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Arbuscular mycorrhizal association of *Bentinckia condapanna*, Berry ex Roxb., an endemic palm of Western Ghats

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Arbuscular mycorrhizal colonization and spore count of *Bentinckia condapanna* growing in the natural habitats were determined. *Glomus intraradices* was found to be the most frequent fungus in the rhizosphere. Inoculation studies were carried out with this fungus alone and with the addition of 'P' as KH_2PO_4. AM inoculation enhanced the growth performance of plants. 'P' addition has not reduced the dependency of the plants to AM fungus.

Key words : Arbuscular mycorrhiza, Western Ghats, *Glomus intraradices*, phosphorus, mycorrhizal dependency

INTRODUCTION

Of the wide array of mycorrhizas, the arbuscular mycorrhiza (AM) formed between plants and Zygomycetous fungi in the Glomales, is certainly the most ancient and wide spread mycorrhizas (Allen, 1996). The beneficial effect of AM association in improving plant growth and nutrient absorption have been demonstrated by various research workers (Nurlaeny, 1995; Clark and Zeto, 1996). Investigation on the occurrence of mycorrhizal association in tropical habitats pointed out that majority of tropical plants grew well in association with mycorrhizal fungi (Chr. Diederichs and Moawad, 1993). They can benefit forest trees in a number of ways, although the most important is the enhancement of nutrient absorption from soil.

Reports on the occurrence and association of AM endophytes on the Western Ghat plants remain scanty. Rangarajan *et al.*, (1987) and Mohankumar and Mahadevan (1987) recorded the occurrence of AM fungi in the forest of Western Ghats. It is reported that occurrence of AM fungi is more frequent in the communities of high plant species diversity (Torrey, 1992). As the Western Ghats region is one of the megacentres of diversity, it was felt essential to study the AM association with forest trees. The present study deals with mycorrhizal association of *Bentinckia condapanna*, an endemic palm of the Western Ghats and to ascertain the dependency of this plant to AM fungi.

MATERIALS AND METHODS

Bentinckia condapanna growing in the Agasthyamala forest (1080-1240) were screened for AM association. Root and rhizosphere samples were collected from plants growing at different sites in Agasthyamala forests. Rhizosphere soil samples were collected from

five different places. Five replicates of soil from each site were mixed thoroughly and 250 g of this was used for spore isolation. Terminal feeder roots of the plants, from where the rhizosphere samples were taken, were also collected and preserved in F:A:A. AM spores in the soil were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Number of spores in 100 g of air dried soil was estimated and identified following the key of Trappe (1982) and Schenck and Perez (1987). The density and distribution of fungi in the rhizosphere was expressed in terms of percentage of frequency. The pH and phosphorous content in the soil were also estimated. The root sample collected were stained with Trypan blue following the method of Philips and Hayman (1970). Percentage of root colonization was quantified by grid-line intersect method (Giovanetti and Mosse, 1980).

Inoculation experiments were conducted using *Glomus intraradices*, the most frequent fungus in the rhizosphere. Inoculum of *Glomus intraradices* was multiplied in pot cultures of onion (*Allium cepa*). The substrate used for this experiment was a double autoclaved and soil mixture, (30 min. at 121°C for two successive days) with a pH of 5.8 and 10.4 kg `P' ha^{-1}. Surface sterilized seeds of *Bentinckia condapanna* were germinated in 1% water agar medium. The germinated seedlings were transferred into pots and 10 g of inoculum was placed as a layer 5 cm below the seedlings. Aqueous solution of KH_2PO_4 at a rate of 15 mg Kg^{-1} substrate was added to the pots as phosphorus source. Uninoculated seedlings were maintained with and without KH_2PO_4 as controls. The pots were watered with sterilized deionized water on alternate days. Hoagland nutrient solution lacking phosphorus was added to the pots twice in a week.

Six months after inoculation, the plants were uprooted and growth parameters like shoot weight, root weight, root length, and leaf area were estimated. Dry matters were estimated after drying the plant material to constant weight in a hot air oven at 70 °C. Phosphorus content in the plant materrial was also estimated. Root colonization was estimated following the method of Philips and Hayman (1970). From the above variables, root to shoot ratio (R:S), phosphorus utilization efficiency (PUE) and mycorrhizal dependency (MD) were determined. The measured growth parameters were alanysed by ANOVA and LSD Multiple range test.

RESULTS AND DISCUSSION

Variations in AM spore numbers were observed among the soil samples collected from different altitudes of Agasthyamala forests. Higher number of spores were recorded in the soil samples collected from higher altitudes (Table 1). Variations in AM spore density and colonization in native plant community were also reported by Brundrett and Kendrick (1988). Colonization in roots were also different in each site and the highest number was recorded in highest altitudes. Higher spore density was observed by Demars and Boerner (1995) in higher topographic positions. Experimental results showed low level of phosphorus and pH in the soil samples (Table 1). Janos (1983) reported that lower the soil pH, higher the proportion of phosphate in the soil and that becomes fixed and becomes less available for the plant for uptake.

