

# RESPONSE OF LONKIDA (*Nauclea orientalis* L.) TOWARDS MYCORRHIZAL INOCULUM IN WATERLOGGED CONDITION

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## ABSTRACT

Waterlogged condition is one of environmental conditions not favorable for plants and mycorrhizal fungi. The tolerant species are more adaptive to waterlogged condition. The documented effects of Arbuscular Mycorrhizal Fungi (AMF) inoculation on wetland plant performance are inconsistent. Besides increasing the tolerance of plants, AMF also causes plants depression in waterlogged condition. This study was carried out to examine the morpho-anatomical adaptation, survival, growth, biomass and nutrient uptake of lonkida (*Nauclea orientalis* L.) from different habitats inoculated with Arbuscular Mycorrhizal Fungi (AMF) in waterlogged condition for 90 days. Experiment procedures included: seed germination, preparation of inoculums and inoculation of AMF and waterlogged treatment. The results showed that lonkida seedlings formed lenticels (100%), adventitious roots (41%) and aerenchym under waterlogged condition. Seedlings from dry land habitats, both mycorrhizal and non-mycorrhizal, had lower height growth rate than those from other habitat types. The same results happened for relative growth rates for shoot (RGRs), root (RGRr) and total (RGRt) dry weight and the ratio of root shoots. Treatments of temporal swamp habitat without waterlogged condition and with waterlogged condition and treatment in savannah with waterlogged condition had RGRt greater than those in other treatments. In general, plant dry weight (root, shoot and total) and total N in roots and shoots were greater in the interaction between temporal swamp habitat with non-mycorrhizal in waterlogged condition. However, lonkida seedling from dry land habitat required AMF under waterlogged condition to improve biomass and N accumulation in roots. Independently, waterlogged condition increased the pool of average height gain (18%), diameter (46%), leaf area (40%), leaf length (17%) and leaf width (21%). Nonetheless, waterlogged condition treatment decreased the number of leaves (9%) and AMF colonization (71%). The results of this study indicated that seedlings from temporal swamp had good growth and high biomass in condition with or without waterlogged condition.

**Keywords:** Arbuscular Mycorrhizal Fungi (AMF), lonkida, morpho-anatomical adaptations, *Nauclea orientalis* L., swamp, waterlogged condition

## INTRODUCTION

Waterlogged is one of environmental conditions not favorable for plants and mycorrhizal fungi (Helgason & Filters 2009). Waterlogged condition causes anaerobic condition indicated by hypoxia and anoxia (Elzenga & van Veen 2010). These conditions can affect growth, development and adaptability of plant species (Kreuzwieser & Gessler 2010;

Parolin & Wittman 2010) as well as species composition of forest stands (Kozłowski 1984, 1997). Effects of waterlogged condition is highly varied depending on genetic and age of the plants, time and duration of inundation (Kozłowski 1984) as well as depth of inundation (Iwanaga & Yamamoto 2008). Plant can tolerate waterlogged condition through morpho-anatomical and physiological adaptation mechanisms. Physiological adaptation can be done by accumulating reserves in the root in the form of carbohydrates and alcohol fermentation as an

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alternative (Kreuzwieser & Gessler 2010; Parolin & Wittman 2010), while morpho-anatomical adaptation is done by forming lenticels, adventitious roots and aerenchyma (Folzer *et al.* 2006; Parolin & Wittman 2010).

Plant tolerance to waterlogged condition varies and is determined by the species (Kozłowski 1984). The tolerant species are more adaptive to waterlogged condition (Kogawara *et al.* 2006). However, differences in habitat of the species also largely determine the success of the plant to survive in inundation condition (Ferreira *et al.* 2009; Nielsen *et al.* 2010). Several studies have reported that species growing naturally in the inundated area are more tolerant to waterlogged condition, such as *Nyssa sylvatica* (Keeley 1980), female *Populus angostifolia* (Nielsen *et al.* 2010) and *Himatanthus sucuba* (Ferreira *et al.* 2009). Information on tropical species tolerance and adaptation to inundation still needs to be studied (Parolin 2009). In Indonesia, research on tolerant species toward different types of inundated habitat is still very limited.

Lonkida (*Naudea orientalis* L.) is a tropical multipurpose tree species that grows naturally in a variety of habitat types including wetlands (Petty & Douglas 2010; Kartikasari *et al.* 2012) and has natural distribution in Indonesia (Whitmore *et al.* 1997; Keßler *et al.* 2002). This species has potential uses for agroforestry, phytoremediation and rehabilitation of degraded land, including wetlands (Marghescu 2001; Amihan-Vega & Mendoza 2005; Mawaddah 2012) and medicinal plant (Lim 2013). A preliminary study of lonkida response to waterlogged condition in greenhouse scale had been conducted and it was confirmed that this species was tolerant to waterlogged condition (Kurniawati 2011). However, studies related to growth response, adaptation, and plant nutrient uptake ability of lonkida from various habitats under waterlogged condition had not been reported.

