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Interaction Effects of Arbuscular Mycorrhizal Fungi and Different Phosphate Levels on Growth Performance of *Catharanthus roseus* Linn.

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Abstract

Catharanthus roseus L. (*Apocynaceae*), a valuable medicinal plant with potential therapeutic value was inoculated with AM fungi *Glomus fasciculatum* under three different phosphate conditions. *Catharanthus roseus* plants raised in presence of the AM fungi showed increased growth in terms of (shoot length, root length, leaf number, fresh weight and dry weight). Total chlorophyll content and phosphate content of the shoot was found to be significantly higher in AM inoculated plants as compared to non AM *Catharanthus* plants. The activities of phosphatase enzymes were found to be increased in AM inoculated plants as compared to non AM plants. Root colonization percent was significantly higher in AM inoculated plants at zero and at all three phosphate levels after 60, 90 and 120 days of AM inoculation, but decreased at third phosphate level after 120 days of AM inoculation. The study suggests that *Catharanthus roseus* is dependent on the mycorrhizal fungi to a large extent for its growth and survival and also shows the potential of AM fungi *Glomus fasciculatum* in increasing growth and biomass of *Catharanthus roseus* L.

Keywords: AM fungi, *Catharanthus roseus*, *Glomus fasciculatum*, phosphatase enzymes

Introduction

Catharanthus roseus (L.) G. Don (Madagascar periwinkle) belonging to the family *Apocynaceae* is an important and highly exploited medicinal plant from which secondary metabolites used in chemotherapy to treat diverse cancers are extracted. The leaves and stem are the sources of natural dimeric alkaloids vinblastine and vincristine that are essential parts of most anticancer chemotherapies (Van der Heijden *et al*., 2004). Large scale mass production of this important medicinal plant requires commercial cultivation especially in areas that are not suitable for traditional farming systems.

Plant roots interact with a wide variety of microorganisms in the soil, among these arbuscular mycorrhizal fungi form an important component of soil microflora. Root colonization by arbuscular mycorrhizal (AM) fungi has been shown to increase the productivity of several crops, especially important in the context of sustainable agriculture and development (Smith and Read, 1997; Podeszfinski *et al*., 2002). In recent years, due to over exploitation of natural resources, biofertilizers have emerged as important components of integrated nutrient supply system and hold a promise for reducing the production costs, improving the crop yields, quality, nutrientsupplies and sustaining the productivity over a longer period (Gill *et al*., 2002). Beneficial effects of AM symbiosis on plant growth, nutrient uptake, and tolerance to environmental stressors have been extensively reported (Koide and Mosse, 2004; Audet and Charest, 2007). However, these fungi show a preferential colonization to hosts and thus the extent to which the host benefit depends of the fungal species involved in the symbiosis (Miller *et al*., 1987).

The selection of efficient AM fungi is a key prerequisite for inoculation programs since there are different levels of compatibility between host plants and AM fungi (Smith and Read, 1997). The present pot culture study was undertaken to understand the physiological and biochemical response of *Catharanthus roseus* to one of the most efficient AM fungus *Glomus fasciculatum* at three different phosphate levels.

Materials and methods

The seeds of *Catharanthus roseus* were obtained from Government Nursery, University of Pune, Maharashtra India. The experiment was conducted in open natural conditions in Botanical Garden, Department of Botany, University of Pune.

Fifty days old seedlings with uniform height were used in the experiment. Three month old 30 g of mycorrhizal inoculum of *Glomus fasciculatum* (Thaxter) Gerd. and Trappe emend. Walker and Koske containing AM colonized roots, rhizosphere soil having extramatrical mycelium and spores (10-15 spores/g of soil) were used as a source of inoculum for each seedling. The inoculum was directly attached to the roots of the seedlings. The experiment consists of eight treatments, nine replicates of each treatment were grown, a total of 72 pots were arranged in completely randomized block design. The treatments consisted of: non AM control, i.e. zero phosphate level and three different phosphate levels of 50 mg, 100 mg

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and 150 mg respectively and AM inoculated plants with zero phosphate level and three different phosphate levels respectively. Four seedlings were maintained in each polyethylene bag having a size of 12×14″containing eight kg of autoclaved soil (autoclaved for one hour at 121°C) and the seedlings were watered daily according to their needs. K_2HPO_4, dipotassium phosphate (analytical reagent) was used as a source of phosphate. Phosphate treatment was given after 30 days of AM inoculation and after that phosphate was given weekly until the last observation was recorded. Observations were recorded after 60, 90, and 120 days after AM inoculation.

