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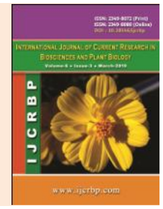

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Growth analysis of jack bean inoculated with *Acaulospora sp.* in nickel-contaminated soil

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ABSTRACT

Growth analysis is a method to determine the response of plant phenotype to the environmental factors affecting the plant growth. The aim of this research was to analyze the growth of jack bean (*Canavalia ensiformis*) inoculated with *Acaulospora* sp. in nickel-contaminated soil. The study was conducted in the Nursery area of Vale Indonesia Corporate, Sorowako, with three treatments of *Acaulospora* sp. isolated from different environments, namely indigenous *Acaulospora* sp., exotic *Acaulospora* sp. and without *Acaulospora* sp. Results showed that jackbean inoculated with indigenous *Acaulospora* sp. had value of average leaf area, leaf area index, net assimilation rate and relative growth rate are higher than those jackbean inoculated with exotic *Acaulospora* sp. and without *Acaulospora* sp. The higher tolerance to environmental conditions with extreme nickel metal concentrations indicates potential of indigenous mycorrhiza that can be used as biological filter agent to promote plant growth.

Introduction

Plant growth is a physiological process as a result of the interaction between genetic and environmental factors (Ncube et al., 2012; Pandey et al., 2017). Growth analysis is a method to determine the response of plant phenotype to the environmental factors affecting growth. Optimum plant growth is positively correlated with the dominant assimilate production occurring on the leaves, therefore, the optimum number of leaves in the plant provides an even distribution of the light reception. The distribution of light reception can be observed by observing leaf area and leaf area index, of which an optimum leaf area index will result in efficient use of resources in plants and lead to a high assimilate production to promote the growth rate of plants (Zakariyya, 2016; Srinivasan et al., 2017). However, according toZaman and Asaeda (2014); Almeida et al. (2017), the formation of leaf assimilates is determined not only by the acceptance of light, but also determined by macro and micro nutrients which play a role in the preparation of the element or the formation of assimilates. Results of research by Kusdianti et al. (2014); Shah et al. (2017); Paunov et al. (2018) revealed that cadmium (Cd) uptake by plants may inhibit the formation of leaf chlorophyll thus affecting plant biomass products. Darmawan (2010) and Anjum et al. (2016) also reported that the chromium (Cr) may decrease the leaf area, the ratio of leaf area, and the rate of net assimilation in the plant.

Post-mining land shows soil conditions with significantly change on physical and chemical properties that have negative effects on plant growth (Ikbal et al., 2016; Mborah et al., 2016). Reported that nickel (Ni) post-mining area was detected to have acidic soil with high nickel concentration (Sariwahyuni, 2012; Netty et al., 2012), therefore, agricultural activities developed in nickel post-mining areas would be a limiting factor which inhibits the growth and production of assimilates, as has been the case in the nickel post-mine area of Pomalaa showing growth of leaf area, volume and diameter of stem plants which lower (Widiatmaka et al., 2010).

Several methods may be used to inhibit the negative impact of heavy metals on plants in contaminated soil, one of which is the biological method. The utilization of indigenous microorganisms, particularly for arbuscular mycorrhiza is an affordable and effective biological method which plays a role in promoting plant growth on heavy metal-contaminated soil. Several strains of arbuscular mycorrhiza reported could tolerate heavy metal stress; such as *Glomus intraradices*, *Glomus mosseae* and *Acaulospora tuberculata* (Adewole et al., 2010; Tuheteru et al., 2017). Thus, this study was aimed to analyze the growth of jack bean inoculated with *Acaulospora* sp. on nickel-contaminated soil.

Materials and methods

The study was conducted in the Nursery Area of Vale Indonesia Corporate, Sorowako, South Sulawesi, Indonesia, with a tropical rainforest climate (Koppen-Geiger), the average annual temperature of the area was 24.2°C with average annual rainfall of 2019 mm, altitude of 415,40 masl, latitude of 2°31'33 "S and longitude of 121°20'50" E.

This study applied three inoculation treatments of *Acaulospora* sp isolated from different environments, namely: indigenous *Acaulospora* sp., exotic *Acaulospora* sp., and without *Acaulospora* sp. (control), prepared with Randomized Block Design.

Indigenous *Acaulospora* sp was isolated from rhizospheres of plant shrubs grows on nickel post-mining rehabilitation land in the Sumasang area, Sorowako village, using wet sieving and decanting techniques by Pacioni (1992) and sucrose centrifugation method by Brundrett et al. (1994); conducted in a microbiology laboratory, Center for Environmental and Forestry Research and Development Makassar, South Sulawesi. While exotic *Acaulospora* sp., using isolate from Kumalawati et al. (2015) was isolated from the non-mining land.