This species was also reported to be colonized by Arbuscular Mycorrhizal Fungi (AMF) *Glomus mossea* in India (Sudha & Ammani 2010) and showed good growth response to the application of AMF in waterlogged condition (Kurniawati 2011). Tolerance to waterlogged condition could be enhanced through AMF inoculation. Association of inundated plants with AMF is part of strategy to improve nutrient uptake and oxygen circulation (Elzenga & van Veen 2010). In

waterlogged condition, AMF association could improve growth (Osundina 1998; Fougnes *et al.* 2007), biomass and nutrient uptake, especially phosphorus (Muok & Ishii 2006; Fougnes *et al.* 2007), the growth of root systems (Qiang-Sheng *et al.* 2013) and the structure of plant communities (Bauer *et al.* 2003). However, colonization, abundance and benefits of AMF in waterlogged condition can vary among plant species (Bauer *et al.* 2003) due to species of AMF used (Secilia & Bagyaraj, 1994; Sah *et al.* 2006), availability of phosphorus (Stevens *et al.* 2002; Garcia *et al.* 2008) and distance from the AMF rhizosphere (Keeley 1980). Biomass of mycorrhizal *Nyssa sylvatica* seedlings from inundated seed sources were higher than that from dry areas (Keeley 1980).

The purpose of this study was to examine the morpho-anatomical adaptation, survival, growth, biomass production and nutrient uptake of AMF-inoculated lonkida originated from different habitat types in waterlogged condition.

## MATERIALS AND METHODS

### Experiment Procedures

#### *Seed germination*

Lonkida seeds were collected from mother trees grown in different habitat types (swamps, temporary swamps, temporary swamps savannah and dry land areas) in Kendari City, Konawe and South Konawe District (Fig. 1), Southeast Sulawesi Province. Lonkida seeds were germinated in plastic boxes having dimension of 20x20x5 cm containing sterile soil media ( $\pm 1$  kg) and were grown until reaching seedling stage at age of 150 days before being used in this experiment. There were 144 seedlings ready to be used in this experiment.

#### *Inoculum preparation and AMF inoculation*

AMF inoculum used was mycofer IPB containing 4 (four) species of AMF, namely *Glomus etunicatum*, *Glomus manihotis*, *Acaulospora tuberculata* and *Gigaspora margarita*. The number of spores per 5 g of inoculum was 57-125 spores. Before AMF inoculation, polybags having size of 15x20 cm were filled with sterile soil media (1.5 kg soil media/bag). There were 72 lonkida seedlings having age of 150 days inoculated with AMF inoculum and subsequently were called mycorrhizal seedlings. The other 72 lonkida

