and the seeds were air-dried and stored in a cooler to maintain their viability. After that, the seeds are ready to be sown/planted in the sprout tub.

Weaning of seedlings and inoculation of mycorrhizal fungi. It was done when the first or second leaves were fully open. The seedlings were weaned into polybags containing soil, sand, and husk charcoal (6:1:3) and inoculated with mycorrhizal fungi. Each polybag was given 5 grams of AMF and buried in the soil under the roots.

Salinity treatment. Seedlings that had been weaned were grown for six weeks and then treated with salinity by watering with NaCl solution at levels of 0, 50, 100, 150, and 200 mM as much as 49 mL/polybag. Each treatment level is equivalent to 0 grams, 2,925 grams, 5.85 grams, 8.775 grams, and 11.7 grams of NaCl/Liter of water. The following is a formula for converting the level of NaCl solution into grams/liter of water:

$$M=\frac{m}{\text{Mr NaCl}} \text{ X } \frac{1000}{V}$$

Note :
M = Molaritas
m = Massa NaCl
Mr Nacl = Molekul Relatif NaCl (58,5)
V = Volume Pelarut

Watering of NaCl solution on plants is carried out every day for the first week. NaCl is given once a week for two months in the following week. Before applying salinity the following week, regular watering is carried out first to all polybags of about 50 mL/polybag every day to prevent build-up salt outside the experimental concentration.

Analisis data. The results of observations on each unit of observation will be analyzed in advance by analysis of variance (F test). If the test results show a real effect then different treatments will be conducted according to the Duncan Multiple Range Test (DMRT) at a 95% significant level.

RESULT AND DISCUSSION

Research result

The results of the variance of the effect of AMF treatment and salinity on the observed variables are presented in Table 1. Table 1 shows that the interaction of AMF inoculation and salinity did not significantly affect all observed variables except root colonization and mycorrhizae inoculation effect (MIE). The provision of AMF inoculation had a very significant effect on all observed variables. Meanwhile, salinity has a very significant effect on all observation variables except the seed quality index.

Table 1. Recapitulation of Print Results for Variable Observations

No.	Observation Parameter	AMF (A)	Salinity (B)	A*B	KK (%)
1	height	**	**	ns	17.29
2	Diameter	**	**	ns	15.69
3	Number of Leaves	**	**	ns	13.59
4	Shoot Dry Weight	**	**	ns	5.28
5	Root Dry Weight	**	**	ns	9.95
6	Total Dry Weight	**	**	ns	9.83
7	Root Shoot Ratio	**	**	ns	11.55
8	Relative Growth Of Shoots(RGRs)	**	**	ns	0.04
9	Relative Growth Of Roots(RGRr)	**	**	ns	0.17
10	Relative Growth OfTotal (RGRt)	**	**	ns	0.20
11	*Mycorrhizae inoculation effect* (MIE)	**	**	**	11.59
12	Seed Quality Index	**	*	ns	4.72
13	Colonization	**	**	**	15.92

Description: * =Significant effect
** = Very significant effect
ns = No significant effect

Plant growth

Inoculation of *Acaulospora*and *C. etunicatum* AMF inoculations significantly increased the diameter and number of five-month-old lonkida leaves and significantly different from the control (Table 2). Treatment with 0 mM salinity significantly increased growth in height, diameter and number of leaves and was significantly different from other treatments except for 50 mM salinity in stem diameter. The salinity treatment on the variables of height, diameter and number of leaves showed that the susceptibility index had a negative effect on all treatments

Table 2. Effect of Treatment on Growth of Lonkida (*N. orientalis*) Seedlings Age five months.

