Effect of Arbuscular Mycorrhizal Colonization on Nitrogen and Phosphorus Uptake and Growth of *Aloe vera* L.

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Abstract. The effect of arbuscular mycorrhizal (AM) colonization on nitrogen (N) and phosphorus (P) uptake and shoot growth of *Aloe vera* was investigated. Plants were inoculated with one of two AM fungi, *Glomus clarum* or *Gigaspora decipiens*. Control plants were not inoculated. Plants were grown under glasshouse conditions in a peat land soil without fertilizers for 12 months. Inoculated *A. vera* plants were colonized with AM fungi. Total length of leaves and number of leaves were higher in inoculated plants than uninoculated plants 12 months after inoculation. Shoot N and P concentrations were higher in inoculated plants than uninoculated plants. Shoot fresh weight was increased by AM colonization. This result suggests that AM colonization can increase the nutrient uptake and growth of *A. vera*.

Aloes are xerophytes in the Aloeaceae in the Liliales that are cultivated for medicinal, vegetable, and cosmetic purposes in Africa, North America, and Southeast Asia. Approximately 400 species have been described in the genus *Aloe*. *Aloe vera* is the most commonly grown aloe. Although there have been a few studies on tissue culture propagation of *A. vera* (Aggarwal and Barna, 2004; Richwine et al., 1995; Roy and Sarkar, 1991;), little work has been done on the cultivation of *A. vera* in the field. *Aloe vera* grown under full sunlight produced more dry weight than those grown under partial shade (Paez et al., 2000). Application of nitrogen (N) and phosphorus (P) fertilizers increased the yield of *A. vera* (Pareek et al., 1999). *Aloe vera* has been cultivated in peat soil along with vegetables in Indonesia. The conditions in tropical peat land include low pH and poor nutrition, and it is difficult for *A. vera* to grow in peat soil without soil amendments. The ash produced after burning shrubs is usually applied to the

soil as a mineral source for *A. vera*. Farmers also use chemical fertilizer and lime for increasing the growth of *A. vera* in these soils.

Arbuscular mycorrhizal (AM) symbiosis has been shown to increase nutrient uptake and growth of horticultural plants. Extension of extraradical hyphae of mycorrhizal fungi into soil increases the surface area for nutrient uptake and, therefore, increases uptake of P, a nutrient that is often depleted in rhizosphere soil solution (Smith and Read, 1997). Many plant species in Liliales, to which Aloes belong, are known to be mycorrhizal-dependent (Tawaraya, 2003). However, there has been no report on mycorrhizal colonization and its effect on nutrient uptake and growth of plant species in Aloeaceae.

The objective of this study was to determine the effects of AM colonization of roots of *A. vera* on nutrient uptake and growth in peat soil.

Materials and Methods

Peat soil was collected at a depth of 20 cm from a vegetable field at Kalamangan, Central Kalimantan, Indonesia. Chemical properties of the soil were as follows: pH (H₂O), 3.05; organic carbon, 500 g C/kg (Tyurin, 1931); KCl-extractable nitrogen, 172 mg N/kg (1 M KCl extraction); available P, 254 mg P/kg (Bray I); cation exchange capacity (CEC), 148 cmol(+)/kg. The soil was

sieved to pass through ≈2 mm and sterilized at 121 °C for 30 min.

Spores of AM fungi were propagated from the peat soils of Kalamangan, Central Kalimantan, by a trap culture with *Allium cepa*. Successfully propagated spores were inoculated to *A. cepa*. Two isolates were selected based on their ability to improve growth of *A. cepa*. These isolates were identified as *Glomus clarum* and *Gigaspora decipiens* with morphological characters of spores, respectively (Tawaraya, unpublished data). These species were propagated in pot cultures of *Pueraria javanica*. Plastic pots were filled with 175 g sterilized zeolite and 5 g AM inoculum. Two 6-day-old *P. javanica* seedlings were transplanted into pots and were grown under natural light glasshouse conditions with no temperature and humidity control for 90 d. Zeolite inoculum consisted of colonized roots, external hyphae, and spores.