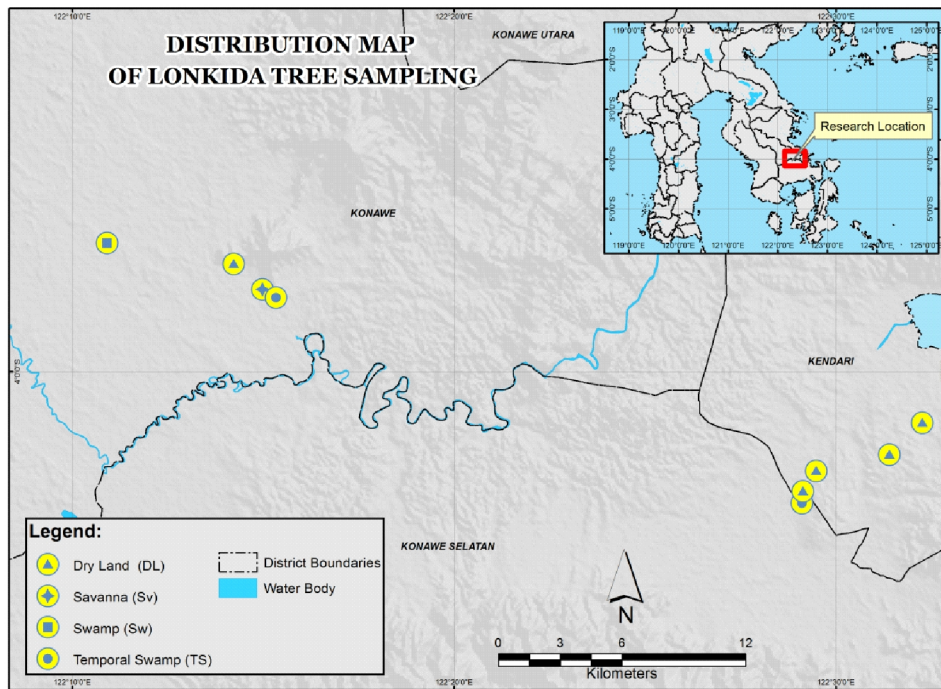


Figure 1. Distribution map of lonkida tree sampling

seedlings were not inoculated with AMF inoculum and served as control treatment; subsequently were called non-mycorrhizal seedlings. Chemical properties of the sterile soil media were pH 5.2, organic C (Walkey & Black) 0.27%, total N (Kjeldahl) 0.06%, C/N ratio 5, available P (Bray I/II) 2.5 ppm, exchangeable Ca, Mg, K, Na, and CEC were 10.32 cmol/kg, 1.49 cmol/kg, 0.66 cmol/kg, 1.76 cmol/kg and 19.79 cmol/kg (Extract Buffer 1.0 N  $\text{NH}_4\text{OAc}$  pH 7.0), respectively.

#### Waterlogged treatment

Mycorrhizal and non-mycorrhizal seedlings at age of 150 days after planting were then treated with waterlogged condition. Each seedling was placed in a polybag having size of 26.5x23 cm containing sterile soil media. A total of 72 seedlings inside polybags were immersed in water, while the other 72 seedlings inside polybags were not immersed, but watered every day as control (Garcia *et al.* 2008). Water level was monitored every 3 days.

#### Experimental design

This study used factorial in a completely randomized design (CRD) consisting of 3 factors:

1. Treatment A = habitat origin of lonkida consisted of:
  - $A_0$  = dry land;
  - $A_1$  = swamp;

$A_2$  = temporal swamp;

$A_3$  = temporal swamp savannah.

2. Treatment B = AMF inoculum treatment consisted of:

$B_0$  = uninoculated (non-mycorrhizal);

$B_1$  = inoculated with AMF inoculum (mycorrhizal).

3. Treatment C = waterlogged treatment consisted of:

$C_0$  = control (non-waterlogged);

$C_1$  = waterlogged.

Each treatment was replicated 3 times and each replication consisted of 3 test plants. The experiment was conducted for 90 days. At the end of experiment, the seedlings' age was 240 days old.

#### Data Collection

##### Growth

Measurements were conducted at the end of the experiment (at the end the 90-day experiment) for seedling height, seedling diameter, number of leaves per seedling and plant survival. Measurement of leaf area was conducted using green leaf area meter GA-5 model. Relative growth rates (RGR) for total (RGTt), shoots (RGTs) and roots (RGT<sub>r</sub>) dry mass for control and waterlogged condition were then calculated using the formula of Mendoza *et al.* (2005):

$$RGR_i = (\ln Wt_i - \ln Wt_0) / (t_i - t_0)$$

where:

RGR= relative growth rate of plants;

i= total dry weight, shoots and roots;

Wt<sub>i</sub> = plant dry weight (total, shoots and roots) at the end of the experiment (seedlings' age was 240 days old);

Wt<sub>0</sub> = plant dry weight at the beginning of experiment period (seedlings' age was 150 days old);

t<sub>i</sub> - t<sub>0</sub> = the difference between the initial and the total period of growth (experiment period was 90 days).

#### *Dryweight and nutrients in plant tissue*

At the end of 90 days, the shoots and roots were harvested. Fresh shoots and roots were oven-dried at 70 °C for 48 hours (Garcia *et al.* 2008). Determination of P was carried out using HNO<sub>3</sub>-HClO<sub>4</sub> method, and N by Kjeldahl (Balai Penelitian Tanah 2009). For N and P, two expressions of nutrient uptake efficiency was calculated i.e. concentration per unit of dry weight and total (accumulation) uptake per unit of dry weight.

#### *Morpho-anatomical adaptation*

Observation and counting of the number of lenticels and adventitious roots were done every week using non-destructive samples. The data of lenticels and adventitious roots were obtained at the end of experiment. The proportion data of lenticels number and adventitious roots were the ratio between the number of samples forming lenticels and adventitious roots. Aerenchyma observations for the lenticels and adventitious roots were made by following the method of freezing microtome (Longstreth 1879). Roots of 3 cm long (diameter < 3 mm) were immersed in 70% alcohol solution (24 hours). The roots were then cut using scissors into root pieces of 0.5 cm long. The root pieces were then put in Yamato Mc-802A Electro Freezer for 2-3 minutes at -15 °C. The roots were then cut using Yamato RV-240 microtome with thickness of 20 µm. Before the roots were placed on object glasses, several drops of 20% glycerol solution were applied on the object glasses, while the roots were stained with safranin. Aerenchyma observation was made

using Axio Imager microscope A1m/Axiocam MRC5 (200x magnification).