Growth measurement

Plants were harvested after 60, 90 and 120 days of AM inoculation and were then analyzed for morphological parameters such as shoot length, root length, leaf number, fresh weight and dry weight.

AM colonization

The percentage of AM colonization in roots was analyzed by clearing and staining of roots by the method of Phillips and Hayman (1970) and percent AM colonization in root was determined by gridline intersect method Giovannetti and Mosse (1980).

Physiological and biochemical parameters

Leaf chlorophyll content was determined by the method of Arnon (1949). The amount of total chlorophyll was expressed in mg/g fresh weight. Total P content was measured as described by Fiske and Subbarao (1925) method. Amount of phosphorus was expressed in mg/g dry weight.

Acid and alkaline phosphatase activity was measured by the method of Lowry *et al*. (1954).

Absorbance was read at 405 nm. The amount of phosphatase activity was expressed in moles of para-nitro phenol (PNP) released/minutes/g of fresh weight of roots.

Statistical analysis was done with the help of statistical package for social sciences (SPSS) software followed by Duncan's Multiple Range Test (DMRT) and different small alphabetical letters indicate significant differences at $p \leq 0.05$ level.

Results and discussion

In the present investigation AM fungi (*Glomus fasciculatum*) was found to have a significant effect on the growth and development of *Catharanthus roseus*. The difference between the mycorrhizal and non mycorrhizal *Catharanthus* plants was visible just after 20 days of AM inoculation, before phosphate was being applied. AM inoculated plants flowered two weeks before the control and produced more flowers at the first flush. As shown in Tab. 1, significant increase was observed in various morphological parameters like shoot length, root length and leaf number in *Glomus fasciculatum* inoculated *Catharanthus* plants after 60, 90 and 120 days as compared to non

Tab. 1. Morphological parameters of *Catharanthus roseus* L. after 60, 90 and 120 days of AM inoculation under three different phosphate conditions

Treatments	Shoot length (cm)			Root length (cm)		
	60 days	90 days	120 days	60 days	90 days	120 days
C	8.33±1.53f	12.00±1.00f	13.00±1.00f	7.67±1.53e	12.67±1.15d	13.00±1.00c
C+1P	10.00±2.00ef	14.00±1.00ef	16.00±1.00e	10.00±1.00de	21.00±2.00c	24.33±2.08c
C+2P	13.33±1.53d	16.33±1.53e	21.00±1.00d	12.00±1.00de	23.67±1.53c	27.00±2.00c
C+3P	16.67±1.53d	25.00±1.00d	31.00±1.00c	14.00±4.00d	32.00±3.61b	38.00±2.00b
Gf	24.00±2.00c	33.00±2.65c	36.67±1.53b	23.67±4.04c	34.00±5.29b	39.33±2.52b
Gf+1p	28.00±2.00b	38.00±2.00b	40.33±2.52a	28.67±3.21bc	41.00±2.00a	45.33±1.53a
Gf+2p	33.67±2.52a	41.00±1.00a	43.00±1.00a	33.67±3.06ab	43.33±2.08a	46.33±1.53a
Gf+3p	36.67±3.51a	42.33±1.53a	43.00±2.00a	37.67±4.51a	45.67±1.53a	47.67±3.06a

Tab. 2. Fresh weight and dry weight of *Catharanthus roseus* L. after 60, 90 and 120 days of AM inoculation under three different phosphate conditions