Nickel-contaminated overburden soil was obtained from mining area of Rante location, Soroako, South Sulawesi, Indonesia. The soil was analyzed in the Laboratory of Chemistry and Soil Fertility, Department Soil Science Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. Soil texture was determined using hydrometer method, pH of soil was measured using pH meter, base cation (BC) with ammonium acetate extracted in pH 7 then was measured with an atomic absorption spectrometer, base saturation calculated with % BC=(bases cation/CEC)x100%, Cation exchange capacity (CEC) with 1 M NH4OAc, C-organic with Walkley and Black method. The concentration of Ni was measured in the laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using the manual book of X-Ray Florence Spectrophotometer/Bruker/S2 Ranger. The characteristics of soil properties are shows in Table 1.

Soil media insert into a container measuring 30 cm x 40 cm was 80%. Jack bean seedlings were grown by adding 24 g propagules of indigenous *Acaulospora* sp. and 6 g propagules of exotic *Acaulospora* sp. which containing 22 spores of *Acaulospora* sp. respectively. Synthetic fertilizer was treated at 7 days after planting with a dose 2 g per plant; irrigation was done every day at 10 am; pest and disease control was performed when the attack symptoms appear; weed control was carried out every week.

Growth analysis components observed were leaf area (LA), leaf area index (LAI), net assimilation rate (NAR), and relative growth rate (RGR) using the mathematical equations by Gardner et al., (1985); Sitompul and Guritno (1995). Variance analysis test was performed to see the effect of treatment used Microsoft excel 2010 software. Orthogonal and Duncan test was performed when the treatments had a significance level of 5% or 1%.

Results

Results of variance analysis on the growth analysis component of jack bean treated with *Acaulospora* sp treatment suggested no significant effect on LA, LAI, NAR, and RGR. However, based on the average values of LA, LAI, NAR, and RGR, it showed that the treatment of indigenous *Acaulospora* sp has the highest average values of growth analysis component compared to exotic *Acaulospora* sp and without *Acaulospora* sp. Jack bean inoculated with *Acaulospora* sp. had an LA value of 19.48% which was higher than that without *Acaulospora* sp (Fig. 1A). It is assumed that in addition to jack bean has a wide adaptability, it is also due to the jackbean inoculated with indigenous *Acaulospora* sp. does not experience nickel stress, particularly at the beginning of plant growth or at 21 days after planting (Fig. 2) due to the defense of indigenous *Acaulospora* sp that has been adapted and associated with plant roots so that high concentrations of nickel can be inhibited by reducing the rate of metal transported to the canopy top of the plant. According to Delvian (2006); Muryati et al. (2016) indigenous mycorrhiza has a high potential for extensive infection as it has a higher tolerance to the high stress-environmental conditions. However, Rainiyati et al. (2009) and Berruti et al. (2016) stated that the effectiveness of mycorrhiza is highly dependent on the suitability between the factors of mycorrhizal species, host plants, soils, and the interactions of those three factors.

Exotic *Acaulospora* sp. inoculated into jack-bean was suspected to be intolerant and required time to adapt to the new environment with nickel metal concentrations of up to 14,200 ppm (Table 1), thus not functioning as a biological filter against nickel metal and causing jack-bean experienced poisoning as occurred in the control treatment (Fig. 2). Al-Qurainy (2009) reported that nickel content with a concentration of 150 $\mu g \cdot g^{-1}$ on soil greatly reduces plant height and leaf area on beans (*Phaseolus vulgaris L.*), also found that in maize (*Zea mays* L.) (Ghasemi et al., 2012), excess nickel naturally affects the complex photosynthetic protein and Hill reaction rate decreases with increasing concentration of nickel metal.

Table 1. Physical and chemical properties of overburden soil in mining area of Rante Location, Soroako, South Sulawesi, Indonesia.

Characteristics	Value
Sand (%)	32
Silt (%)	35
Clay (%)	33
pH (H_2O)	5,62
C-Organik (%)	1,88
CEC (cmol (+) kg^{-1}	9,63
BC (%)	69
Al (ppm)	58,7
K (ppm)	4,4
P (ppm)	1,3
Ni (ppm)	14.200