#### *Susceptibility Index (SI)*

For all measured plants and soil variables, Susceptibility Index (SI) was calculated using the method of Hiler *et al.* (1972):

SI = 1 - (waterlogged plant / non-waterlogged plant)  
SI value will be positive if the waterlogged condition decreases the variables measured. On the other hand, SI value will be negative if waterlogged condition increases the variables measured.

#### *AMF spore density and colonization*

Thirty grams of soil samples were collected from soil medium in polybags used as mycorrhizal lonkida medium. Spores were extracted from soil using wet sieving and decanting method (Gerdemann & Nicolson 1963) followed by centrifugation; supernatant acquired was added with 50% sugar solution (Walker *et al.* 1982). AMF spores extracted were observed and counted under a dissecting microscope with 35x magnification. Root colonization was observed by using root staining technique of Brundrett *et al.* (1996). Fresh roots were cleaned in 10% KOH for 2 days, then were immersed in H<sub>2</sub>O<sub>2</sub> for 10-20 minutes and were rinsed thoroughly with water. The roots were then soaked in HCl 0.2% for 20 minutes and then were stained with trypan Blue solution (0.05%). Ten root samples (1 cm long) from each plant were observed under a microscope with 200x magnification. Root colonization was calculated using the formula of Brundrett *et al.* (1996):

% root colonization =

$$\frac{\text{Number of fields-of-view containing mycorrhizae}}{\text{Total observed fields-of-view}} \times 100\%$$

#### **Data Analysis**

Data were analyzed using analysis of variance (ANOVA). Abnormal data were normalized using logarithmic transformation. The data were further analyzed using Duncan's multiple range test (DMRT) for means comparison. Data analyses were computed using SAS 9.1.3 portable program.

## RESULTS AND DISCUSSION

### Morpho-Anatomical Adaptation

Seedlings of lonkida have morpho-anatomical adaptation mechanisms to survive in waterlogged condition. The results showed that lonkida

seedlings produced adventitious roots, lenticels and aerenchyma (Fig. 2 and Table 1). Lenticels began to form within 3 to 5 days after being in the waterlogged condition. All seedlings under waterlogged condition formed 100% lenticels (Table 1). Lonkida seedlings grown in

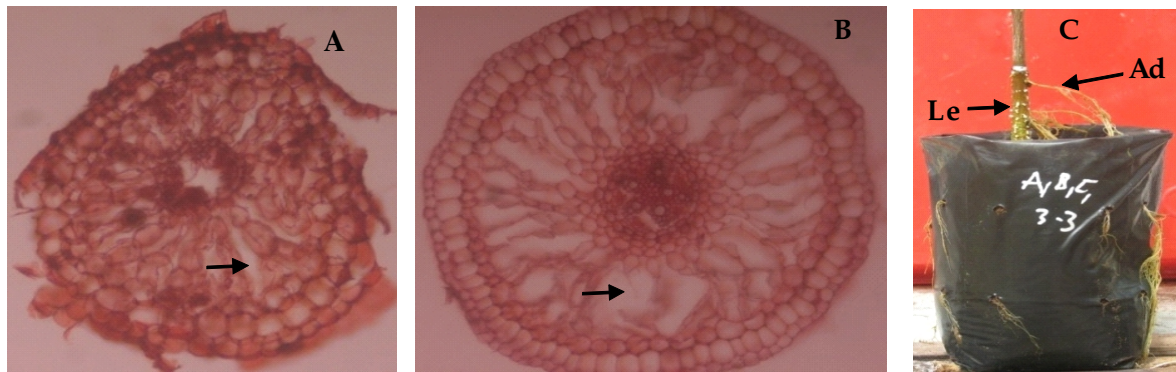


Figure 2. Cross-section of lonkida root tissue (x200 magnification) showing the presence of aerenchyma in (A) non-waterlogged condition; (B) waterlogged condition; (C) formation of lenticels (Le) and adventitious roots (Ad)

Table 1. Measurement of the observed variables for lonkida seedlings grown in waterlogged condition and in non-waterlogged condition