Treatment	Height (cm)	Diameter (mm)	Number of leaves
AMF Inoculum (A)			
Control	12.79 ± 1.46^{b}	3.30 ± 0.26^{b}	7.93 ± 0.76^{b}
Acaulospora sp1.	21.82 ± 1.34^{a}	6.61 ± 0.20^{a}	10 ± 0.58^{a}
C. etunicatum	22.40 ± 1.30^{a}	6.07 ± 0.31^{a}	9.93 ± 0.64^{a}
Salinity (B)			
Control	26.15 ± 1.74^{a}	6.3 ± 0.54^{a}	13.61 ± 0.58^{a}
50 Mm	$19.78 \pm 2.63^{b(-)}$	$5.54 \pm 0.74^{ab(-)}$	$8.77 \pm 0.55^{b(-)}$
100 Mm	$18.41 \pm 1.69^{bc(-)}$	$5.13 \pm 0.58^{b(-)}$	$8.33 \pm 0.55^{b(-)}$
150 mM	$15.73 \pm 2.10^{cd(-)}$	$4.91 \pm 0.61^{b(-)}$	$8.22 \pm 0.58^{b(-)}$
200 mM	$14.96 \pm 1.19^{d(-)}$	$4.76 \pm 0.42^{b(-)}$	$7.50 \pm 0.42^{b(-)}$
CV	17.29	15.69	13.59

Note: The same letter in the same column shows no significant difference according to the DMRT test (α = 0.05), CV (Coefficient of variant), ± Standard Error, mM (Molar Mass), Sign (+) indicates that the susceptibility index has an effect when salinity treatment lower the value of the measured variable compared to the control, and vice versa (-).

Plant Dry Weight

The results showed that *C. etunicatum* AMF inoculation increased root, shoot and total dry weight of N. orientalis seedlings compared to controls and was not significantly different from Acaulospora sp1 except for root dry weight variables (Table 3). The 0 mM salinity treatment increased root dry weight, shoot dry weight and total dry weight variables and was significantly different from other treatments except for 50 mM salinity treatment for root dry weight variables. Salinity treatment on the variables of shoot dry weight, root and total dry weight showed that the susceptibility index had a negative effect on all treatments.

Table 3. Effect of AMF Types of Treatment and Salinity on Dry Weight Variables of Lonkida (N. orientalis) Seedlings at five Months Age.

Treatment	Plant Dry Weight (g)		
	Root	Shoots	Total
AMF Inoculum (A)			
Control	0.37 ± 0.05^{c}	1.01 ± 0.16^{b}	1.38 ± 0.21^{b}
Acaulospora sp1.	0.56 ± 0.02^{a}	2.07 ± 0.14^{a}	2.63 ± 0.16^{a}
C. etunicatum	0.48 ± 0.02^{b}	2.14 ± 0.19^{a}	2.62 ± 0.20^{a}
Salinity (B)			
Control	0.59 ± 0.03^{a}	2.63 ± 0.24^{a}	3.23 ± 0.25^{a}
50 Mm	$0.49 \pm 0.04^{ab(-)}$	$1.79 \pm 0.26^{b(-)}$	$2.28 \pm 0.29^{b(-)}$
100 mM	$0.48 \pm 0.04^{b(-)}$	$1.66 \pm 0.17^{bc(-)}$	$2.14 \pm 0.20^{b(-)}$
150 mM	$0.36 \pm 0.06^{c(-)}$	$1.29 \pm 0.26^{d(-)}$	$1.66 \pm 0.32^{c(-)}$
200 mM	$0.43 \pm 0.03^{bc(-)}$	$1.31 \pm 0.20^{cd(-)}$	$1.75 \pm 0.22^{bc(-)}$
CV	5.28	9.95	9.83

Note: The same letter in the same column shows no significant difference according to the DMRT test ($\alpha = 0.05$), CV (Coefficient of variant), ± Standard Error, mM (Molar Mass), Sign (+) indicates that the susceptibility index has an effect when salinity treatment lower the value of the measured variable compared to the control, and vice versa (-).