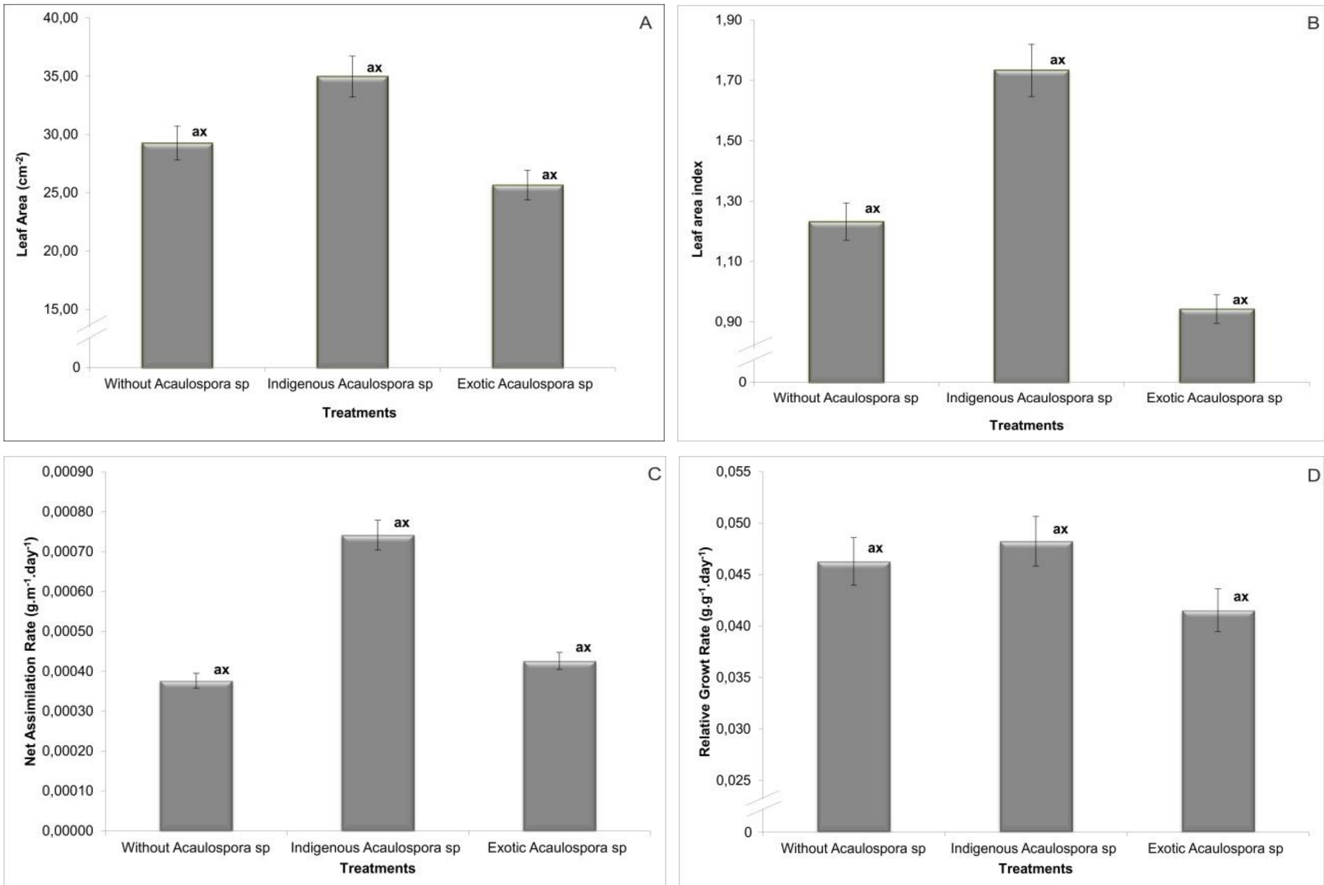

Fig. 1: The average values of leaf area (A), leaf area index (B), net assimilation rate (C) and relative growth rates (D) of jackbean inoculated with *Acaulospora* sp. The columns followed by the same symbols show no differences between treatments based on the Duncan (a, b, c) and Orthogonal (x, y, z) tests at 5% level.

Fig. 2: Symptoms of nickel toxicity in jack bean inoculated with *Acaulospora* sp at 21 days after planting. Note: Perlakuan Aca-0 is without *Acaulospora* sp (control); Perlakuan Aca-1 is indigenous *Acaulospora* sp and Perlakuan Aca-2 is exotic *Acaulospora* sp. treatments.