Variables	Measurement for lonkida seedlings grown in waterlogged condition (mean±SE)	Measurement for lonkida seedlings grown in non-waterlogged condition (mean±SE)	SI	Effect of waterlogged condition
<i>Plant growth variables</i>				
Survival	100±0.00 a	100±0.00 a	ns	ns
Height (cm)	8.13±2.45 a	6.18±1.177 b	-0.32	+
Diameter (mm)	1.81±0.31 a	1.24±0.25 b	-0.46	+
Total of leaves (blade)	13.1±0.838 b	14.3±1.183 a	0.09	-
Leaf area (cm <sup>2</sup> )	9.8±3.045 a	7.0±0.659 b	-0.40	+
Leaf length (cm)	8.2±0.878 a	7.0±0.363 b	-0.17	+
Leaf width (cm)	2.9±0.431 a	2.4±0.132 b	-0.21	+
RGRs (per day)	0.0084±0.0032 a	0.0060±0.0015 b	-0.42	+
RGRr (per day)	0.0075±0.0035 a	0.0069±0.0020 a	ns	ns
Shoot/root ratio	1.89±0.25 a	1.77±0.32 a	ns	ns
<i>Adaptation to waterlogged condition</i>				
Total adventitious root	1.16±0.552 a	0.0±0.00 b	-	+
Adventitious root (%)	48.6±17.005 a	0.0±0.00 b	-	+
Total lenticels	30.9±2.91 a	1.3±0.35 b	-22.7	+
Lenticels (%)	100±0.00 a	81±16.95 b	-0.23	+
<i>AMF symbiosis</i>				
AMF colonization (%)	11.5±2.64 b	29.1±6.25 b	0.61	-
<i>Nutrient uptake</i>				
N in root (%)	1.091±0.13 a	1.185±0.10 a	ns	ns
N in shoot (%)	0.73±0.059 b	0.83±0.061 a	0.12	-
P in root (%)	0.208±0.14 a	0.203±0.016 a	ns	ns
Total P in root (mg)	0.255±0.05 a	0.199±0.033 b	-0.28	+
P in shoot (%)	0.124±0.02 a	0.108±0.013 a	ns	ns
Total P in shoot (mg)	0.276±0.11 a	0.178±0.029 b	-0.55	+

Notes:

- 1) Figures followed by the same letters in different columns are not significantly different according to DMRT at 95 % confidence level
- 2) ns= not significantly different
- 3) SI/Susceptibility index= 1-(waterlogged plant/non-waterlogged plant)

## RESULTS AND DISCUSSION

### Morpho-Anatomical Adaptation

Seedlings of lonkida have morpho-anatomical adaptation mechanisms to survive in waterlogged condition. The results showed that lonkida seedlings produced adventitious roots, lenticels and aerenchyma (Fig. 2 and Table 1). Lenticels began to form within 3 to 5 days after being in the waterlogged condition. All seedlings under waterlogged condition formed 100% lenticels (Table 1). Lonkida seedlings grown in waterlogged condition had an increase in lenticels and adventitious roots compared with lonkida seedlings grown in non-waterlogged condition (Table 1). Aerenchyma was formed in the root cortex of lonkida in both waterlogged and non-waterlogged conditions. Aerenchyma proportion in root tissue of lonkida grown in waterlogged condition was greater than that of lonkida grown in non-waterlogged condition (Fig. 2).

Seedlings of lonkida adapted to waterlogged condition by modifying the morphology and anatomy of the body through lenticels, adventitious root and aerenchyma formations. In the treatment where lonkida seedlings were grown in non-waterlogged condition and swamp habitat, the lenticels percentage was below 100 %. The same finding was found in aerenchyma in root tissue. Adventitious root formation were absent in treatment where lonkida seedlings were grown in non-waterlogged condition. The adaptation mechanism improves the oxygen uptake ability and oxygen transfer to plant tissues in waterlogged condition (Yin *et al.* 2012). Thus, morphological and anatomical adaptations could reduce hypoxic condition and contribute to the restoration and maintenance of aerobic respiration of seedlings in waterlogged condition (Ashraf 2012). Lenticels were crucial in the diffusion of oxygen and anaerobic metabolism resulting products (ethanol, CO<sub>2</sub> and CH<sub>4</sub>) and played a role in plant water homeostasis (Ashraf 2012). The adventitious root of plants tolerant to waterlogged condition was formed to play the role as the main root replacement to maintain water and mineral supplies when the main roots were not functioning normally, to maintain aerobic respiration of seedlings and to initiate stomata opening (Kozłowski 1997; Ashraf 2012). Network of aerenchyma contributed to the life

and growth of plants in a long term waterlogged condition (Ashraf 2012).

Adaptation mechanisms mentioned above were found both in plants tolerant or intolerant to waterlogged condition. Lenticels and adventitious root were found in woody plant, such as *Calophyllum brasilienses* Camb (de Oliveira & Joly 2010), *Erythrina speciosa* (Medina *et al.* 2009), *Larix laricina* (Islam & Macdonald 2004), *Populus deltoids* (Béjaoui *et al.* 2012), *Salix gracilistyla* (Nakai *et al.* 2010) and *Salix martiana* (Parolin 2009). Aerenchyma formation in root also occurs in *Erythrina speciosa* (Medina *et al.* 2009), *Melaleuca cajuputi* (Tanaka *et al.* 2011) and *Quercus petraea* (Folzer *et al.* 2006). In addition to the above mechanisms, the roots of lonkida seedlings also appeared on the surface of the media and out through the holed polybags. These facts indicated that the roots could grow well in waterlogged condition and could absorb oxygen and nutrients for the plants. The formation of leaves and roots in waterlogged condition was thought to support growth through the activity of oxygen uptake, increased water and nutrient contents and increased photosynthesis activity (Kozłowski 1997). Increased seedling growth in waterlogged condition indicated that lonkida seedlings had high efficiency in the improvement of biomass per each nutrient absorbed and maintained aerobic respiration (Kozłowski 1997; Tanaka *et al.* 2011).