Colonization and Mycorrhizae Inoculation Effect (MIE)

The highest AMF colonization was found in the interaction of *C. etunicatum* AMF and 50 mM salinity, which was 59.03% and significantly different from other treatments (Table 4). Except for the interaction of *Acaulospora* sp1 AMF and 150 mM salinity, the highest value for the MIE variable was 80.98% and significantly different from other treatments except for the *C. etunicatum* AMF interaction and 150 mM salinity.

Table 4. Observations of the Interaction of AMF and Salinity on Colonization Variables and Mycorrhizae Inoculation Effect of N. orientalis five months old.

Treatment		Colonization	MIE (%)
AMF Inoculum (A)	Salinity (B)		
Control	0 mM	0.00[h]	-
	50 mM	13.06±4.35[f]	-
	100 mM	4.90±3.92[g]	-
	150 mM	4.30±2.87[g]	-
	200 mM	17.63±7.28[def]	-
Acaulospora sp1.	0 mM	24.40±6.16[cde]	32.26[d]
	50 mM	25.26±1.83[cd]	46.80[c]
	100 mM	29.80±3.14[bc]	38.04[d]
	150 mM	17.26±3.93[def]	80.98[a]
	200 mM	13.46±2.36[ef]	44.39[c]
C. etunicatum	0 mM	30.96±2.67[bc]	31.85[d]
	50 mM	59.03±5.07[a]	54.17[b]
	100 mM	36.93±5.26[bc]	37.46[d]
	150 mM	43.00±5.46[ab]	78.72[a]
	200 mM	10.83±3.45[f]	34.76[d]

Note: The same letter in the same column shows no significant difference according to the DMRT test (α = 0.05), CV (Coefficient of variant), ± Standard Error, mM (Molar Mass).

Root Shoot Ratio (NPA) and Lonkida Seed Quality Index (IMB)

AMF inoculum type *C. etunicatum* was able to increase NPA higher than the control and was not significantly different from Acaulospora sp1 (Table 5). Inoculation of *Acaulospora* sp1 AMF inoculation increased IMB variables better and significantly different than all treatments. The 0 mM salinity treatment increased the root shoot ratio and seed quality index. However, it was not significantly different from the 50 mM and 100 mM salinity treatments on the IMB variable. Treatment of 0 mM salinity showed significantly different results to all treatments on the NPA variable. The salinity treatment on the NPA and IMB variables showed that the susceptibility index had a negative effect on all treatments.

Tabel 5. Effect of Treatment on Root Root Ratio (NPA) and Seed Quality Index (IMB) of Lonkida Seedlings Age five months.

Treatment	NPA	IMB
AMF Inoculum (A)		
Control	2.47±0.27[b]	0.21±0.03[c]
Acaulospora sp1.	3.62±0.19[a]	0.38±0.02[a]
C. etunicatum	4.32±0.25[a]	0.32±0.02[b]
Salinity (B)		
Control	4.44±0.34[a]	0.37±0.03[a]
50 mM	3.48±0.41[b(-)]	0.31±0.04[ab(-)]
100 mM	3.44±0.21[b(-)]	0.30±0.03[ab(-)]
150 mM	2.99±0.50[b(-)]	0.25±0.04[b(-)]
200 Mm	2.98±0.31[b(-)]	0.27±0.03[b(-)]
CV	11.55	4.72

Note: The same letter in the same column shows no significant difference according to the DMRT test (α = 0.05), CV (Coefficient of variant), ± Standard Error, mM (Molar Mass), Sign (+) indicates that the susceptibility index has an effect when salinity treatment lower the value of the measured variable compared to the control, and vice versa (-).

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Plant Relative Growth

Inoculation of both types of AMF significantly increased the relative growth of plants compared to control (Table 6). The 0 mM salinity treatment significantly increased the relative growth of plants and was significantly different from the other treatments, except for the 50 mM and 100 mM salinity treatments on root dry weight variables. Treatment of salinity on the relative growth of shoots, roots and total relative growth showed that the susceptibility index had a negative effect on all treatments.