Aloe vera seedlings were obtained from the field of the National Center for Assessment and Development of *Aloe vera* under the Agency for Assessment and Application of Technology, Pontianak, West Kalimantan, Indonesia. The seedlings had three or four roots (≈3 cm long) and two or three leaves. Before transplanting, the roots of seedlings were soaked 1 h in water, surface sterilized by shaking for 5 min in 5% NaClO, and thoroughly rinsed twice in sterilized, distilled water. Seedlings were analyzed to control mycorrhizal colonization before inoculation and there was no mycorrhizal colonization in the seedlings.

Plastic pots (15-cm depth, 12 cm wide) were filled with 1200 g sterilized peat soil. AM fungal inoculation was achieved by placing 10 g of inoculum near the roots. One *A. vera* seedling was transplanted into the pot. Application of 10 g of inoculum did not show any fertilizing effect in preliminary experiment. Control seedlings were not inoculated. Seedlings were watered daily with tap water to maintain a field capacity (50% of maximum water-holding capacity). The seedlings were grown in a glasshouse of the Forest and Nature Conservation Research and Development Center (6°36' S, 106°45' E), Bogor, West Java, Indonesia, for 12 months. The pots were arranged in a completely randomized design with 10 replications for each treatment and harvest.

Total length of leaves and number of leaves were measured 92, 151, 212, and 365 d after transplanting. Plants were harvested 6 (180 d) and 12 (365 d) months after transplanting and shoots and roots were separated. Shoot fresh weight was measured. A subsample of shoot tissue was digested in H₂SO₄ and H₂O₂ solution (3:1, by volume). The N and P concentrations in the digested solution were determined by the semimicro Kjeldahl method (Jones et al., 1991) and the vanado molybdate yellow method (Olsen and Sommers, 1982), respectively, and expressed as a percentage of dry weight.

Roots of *A. vera* were gently washed over a 2-mm sieve under running tap water to

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Table 1. Mycorrhizal colonization (% root length colonized), shoot nitrogen and phosphorus concentration, and shoot fresh weight of *Aloe vera* inoculated with or without control arbuscular mycorrhizal fungi, *Glomus clarum*, or *Gigaspora decipiens* 6 and 12 months after inoculation.

Treatment	Mycorrhizal colonization (%)	N concn. (mg/g)		P concn.(mg/g)		Shoot fresh wt. (g/plant)	
		6 mo	12 mo	6 mo	12 mo	6 mo	12 mo
Control	6 a ²	0.57 a	1.24 a	0.13 a	0.17 a	185.41 a	203.50 a
<i>G. clarum</i>	96 b	0.72 a	3.05 b	0.19 c	0.41 b	265.84 b	301.61 b
<i>G. decipiens</i>	92 b	0.69 a	3.35 b	0.15 b	0.45 b	216.62 a	453.84 c

²Means followed by different letters in the column are significantly ($P < 0.05$) different as determined by the least significant difference test.

separate them from soil particles. Younger and fresh roots were selected randomly and the roots were cleared in 100 g·L⁻¹ potassium hydroxide for 1 hour, acidified with diluted HCl, and stained with 500 mg·L⁻¹ trypan blue in lactoglycerol. Roots were observed under a compound microscope at ×200 magnification. The percentage root length colonized by AM fungi was calculated by the grid line intersect method (Giovannetti and Mosse, 1980).

Data were statistically analyzed using analysis of variance with StatView 5.0 (SAS Institute, Cary, NC). Comparison of means was done using the least significant difference where the F-value was significant.

Results and Discussion

Roots in the treatments of both AM fungi were colonized up to 95% 12 months after transplantation (Table 1). Roots in the control treatment showed 6% colonization. It is likely that AM colonization in the control treatment originated from the soil underneath the plastic posts because the pots were placed directly above the soil surface. Many plant species in Liliales, of which *A. vera* is a member, are known to form AM colonization (Harley and Harley, 1987). There is little information about mycorrhizal colonization of the Aloeaceae. Muthukumar and Udaiyan (2000) observed AM colonization in roots of *A. vera* growing in southern India. However, there has been no report on mycorrhizal colonization and its effect on nutrient uptake and growth of plant species in Aloeaceae.