### Plant Growth

Height of lonkida seedlings in temporal swamp and savannah habitats without mycorrhiza showed significant difference from other treatments, except for treatment in swamp habitats either with or without mycorrhiza (Fig. 3a). Lonkida seedlings grew better in waterlogged condition compared with those in non-waterlogged condition, with height growth differences of 32%, diameter differences of 46%, leaf area differences of 40%, leaf length differences of 17% and leaf width differences of 21% between the two treatments (Table 1). Nonetheless, waterlogged condition reduced the number of leaves by 9%. Temporal swamp habitat on waterlogged condition had the highest RGRt value (0.0125 g/day) and the lowest RGRt value occurred in the dry land under waterlogged condition (0.0047 g/day) (Fig. 3b). Treatment of

waterlogged condition did not significantly affect RGRr, but RGRs increased by 42% (Table 1). Non-mycorrhizal lonkida seedlings had higher RGRs, RGRr and RGRt compared with mycorrhizal lonkida seedlings (Table 3). The results of this study indicated that waterlogged condition did not inhibit vertical growth (root and shoot) and horizontal growth (diameter) of plant.

Lonkida seedlings originated from dry land habitats have lower height growth rate, RGRs, RGRr, RGRt and root shoot ratio compared with lonkida seedlings originated from other habitat types, for both mycorrhizal and non-mycorrhizal treatments. The results of this study indicated that lonkida seedlings originated from dry land habitat were not suitable to be planted in waterlogged condition. Several studies have reported the same results, such as study on the female *Populus angustifolia* that grew naturally in inundated area was more tolerant to waterlogged condition within 15 weeks compared to male *P. angustifolia* male which originated from the dry land (Nielsen *et al.* 2010). Ferreira *et al.* (2009) reported that *Himatanthus sucuba* originated from non-flooded areas (terra-firme) was intolerant to waterlogged condition compared with that originated from inundated area (Varzea) in Amazonian flood plain. However, shoot root ratio of seedlings originated from dry habitats were smaller than those from other habitats. The low shoot root ratio was assumed as being a protective mechanism from dehydration through transpiration (van Splunder *et al.* 1996). Another strategy that might occur was that seedlings from dry land habitats had more C translocation to the roots than to the shoots (Martinez *et al.* 2012).

In contrast to the dry habitats, lonkida seedlings originated from temporal swamp habitat in waterlogged and non-waterlogged conditions and lonkida seedlings originated from savannah habitat in waterlogged condition had greater RGRt compared with RGRt in other treatments. The average height growth of lonkida seedlings from temporal swamp and savannah habitats were quite high, indicating that lonkida seedlings from both habitats were tolerant to waterlogged condition; which also indicated that the physiological adaptation mechanism of the seedlings was not disrupted and morpho-anatomical change was formed well in the

waterlogged condition. In addition, lonkida seedlings in the temporal swamp grew well in dry condition and in waterlogged condition. Parolin (2009) explained that the differences in the type of tolerance to waterlogged condition were strongly associated with ecotype differences among habitats due to natural selection. The results of this experiment indicated that lonkida is a species having relatively broad ecological distribution, compared to most species from inundated area, which generally had relatively small ecological amplitude (Parolin 2009).

### Plant Biomass

Table 2 shows significant difference on shoot dry weight was shown for mycorrhizal lonkida seedlings originated from dry land in waterlogged condition, non-mycorrhizal lonkida seedlings originated from swamp in waterlogged condition, non-mycorrhizal lonkida seedlings originated from savannah in waterlogged condition and mycorrhizal lonkida seedlings originated from savannah in non-waterlogged condition. The same table also shows that root dry weight of mycorrhizal lonkida seedlings originated from dry land in waterlogged condition was significantly different from all other treatments. Significant difference on total dry weight was shown for mycorrhizal lonkida seedlings originated from dry land in waterlogged condition, non-mycorrhizal lonkida seedlings originated from savannah in waterlogged condition and mycorrhizal lonkida seedlings originated from savannah in non-waterlogged condition (Table 2). Shoot-root ratio of non-mycorrhizal lonkida seedlings was higher (1.95) than that of mycorrhizal lonkida seedlings (1.65) (Table 3).