Table 6. The Effect of AMF Inoculation Treatment and Salinity on the Relative Growth of 5 Months Lonkida Plants.

Treatment	RGRs	RGRr	RGRt
AMF Inoculum			
Control	0.0042^{b}	0.0016^{c}	0.0058^{b}
Acaulospora sp1.	0.0086^{a}	0.0023^{a}	0.0109^{a}
C. etunicatum	0.0090^{a}	0.0020^{b}	0.0110^{a}
Salinity (B)			
Control	0.010^{a}	0.0024^{a}	0.013^{a}
50 Mm	$0.007^{b(-)}$	$0.0020^{ab(-)}$	$0.009^{b(-)}$
100 mM	$0.0068^{bc(-)}$	$0.0020^{ab(-)}$	$0.008^{bc(-)}$
150 mM	$0.0056^{c(-)}$	$0.0018^{c(-)}$	$0.007^{c(-)}$
200 Mm	$0.0054^{c(-)}$	$0.0015^{c(-)}$	$0.0074^{c(-)}$
CV	0.17	0.04	0.2

Note: The same letter in the same column shows no significant difference according to the DMRT test (α = 0.05), KK (Coefficient of variant), ± Standard Error, mM (Molar Mass), Sign (+) indicates that the susceptibility index has an effect when salinity treatment lower the value of the measured variable compared to the control, and vice versa (-).

Discussions

The results showed that five-month-old Lonkida (Naucleaorientalis L.) seedlings were colonized by Arbuscular Mycorrhizal Fungi (AMF). AMF treatment gave a better effect than treatment without AMF. AMF colonization is characterized by AMF structures in the form of external and internal hyphae in five-month-old Lonkida seedlings. This structure results from fungal infection in plant roots (Sastrahidayat, 2011). Based on the percentage of observations, AMF colonization could increase the growth of 5-month-old Lonkida seedlings compared to controls. Treatment colonization ranged from 0-59.03%. The highest colonization percentage was found in the interaction of *C. etunicatum* AMF and 50 mM salinity of 59.03% and belonged to the high category (Rajapakse and Miller, 1992). The high rate of AMF colonization in the 50 mM salinity treatment was thought to be due to the higher soil moisture factor than other treatments. This is in accordance with the opinion of Manurung and Kristina (2018) that the percentage of AMF colonization on plant roots generally increases when they are in wet conditions or high humidity. The higher the salinity level in the plant, the faster the plant will experience oxidative stress or water loss. Each type of plant has a different response to AMF. AMF type *C. etunicatum* contributed significantly to the growth of lonkida plants.

The interaction of AMF treatment and salinity was not effective in increasing the variables of height, diameter, number of leaves, and dry weight of lonkida plants. However, the difference in the salinity treatment level affected the plant's growth and dry weight. Lonkida plants without salinity treatment had higher total plant relative growth (RGRt) than other treatments. The average relative growth of total Lonkida seedlings in the treatment without salinity showed that Lonkida did not have a high tolerance for salinity stress conditions. This is different from the research results conducted by Plenchette and Duponnis (2005), which stated that AMF inoculated plants had better growth and biomass than non-mycorrhizal plants, especially under stress conditions such as salinity (Al-Karaki, 2000; Feng *et al*., 2002). This is thought to be due to the ability of AMF to reduce oxidative stress caused by salt, causing a reduction in root colonization, growth, leaf area, and plant chlorophyll content (Latef and Chaoxing, 2011).