The adaptation of mycorrhiza is associated with two factors; the first is the gradual decrease in metal concentration due to the immobilization process occurring in the rhizosphere (Laghlimi et al., 2015); The second is the gradual change of the structure of the microbial community is based on gradual changes in the structure of the community over the profile of phospholipid fatty acids that affect more tolerant organisms (Escobar et al., 2015). Although metals may cause changes in the microbial community, microorganisms are more resistant to metals (Suharno et al., 2014). Revealed that certain types of Glomus such as *G. mosseae* and *G. intraradices* were able to increase tolerance for heavy metal absorption, particularly cadmium and lead (Adewole et al., 2010). The evolution process of metal tolerance can occur quickly. Some mycorrhizal arbuscular strains are tolerant within a year or two (Sudova and Vosatka 2007). Furthermore, the use of mycorrhiza from polluted sites which is tolerant to metal toxicity and able to adapt well can be potentially developed as an inoculant source (Sudova and Vosatka, 2007).

In addition to increase the rate of nutrient transfer at host roots, mycorrhiza also increases resistance to biotic and abiotic stress. Moreover, mycorrhiza also helps to maintain plant growth stability in contaminated stress conditions (Arisutanti and Purwani, 2013). The mechanisms of protection against heavy metals and toxic substances provided by mycorrhizas can be through filtration effects, chemically deactivating or accumulating these elements in hyphae (Aprilia and Purwani, 2013). In response to heavy metals, plants are equipped with a repertoire of mechanisms to counteract the toxicity of heavy metals (Emamverdian et al., 2015). Important elements of this chelating metal form the metal complexes of phytochelatin or metallothionein at intercellular and intercellular levels, followed by the loss of heavy metal ions from sensitive areas or from ligand-metal complexes stored in vacuolar sequestration of the ligand-metal complex. Non-enzymatic synthesized compounds such as proline (Pro) are able to strengthen the metal detoxification capacity of antioxidant enzymes at the intracellular level.

Colonization of the mycorrhiza will give a positive role in the supply of nitrogen, phosphor, and water, thereby spurring growth starting from the provision of carbohydrates from the photosynthetic organ as well as the provision of water and nutrients by roots to the synthesis of plant biomass (Upadhyaya et al., 2010; Sianipar et al., 2016). Leaves are the most important photosynthetic organ in light absorption. After the germination phase, the plant leaves more than one strand with an unequal location on each plant, and then the amount of light that reaches the leaf surface may differ among individual leaves. Leaves that lie on the lower layer of the canopy will receive less light than those on the top layer. This difference will increase with the increasing number of leaves due to the increase of plant age. Under certain conditions, the leaves in the lower layer will receive light below the point of light compensation because leaves shade each other and lead to inhibit plant growth.

The LAI value of jack bean inoculated with *Acaulospora* sp (Fig. 1B) was higher than the treatment without *Acaulospora* sp. The LAI> 1 score indicates the occurrence of cross shading between the leaves, it lead the shaded leaf in the lower layer of the canopy getting less light so it may have a lower photosynthetic rate than those unopened leaves (Sitompul and Guritno, 1995). The finding results showed that shaded plants yield a biomass reduction in colesom crops (Afa and Sudarsono, 2014). However, this phenomenon does not occur in induced indigenous jack bean *Acaulospora* sp that has LAI value of 40.65% higher than the jack bean without *Acaulospora* sp (Fig. 1B), this is probably because the LAI value has not reached a critical value. That soybean plants had a critical LAI of 2.20 and optimum LAI of 2.20 to 4.50 for plant growth (Indradewa, 1997; Cox and Cherney, 2011).

Jack-bean is one of the legume plants belonging to the C3 type. In the metabolic process of photosynthetic, rubisco enzyme that unites CO_2 with RuBP (Ribulose bisphosphate, the substrate for carbohydrate formation in photosynthesis process) in the initial assimilation process may also bind O_2 at the same time to process photorespiration and impact on competition between CO_2 and O_2 in using RuBP (Sutoyo, 2011) which furtherly impact on net photosynthesis of C3 plants are lower than C4 plants (Ramadhani et al., 2013; Kumar et al., 2017). This adverse effect did occur in jack-bean plants inoculated by indigenous *Acaulospora* sp. with a higher NAR value of 94.74% (Fig. 1C) with an RGR value of 4.35% higher than control treatment (Fig. 1D); these might because by LAI value which not reached critical step and possibly also because jack bean is able to perform nyctinasty when high temperature occurred on daytime: that is by changing leaves position from vertical to the horizontal position so as to minimize the occurrence of shade between leaf blades and efficient use of light. Research findings by Widyaswari et al. (2017) and Ko et al. (2017) showed that the LAI values of some rice varieties with a horizontal leaf position could reach LAI value from 5.00 to 6.50 and had not affected the assimilates production.

Conclusion

The results of the study shows that indigenous *Acaulospora* sp. can contribute to jack bean growth in nickel contaminated soil, as shown on the average value of the growth analysis components. This also indicates that indigenous *Acaulospora* sp. may act as biological filter agents against heavy metals in post-mining lands.

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