The results of this study showed that lonkida seedlings had various adaptation strategies to overcome environmental stress. Seedlings from dry habitats required mycorrhizae fungi in waterlogged condition. Some studies also indicated that AMF improved plant tolerance in waterlogged condition (Osundina 1998; Neto *et al.* 2006; Fougnes *et al.* 2007). The role of AMF in waterlogged condition can be seen from increase of nutrient uptake especially P (Stevens *et al.* 2002; Muok and Ishii 2006; Fougnes *et al.* 2007; Garcia *et al.* 2008) in enhancing growth (Secilia &

Table 2. Interactions effect among habitat, mycorrhizae application, and waterlogged condition on the observation variables

Treatment	Variable							
	AMF Spore (mean±SE)	Vesicle (mean±SE)	Shoot dry weight (g) (mean±SE)	Root dry weight (g) (mean±SE)	Total dry weight (g) (mean±SE)	Total N in root (mg) (mean±SE)	Total N in shoot (mg) (mean±SE)	
Dry land	non-mycorrhizal non-waterlogged	0.00±0.00e	0.00±0.00 c	1.66±0.25 a	0.91±0.22 a	2.57±0.46 a	9.4±1.93 a	13.5±0.95 a
	non-mycorrhizal waterlogged	0.00±0.00 e	0.00±0.00 c	1.55±0.23 a	1.10±0.04 a	2.65±0.21 a	11.4±0.43 a	11.1±1.40 a
	mycorrhizal non-waterlogged	279±15.67 c	9.70±0.33 a	1.31±0.06 a	1.04±0.03 a	2.35±0.05 a	11.5±0.20 a	10.8±1.40 a
	mycorrhizal waterlogged	169±13.17 cd	11.1±0.94 a	2.19 ±0.28ab	1.70±0.14 ab	3.89±0.35 ab	21.5±5.28 b	15.4±1.34 a
Swamp	non-mycorrhizal non-waterlogged	0.00±0.00 e	0.00±0.00 c	1.76±0.21 a	0.84±0.11 a	2.60±0.31 a	9.4±0.68 a	14.8±1.79 a
	non-mycorrhizal waterlogged	0.00±0.00 e	0.00±0.00 c	2.41±0.28 ab	1.12±0.07 a	2.53±0.34 a	11.9±0.73 ab	16.0±2.26 ab
	mycorrhizal non-waterlogged	347±48.31 b	3.30±0.88 b	1.49±0.17 a	1.00±0.13 a	2.49±0.27 a	12.6±0.98 ab	12.4±0.89 a
	mycorrhizal waterlogged	155±36.47 b	4.40±0.73 b	1.82±0.19 a	1.06±0.16 a	2.88±0.34 a	10.9±1.62 a	14.5±1.76 a
Temporal swamp	non-mycorrhizal non-waterlogged	0.00±0.00 e	0.00±0.00 c	1.64±0.14 a	0.99±0.33 a	2.63±0.48 a	10.7±2.26 a	13.6±1.48 a
	non-mycorrhizal waterlogged	0.00±0.00 e	0.00±0.00 c	3.32±0.38 b	1.72±0.16 b	5.04±0.54 b	16.3±1.07 ab	25.0±3.66 b
	mycorrhizal non-waterlogged	652±31.65 a	3.80±0.77 b	1.43±0.09 a	1.15±0.17 a	2.58±0.18 a	13.4 ±1.84 ab	12.6±0.80 a
	mycorrhizal waterlogged	128±15.96 d	4.60±0.47 b	1.98±0.26 a	1.15±0.13 a	3.13±0.36 a	13.0±1.35 ab	14.1±2.58 a
Savannah	non-mycorrhizal non-waterlogged	0.00±0.00 e	0.00±0.00 c	1.56±0.16 a	0.81±0.07 a	2.37±0.23 a	10.5±0.84 a	13.5±0.38 a
	non-mycorrhizal waterlogged	0.00±0.00 e	0.00±0.00 c	2.34±0.43 ab	1.16±0.18 a	3.49±0.60 ab	14.1±2.49 ab	15.4±1.47 a
	mycorrhizal non-waterlogged	449±56.33 b	1.00±0.74 bc	2.28±0.23 ab	1.18±0.15 a	3.47±0.30 ab	14.2±2.49 ab	16.3±0.80 ab
	mycorrhizal waterlogged	142±17.57 d	9.00±0.61 a	1.71±0.24 a	0.72±0.05 a	2.42±0.27 a	7.2±0.77 a	14.2±3.02 a

Notes:

Figures followed by the same letter in the same column are not significantly different according to DMRT at 95 % confidence level

Table 3. Effect of mycorrhizae application on some variables observed

Variables	Mycorrhizal seedling (mean±SE)		Non-mycorrhizal seedling (mean±SE)	
Diameter (mm)	1.31±0.24 b		1.74±0.30 a	
Shoot root ratio	1.65±0.27 b		1.95±0.28 a	
RGRs (per day)	0.0052±0.0015 b		0.0092±0.0029 a	
RGRt (per day)	0.0052±0.0013 b		0.0092±0.0034 a	
RGRt (per day)	0.0052±0.0012 b		0.0091±0.0028 a	
Cons. P in root (%)	0.196±0.011 b		0.215±0.017 a	

Notes:

Figures followed by the same letters in different columns are not significantly different according to DMRT at 95% confidence level



Bagyaraj 1994; Osundina 1998; Fougnyes *et al.* 2007) and plant biomass (Keeley 1980; Secilia & Bagyaraj 1994).