Treatments	Fresh weight (g)			Dry weight (g)		
	60 days	90 days	120 days	60 days	90 days	120 days
C	0.98±0.23d	1.82±0.16h	2.05±0.43e	0.24±0.06g	0.42±0.05g	0.67±0.08g
C+1P	1.87±0.67d	2.74±0.16g	3.02±0.32e	0.37±0.07g	0.59±0.03f	0.89±0.15f
C+2P	2.33±0.28d	4.22±0.23f	5.71±0.78d	0.54±0.10f	1.25±0.11e	1.48±0.07e
C+3P	4.17±0.66c	8.22±0.16e	9.24±1.16c	0.74±0.07e	1.54±0.09d	2.48±0.17d
Gf	7.85±0.96b	10.26±0.12d	11.84±1.06b	1.82±0.08d	2.73±0.06c	3.13±0.06c
Gf+1p	8.04±1.22b	11.46±0.11c	2.98±0.57b	2.07±0.13c	3.11±0.08b	3.54±0.08b
Gf+2p	10.01±0.32a	11.99±0.17b	14.59±0.59a	2.16±0.09b	3.24±0.10ab	3.95±0.09a
Gf+3p	11.20±1.08a	12.83±0.16a	15.51±1.22a	2.33±0.11a	3.33±0.07a	3.98±0.15a

Tab. 3. Acid and alkaline phosphatase activity in *Catharanthus roseus* L. after 60, 90 and 120 days of AM inoculation under three different phosphate conditions

Treatments	Acid phosphatase activity μ mole pnp-released/g fresh weight			Alkaline phosphatase activity μ mole pnp-released/g fresh weight		
	60 days	90 days	120 days	60 days	90 days	120 days
C	0.422±0.027f	0.713±0.025f	0.790±0.033e	0.767±0.056f	0.931±0.036f	1.098±0.072f
1P	0.514±0.049e	0.834±0.019e	0.929±0.014d	0.811±0.049de	1.034±0.032e	1.179±0.035ef
2P	0.649±0.032d	0.989±0.024d	1.141±0.025c	0.913±0.030d	1.122±0.073e	1.257±0.057de
3P	0.691±0.030d	1.197±0.036c	1.212±0.073c	1.099±0.027c	1.211±0.089d	1.354±0.064d
GF	0.773±0.022c	1.249±0.031b	1.304±0.022b	1.261±0.071b	1.643±0.021c	1.709±0.065c
Gf+1P	0.868±0.037b	1.288±0.040b	1.323±0.052b	1.269±0.089b	1.740±0.022b	1.823±0.027b
Gf+2P	1.129±0.031a	1.396±0.026a	1.436±0.036a	1.396±0.102a	1.826±0.019ab	1.958±0.045a
Gf+3P	1.168±0.027a	1.425±0.020a	1.447±0.051a	1.461±0.042a	1.879±0.085a	1.971±0.064a

mycorrhizal *Catharanthus* plants at all levels of phosphate. Increase in growth parameters due to mycorrhizal inoculation has been reported earlier in other medicinal plants (Earanna *et al*., 2002; Sena and Das, 1998). Similar results were obtained by Karthikeyan *et al*. (2008) in *Catharanthus roseus* inoculated with *Glomus mosseae*. Similar report was presented by Matsubara and Sakurai (2000) who also reported that plant production was highly variable with or without mycorrhiza. After 60, 90 and 120 days of AM inoculation, fresh weight and dry weight in mycorrhizal *Catharanthus* plants at all three levels of phosphate was found to be significantly higher as compared to non mycorrhizal *Catharanthus* plants. This may be due to the formation of external mycelium around the roots by VAM fungi and the increase in photosynthetic rate. Increase in plant biomass because of AM fungal inoculation has been reported in other medicinal plants like *Palmarosa* (Gupta and Janardhanan, 1991) and *Coleus forskholii* (Boby and Bagyaraj, 2003).