Shoot N concentration in control plants was 0.566 mg·g⁻¹ 6 months after transplantation and was not different among treatments (Table 1). Shoot N concentration in the plants inoculated with *G. clarum* and *G. decipiens* was higher than that of control plants 12 months after transplanting. Shoot P concentration in the plants inoculated with *G. clarum* and *G. decipiens* was higher than that of controls 6 and 12 months after transplanting. P and N fertilization increased the vegetative growth and yield of *A. vera* (Pareek et al., 1999; Van Schaik and Struik, 1997), suggesting the importance of P and N nutrition for aloe growth.

Number of leaves increased with time and was higher in plants inoculated with *G. clarum* and *G. decipiens* than that of control plants 212 d after transplanting (Fig. 1). This difference continued until 365 d after transplanting. Total leaf length was not different among treatments 212 d after transplanting (Fig. 2). Total leaf length was longer in plants

Fig. 1. Number of leaves of *Aloe vera* inoculated with or without (control, circle) arbuscular mycorrhizal fungi, *Glomus clarum* (triangle), or *Gigaspora decipiens* (square). Means followed by different letters are significantly ($P < 0.05$) different as determined by the least significant difference test.

inoculated with *G. clarum* and *G. decipiens* than that of control plant 12 months after transplanting.

Shoot fresh weight was higher in plants inoculated with *G. clarum* 6 months after transplanting (Table 1). Shoot fresh weight was higher in plants inoculated with *G. clarum* and *G. decipiens* than that of controls 12 months after transplanting. There were significant increases in number of leaves 212 d after transplanting and in shoot fresh weight 365 d after transplanting. It is considered that growth response to mycorrhizal colonization appeared 6 months after inoculation because growth of *A. vera* was slow. Shoot fresh weight of plants inoculated with *G. decipiens* was twofold that of control plants. This means that mycorrhizal colonization can increase the yield of *A. vera*.

Mycorrhizal dependency calculated with data of shoot fresh weight was 33% and 55% in plants inoculated with *G. clarum* and *G. decipiens*, respectively. These values are higher than those of other non-Liliales crop plant species but lower than that of Alliaceae (92%) and Agavaceae (87%), Liliales (Tawaray, 2003). *Aloe vera* may be less dependent on AM fungi than other Alliaceae plants. It is interesting that plants inoculated with *G. decipiens* were 50% bigger than those inoculated with *G. clarum*, although both AM fungi promoted the same size and number in leaves and similar N and P concentrations. These two AM fungi increased shoot growth of *Dyera polyphylla* and *Aquilaria filaria* equally (Turjaman et al., 2006). This effect

Fig. 2. Total length of leaves of *Aloe vera* inoculated with or without (control, circle) arbuscular mycorrhizal fungi, *Glomus clarum* (triangle), or *Gigaspora decipiens* (square). Means followed by different letters are significantly ($P < 0.05$) different as determined by the least significant difference test.

may be specific to the combination of *A. vera* and *G. decipiens*. Mechanism of this specific effect needs to be clarified.

Production of leaves is important for determining the yield and market value of *A. vera* (Eshun and He, 2004). When the leaves of Aloe are cut, an exudate arises from the cells of the leaves (Reynolds, 2004). This exudate contains several compounds that have medical, cosmetic, and nutraceutical values. Mycorrhizal colonization significantly increased leaf development and increased leaf biomass (Figs. 1 and 2; Table 1). Application of P and N fertilizer can increase the growth of Aloe (Pareek et al., 1999). Inoculation with mycorrhizal fungi could improve the ability of Aloe to acquire P and N from soil and decrease application of P and N fertilizer if indigenous AM fungi are ineffective or are low attributable to disturbance. Moreover, inoculation with mycorrhizal fungi would stimulate the efficient use of P resources and reduce pollution incited by runoff from P and N fertilization.

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