### Nutrient Uptake

The highest total N in root occurred in mycorrhizal lonkida seedlings originated from dry land habitat in waterlogged condition. The highest total N in shoot occurred in non-mycorrhizal lonkida seedlings originated from temporal swamp in waterlogged condition (Table 2). The results of this study revealed that AMF was needed by seedlings of dry land habitat in waterlogged condition. N uptake by AMF in waterlogged condition had also been reported in *Aster tripolium* (Neto *et al.* 2006). In contrast to the dry habitats, seedlings of temporal swamp habitat did not require mycorrhizae in waterlogged condition. Non-mycorrhizal lonkida seedlings from temporal swamp in waterlogged condition had higher N accumulation in leaves and roots. Total P in root of lonkida seedlings grown in waterlogged condition was higher than that of lonkida seedlings grown in non-waterlogged condition (Table 1) which was in line with research on plants of *Lotus Glaber* Mill. (Mendoza *et al.* 2005; Garcia *et al.* 2008).

### Arbuscular Mycorrhizal Fungi (AMF) Colonization and Spore Density

Arbuscular mycorrhizal fungi (AMF) colonization in waterlogged condition was 61% lower than that in non-waterlogged condition (Table 1). AMF structures found in the roots of lonkida were internal hyphae, external hyphae, hyphal coils, vesicles and arbuscular. The internal structure of the AMF hyphae was commonly good without waterlogged condition. The numbers of vesicles per 1 cm roots were more in lonkida seedlings grown in waterlogged condition (Table 2). Waterlogged condition inhibited colonization and spore density of AMF. AMF colonization was believed to decrease the density of spores in waterlogged condition due to anaerobic environment (Escudero & Mendoza 2005; Mendoza *et al.* 2005; Garcia *et al.* 2008; Stevens *et al.* 2011; Wu *et al.* 2013). The low number of spores in this study attributed to the fact that the species of AMF used were not originated from areas with waterlogged condition (Qiang-Sheng *et al.* 2013), or oxygen -deficient

condition (Kozlowski 1997), or had limited supply of C from the host (Neto *et al.* 2006). Thus, the AMF was not able to sporulate and had limited external hyphae resulting from waterlogged condition (Secilia & Bagyaraj 1994; Sah *et al.* 2006). Nevertheless, other studies showed higher colonization of AMF in waterlogged condition (Sah *et al.* 2006; Neto *et al.* 2006; Faougnies *et al.* 2007). In addition to the species of AMF, plant species (Bauer *et al.* 2003), availability of P (Stevens *et al.* 2002; Garcia *et al.* 2008), distance from the AMF rhizosphere (Keeley 1980) as well as depth and duration of waterlogged condition also contributed to the AMF symbiosis (Graham *et al.* 2013).

Arbuscular mycorrhizal structure found were the internal and external hyphae, vesicles, arbuscular, coil and spores. Arbuscules found and the numbers of vesicles per cm root were more in waterlogged condition than in non-waterlogged condition. Arbuscule is a structure of AMF in the root that acts as a terminal for the transfer of nutrients from the host to the AMF and the transfer of carbon to fungi (Smith & Read 2008). Vesicles are storage structures and can support the growth of the internal hyphae (Smith & Read 2008). In waterlogged condition, vesicle structures were found in large quantities. The results of this study indicated that the AMF saved a lot of energy in the vesicles needed to adapt to the waterlogged condition (Garcia *et al.* 2008). This fact had also been reported in the roots of *Lotus tenuis* (Mendoza *et al.* 2005) and *Citrus junos* (Qiang-Sheng *et al.* 2013). Low number of arbuscules in the root of lonkida also showed that the transfer of C from plants to fungi was low and inhibited sporulation process

## CONCLUSIONS

Lonkida seedlings had high survival rate and adapted to waterlogged condition by modifying the morpho-anatomical through formation of lenticels, adventitious roots and aerenchyma. Lonkida seedlings from inundated habitat tended to have higher growth and biomass. In waterlogged condition, AMF treatment increased biomass and N accumulation in roots of lonkida seedlings originated from dry land habitat. Arbuscular mycorrhizal fungi (AMF) colonization in waterlogged condition was lower

than that in non-waterlogged condition. Based on these results, lonkida has the potential to be used for rehabilitating degraded mining lands.

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