As shown in Fig. 1, the percentage of AM colonization was found to be higher at zero and all three phosphate levels after 60, 90 and 120 days of AM inoculation, but decreased at third phosphate level after 120 days of AM inoculation. No AM colonization was observed in control plants. This result is in accordance with earlier studies which reported an inhibitory effect of P on AM colonization of roots of barley (Khaliq and Sanders, 2000), wheat (Mohammad *et al*., 2004), and maize (Ozcan and Taban, 2000).

As shown in Fig. 2, total chlorophyll content of leaves showed significant increase in AM inoculated *Catharanthus* at all three different levels of phosphate after 60, 90 and 120 days of AM inoculation as compared to non AM *Catharanthus* plants. The increase in total chlorophyll content in inoculated plants may be due to increased uptake of phosphorus, which will increase the photosynthetic activity of *Catharanthus roseus* plants and ultimately the chlorophyll content. The present results are in agreement with several authors who found increased chlorophyll content in mycorrhizal inoculated plants as compared to non inoculated plants (Allen *et al*., 1981; Morte *et al*., 2000; Mathur and Vyas, 2000). The mutuality of symbiotic fungi stimulated the production of more leaf chlorophyll that consequently may lead to an increase in photosynthetic potential of inoculated plants and hence enhanced growth (Ekanayake *et al*., 2004).

As shown in Tab. 3, the activities of phosphatase enzymes (acid phosphatase, alkaline phosphatase) were found to be significantly higher in mycorrhizal treated *Catharanthus* plants as compared to non inoculated *Catharanthus* plants at all levels of phosphate after 60, 90 and 120 days of AM inoculation. Higher activity of acid phosphatase and alkaline phosphatase were found at *Gf*+2p and *Gf*+3p levels. The present results are in agreement with several authors who also observed increased activity of acid phosphatase and alkaline phosphatase in mycorrhizal roots as compared to non mycorrhizal roots. Fries *et al*. (1998) observed that acid phosphatase and alkaline phosphatase activities in maize roots were closely correlated to levels of AMF colonization of roots. Selvaraj (1998) found that due to inoculation of AM fungi, *G. fasciculatum*, acid phosphatase activity increased in leaves and roots of *P. juliflora*. Higher uptake of phosphorus in mycorrhizal *Catharanthus* plants may be due to the increased level of phosphatase enzymes which ultimately resulted in enhanced growth response of *Catharanthus roseus.* Ezawa *et al*. (2001) suggested that ALP in colonized root played an

Tab. 4. Total phosphate content of *Catharanthus roseus* L. shoot after 60, 90 and 120 days of AM inoculation under three different phosphate conditions

Total Phosphate content mg/g of dry weight			
Treatments	60 days	90 days	120 days
C	0.716±0.080e	1.165±0.068e	1.437±0.044e
C+1P	1.011±0.093d	1.949±0.149d	2.091±0.238d
C+2P	1.110±0.158d	2.208±0.246cd	2.404±0.241cd
C+3P	1.440±0.087cd	2.386±0.191bc	2.497±0.168c
Gf	1.250±0.141bc	1.638±0.077b	1.849±0.239bc
Gf+1p	1.659±0.148ab	2.364±0.247b	2.898±0.162ab
Gf+2p	1.784±0.149a	2.545±0.122ab	3.061±0.288a
Gf+3p	1.914±0.302a	2.777±0.257a	3.067±0.359a

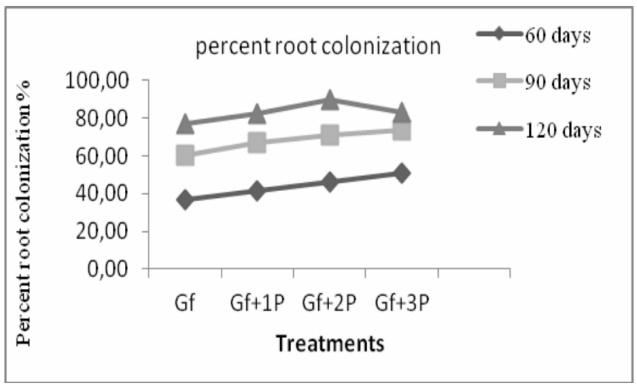

Fig. 1. The percentage of AM colonization in *Catharanthus roseus* L. after 60, 90 and 120 days of AM inoculation