Table 1. Data (mean) showing pH, `P' content and spore number in the rhizosphere and root colonization of *Bentinckia condapanna* in the Agasthyamala forests

Altitude (M)	pH	Soil P ($Kg\ ha^{-1}$)	Root colonization (%)	Spore Number /100 g
1080	5.3	11.8	68	496
1120	5.3	12.6	72	526
1160	5.2	10.6	74	610
1200	5.3	8.4	69	688
1240	5.2	8.8	71	672

The frequency of distribution of different AM fungi isolated from the rhizosphere of *Bentinckia condapanna* is shown in Table 2. Seven different AM fungi belonging to three genera viz. *Acaulospora, Glomus* and *Sclerocystis* were identified. *Glomus* was found to be the most dominant genus among them. *Glomus intraradices* was observed to be the most frequent species followed by *G. mosseae. G. intraradices* was therefore selected for the inoculation studies on *B. condapanna.*

Table 2. Occurrence of AM fungi in the rhizosphere of *Bentinckia condapanna*

AM fungi	Sampling site					Site Frequency (%)
	1	2	3	4	5	
Glomus aggragatum	+	-	+	-	-	40
G. clarum	-	+	+	-	+	60
G. fasciculatus	-	-	+	+	-	40
G. intraradices	+	+	+	+	+	100
G. mosseae	+	+	-	+	+	80
Sclerocystic sp.	-	-	+	+	-	40
Acaulospora sp.	+	+	-	-	-	40

AM infected plants showed distinct growth advantage over non-inoculated plants (Table 3). Inoculated plants showed an increase of 54% and 36% in dry weight of shoot and root respectively. AM inoculation resulted in the enhancement of above ground system compared to the root system. Considerably higher shoot biomass indicated that AM fungi enhance shoot growth more than that of the root. Significant increase in leaf area was also observed in inoculated plants. Tewari *et al.* (1993) have reported that mycorrhizal infection increased the leaf area and phosphate concentration, which in turn helped the plant to accumulate more dry matter. Mycorrhizal infection also increased the total root lenght. Similar finding were reported by Amijee *et al.* (1989).

Both AM inoculation and `P' nutrition had exerted considerable influence on plant growth characteristics. In most mycorrhizal studies percentage of AM infection decreases on the addition of `P' fertilizer to the soil. Abbott and Robson (1982) observed that the rate at which plant growth was stimulated by AM species depend on the rate at which root incection was established. In this study, it was observed that addition of `P' has reduced

Table 3. Effect of phosphorus and AM inoculation on growth parameter of *Bentinckia condapanna*

Treatment	Shoot wt.(g)	Root wt.(g)	Root lenght (cm)	Leaf area (cm^2)	Root/shoot ratio	P (%)	PUE
Control	7.86^{i}	4.88^{i}	2209.20^{i}	135.26^{i}	0.62^{a}	0.19^{e}	0.54^{a}
+P	11.40^{fg}	6.37^{c}	2880.50^{a}	176.46^{fg}	0.56^{ab}	0.29^{c}	0.34^{cd}
+AM	18.12^{a}	7.58^{ab}	2479.54^{fg}	254.21^{a}	0.42^{e}	0.34^{d}	0.29^{de}
+P+AM	17.78^{ab}	7.8^{a}	2572.47^{de}	246.57^{ab}	0.44^{de}	0.38^{a}	0.26^{e}

Means within the same column followed by the same superscript (s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

root colonization from 68% to 56%, but it was not reflected in the accumulation of dry weight of plants. According to Lu and Miller (1993) the reduction in colonization with added `P' had not reduced the absorption of soil `P'. Miyasaka *et al.* (1993) had reported enhancement in the growth of inoculated plants by `P' addition. When the initial concentration of `P' was extremely low in soil, small additions might enhance the infections. Addition of `P' to the non-inoculated plants also resulted in an increase in dry weight and leaf area. It also showed an increase of 23% in root length compared to control plants. The increase in root length by `P' addition according to Amijee *et al.* (1989) was due to the stimulation of initiation of first order lateral roots.

R:S ratio of control plants were found higher compared to other plants. AM inoculation resulted in the enhancement of above ground system and this lowered the R : S ratio. Lower R:S ratio in AM inoculated plants were also observed by Tewari *et al,* (1993). Considerably higher shoot and total plant biomass observed in the present study indicated that, AM fungi enhanced shoot growth more than root growth and also lowered the R:S ratio.

`P' content in the inoculated plants nearly get doubled compared to the control plants. Raju *et al,* (1990) had also reported increased `P' content in AM inoculated plants. Baon *et al.* (1993) observed higher `P' uptake in mycorrhizal plants compared to non-mycorrhizal plants, where no `P' was added. When `P' was supplied to non mycorrhizal plants an increase in internal `P' was observed. Significant difference in `P' content could not be observed between mycorrhizal and non-mycorrhizal plants supplied with `P'. When `P' was supplied to mycorhizal plants, a considerable increase in `P' concentration was observed compared to no `P' added mycorrhizal plants. Similar findings were observed by Lu *et al.* (1994).

Mycorrhizal response appeared primarily be related to phosphorus utilization efficiency (PUE). As the internal `P' concentration increased, the PUE was found decreasing in mycorrhizal and `P' added non-mycorrhizal plants. The PUE of mycorrhizal plants was lower compared to that of the non-mycorrhizal plants (Table 3). Mycorrhizal infection lowered the efficiency of `P' utilization by plants. Similar findings were reported earlier by Stribley *et al.* (1980) and Baon *et al,* (1993).

Mycorrhizal dependency was calculated based on the formula of Plenchette *et al,* (1983). When the R:S ratio decreased, a corresponding increase in mycorrhizal dependency was observed. As the plant gets infected, the enhanced growth resulted in the lowering of R : S ratio and thus increased the dependency of the plant to mycorrhiza. The MD value was 57% for the plants inoculated with AM fungi. The addition of `P' did not reduce the dependency of plants of AM fungi as indicated by 56% of dependency shown by the `P' added plants. This indicated that AM inoculation of the *Bentinckia condapanna* enhances the growth of the plants with improved `P' status, thus confirming its dependency on arbuscular mycorrhiza.

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