Independently, *C. etunicatum* type AMF effectively increased plant height of *N. orientalis* compared to *Acaulospora*sp1. and control. The increase in plant height was thought to be because AMF type *C. etunicatum* could spread to plant roots very quickly to form higher root colonization (Ingleby *et al*., 2007 in Wulandari, 2019). Meanwhile, *Acaulospora* sp1 AMF increased growth parameters such as diameter and number of plant leaves (Table 3). This is in line with research by Delvian (2003), which reported that the application of AMF to *Leucaena leucocephala* seedlings could increase the height (177.61%), diameter (154.54%), canopy dry weight (174.68%), and plant dry weight. (186.59) when compared with plants without AMF administration. The results showed that 0 mM salinity effectively increased plant height, diameter, the number of leaves, and dry weight compared to 50 mM, 100 mM, 150 mM, and 200 mM salinity treatments. Presumably, the higher salinity level in plants can inhibit the growth and production of plant products. Due to a decrease in water storage capacity by plants, excessive Na and Cl toxicity, imbalances in nutrient absorption, changes, and deviations in leaf shape and anatomy, can interfere the process. Plant physiology includes photosynthesis (Van Hoorn *et al*., 2001 in Nasim, 2010; Parida and Das, 2005).

The presence of AMF in plant roots positively influences plant physiological aspects, and this is because AMF can assist plants in maximizing nutrient absorption. The results showed that the highest mycorrhizal dependence was found in *Acaulospora*sp1 AMF (80.98%) and *C. etunicatum* (78.72%) at a salinity level of 150 mM (Table 5). This indicates that the higher the salinity level given, the higher the level of dependence of plants on AMF. According to Setiadi (1992) in Saputri and Suwirmen (2016), plants with a high dependence on AMF inoculation will usually show a significant growth response to AMF inoculation.

On the other hand, plants cannot grow appropriately without symbiosis with AMF. AMF inoculation on five-month-old *N. orientalis* plants effectively increased shoot dry weight. This is in line with the research of Corkidi and Rincon (1997) in Tuheteru*et al*. (2012) that AMF can increase the growth of four types of tropical plants, especially on the dry weight of roots and shoots against the control. This is thought to be caused by AMF, which can absorb nutrients to be used in plant growth processes and plant metabolic processes such as photosynthesis by increasing the amount of leaf chlorophyll and P uptake (Pradyudyaningsih, 2004 In Wulandari, 2019; Tuheteru *et al*., 2012). P uptake can help the photosynthesis process of plants through the supply of energy in the form of ATP and NADPH, CO_2 acceptors, RUBP (Ribulose bisphosphate), and the ratio of sugar biosynthesis (Rychter and Rao, 2005 in Javaid, 2010).

The results showed that the highest shoot-to-root ratio was found in *C. etunicatum* AMF inoculations with an average value of 4.32 (table 6). According to Zahrul (2018), the high value of NPA indicates that the absorption of nutrients and water by plants is translocated to the shoots for the formation of the vegetative parts of plants. In the application of salinity, the highest root shoot ratio was found at 0 mM salinity, which was 4.44 compared to other treatments. This is presumably because high salinity levels can interfere with plant growth processes by reducing water availability and nutrient absorption. This is supported by the opinion of Ghafoor *et al*. (2004) in Sopandie (2006) that salinity disrupts plant growth and development through a decrease in the osmotic potential of the soil solution so that the availability of water for plants is reduced, spurring imbalances in nutrient metabolism and changes in the physical and chemical structure of the soil. Meanwhile, for the seed quality index (IMB), values ranged from 0.21-0.38 where the highest value was found in the *Acaulospora*sp1 AMF treatment of 0.38 (Table 6). As for the salinity treatment, the highest IMB value was found in the 0 mM salinity treatment (control). According to Junaedi*et al.* (2010) in Farida (2019) that seeds with NPA values ranging from (2-5) and IMB values (> 0.09) have met the criteria for planting in the field.

CONCLUSION

Based on the research that has been done, it can be concluded that the interaction of AMF and salinity was not effective in increasing the growth of N. orientalis plants. However, it was effective in increasing the percentage of colonization and the Mycorrhizae Inoculation Effect. AMF inoculation improved AMF inoculation improved plant growth. Independently giving salinity can reduce the variables of height, diameter, number of leaves, plant dry weight, and seed quality index.

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