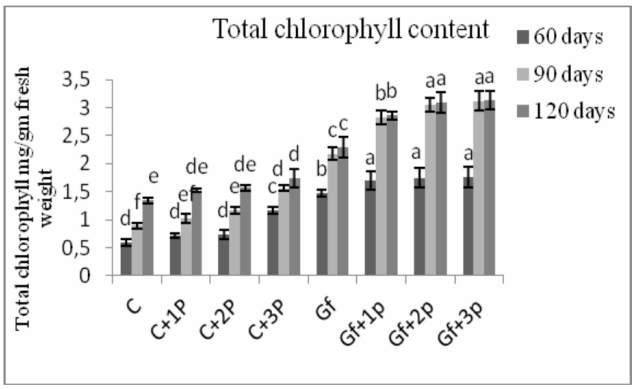

Fig. 2. Total chlorophyll content in *Catharanthus roseus* L. after 60, 90 and 120 days of AM inoculation

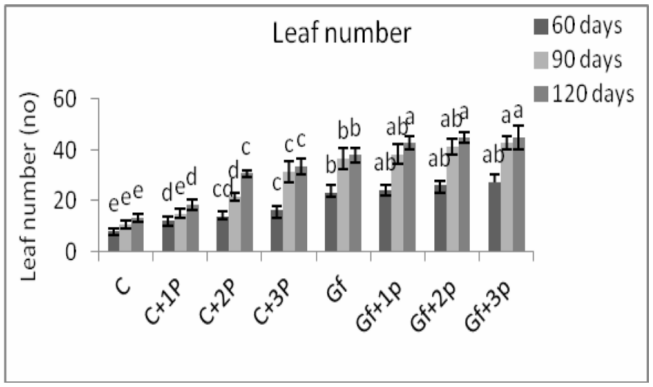

Fig. 3. Number of leaves in *Catharanthus roseus* inoculated with *Glomus fasciculatum* after 60, 90 and 120 days of AM inoculation

important role in polyP degradation and thus the release of inorganic phosphate (Pi) from arbuscules to root cells. Phosphorus translocated from external hyphae which is normally present in the form of polyphosphate can be hydrolysed by alkaline phosphatase.

The major role of AM fungi is phosphate uptake, because it encodes a phosphate transporter gene. In the present investigation, AM inoculated *Catharanthus* contained significantly higher total phosphate content in their leaves as compared to non AM *Catharanthus* at all levels of phosphate after 60, 90 and 120 days of AM inoculation (Tab. 4). The results of the present study coincide with the reported findings of Jackobsen *et al*. (1992) who have reported that the fungal hyphae growing beyond the rhizospheric soil increase the absorptive surface area of the root, which result in a greater efficiency of nutrient absorption, especially slowly diffusing mineral ions like phosphorus (Kothari *et al*., 1991; Li *et al*., 1991). The least uptake of phosphorus was observed in uninoculated control plants. Transport of P into host plants and its release to root cells is an important function of AMF (Ryan *et al*., 2007; Smith *et al*., 2001). Increased P absorption is usually attributed to increased surface area and increased soil exploration by the root-AMF association (Miyasaka and Hobte, 2001)

Conclusions

This work clearly indicates the beneficial effects of AM fungi *Glomus fasciculatum* on the growth and biomass of economically important plant parts (shoot, root) which are often the harvest products in medicinal plants specifically *Catharanthus roseus*. Moreover numbers of days taken for first flowering were also reduced due to inoculation of *Glomus fasciculatum* and application of 100 mg K_2HPO_4 kg^{-1} was equally as good as 150 mg K_2HPO_4 kg^{-1} of soil. This study shows that the application AM fungi controls growth and development of *Catharanthus roseus* to a large extent and inorganic fertilizer (Phosphate) alone is not suitable for *Catharanthus roseus* cultivation.

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