Arbuscular Mycorrhizal fungi and precursor feeding improve protein and trypthopan decarboxylase enzyme content in *Cathaaranthus roseus* L.

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Abstract

To obtain a better understanding of the mycorrhizas as an elicitor, and precursor feeding effects on the protein content and TIAs biosynthesis in *Catharanthus roseus* L., foliar application of four concentrations of tryptophan (0, 150, 250 and 350 mg/l) on some growth parameters and chemical compositions of mycorrhizal plants were investigated. The results revealed that the total protein content in the shoots and roots was higher respectively in *G. etunicatum* and *G. versiforme* mycorrhizal plants treated by 350 mg/l tryptophan, and this enhancement was significant at 5% statistical level. The evaluation of SDS-PAGE obtained results showed that the application of mycorrhizas and tryptophan led to significant accumulation of a 50-55 kDa M.W protein in the shoots and roots which probably could be attributed to TDC enzyme. From this observation we could suggest that the treatment of both mycorrhizas and tryptophan may be resulted in high expression of TDC enzyme.

Keywords: Catharanthus roseus L.; Mycorrhiza; Tryptophan; SDS-PAGE

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Introduction

Arbuscular Mycorrhizal Fungi (AMF) are ubiquitous soil microorganisms that form a mutual association with a wide variety of plants. In general, in this symbiosis relationship, AMF obtain carbon from the plants and provide the phosphorous and the other nutrient for plants from the soil (Kapoor et al., 2008). The benefits of AMF are related to enhancing plant adaptation to stressful conditions by improving the plant growth and nutrient (De la Rosa-Mera, 2011). In general, the symbiosis is significant to life, in both natural and agricultural ecosystems (Kapoor et al., 2008).

Catharanthus roseus (L.) is an important medicinal and ornamental bedding plant belonging to the family apocynaceae (Abdul Jaleel et al., 2008). The major property of this plant is production of the important secondary metabolites, such as terpenoid indole alkaloids (TIAs), vinblastine and vincristine which are used as anticancer drugs. These two bis-indole alkaloids accumulate in trace amount and this promoted efforts to improve their production in plant (Mgnotta et al., 2006). Beside these alkaloids that are produced in the shoot parts of

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the plant, there are also some compounds such as ajmalicine produced in the roots of *C. roseus* plants (De la Rosa-Mera, 2011). The presence of these medicinal alkaloids and ornamental value of this plant, have gained commercial importance for it and many investigations have been carried out to enhance its alkaloids contents.

There are two major metabolic pathways present for the biosynthesis of TIAs. One pathway comes from tryptophan as a precursor where Tryptophan Decarboxylase (TDC) converts it to tryptamine and the other pathway drives from loganine which is converted to secologanin, a mono terpenoid glucoside. The carboxylation of tryptophan is the first step in the formation of TIAs in *C. roseus* L. plants. Tryptamine and secologanine are condensed to strictosidine which is the general precursor of TIAs (Witmer et al., 2002).

In this study, we examined the growth, protein content and presence of the enzymes which involve in the biosynthesis of the alkaloids of *C. roseus* under treatment of plants with some mycorrhizal species and three concentrations of tryptophan, as a precursor.

Materials and Methods

Inoculum production

Pot culture of the arbuscular mycorrhizal fungi, *Glomus etunicatum, Glomus intraradices* and *Glomus versiforme* were initiated on corn in a greenhouse during April to June 2011. Soil used for production of mycorrhizal inoculum was collected from the field and mixed with sand (1:5 w/w) and 100 g fungi inoculum. Soil and sand were autoclaved before mixing at 120 °C for 4 h. Plants were grown at 32 °C under 16 h light and 8 h dark periods and were illuminated by white fluorescent light and sodium lamp with total irradiance of about 75 $\mu Em^{-2}s^{-1}$. Rorison's solution was used as nutrient medium. Finally, roots were removed from the soil, cut and then mixed with the soil. This inoculum included soil/sand mixture, extraradical hyphae, spores and colonized roots.

Plant material and growth conditions

A pot culture of periwinkle plants was carried out at the University of Agricultural Sciences, Sari, Iran. The seeds were surface sterilized in 0.2% $HgCl_2$ solution for 5 min and were sown in the nursery for five days. Resulting seedlings were transferred into plastic pots, which contained sterilized soil:sand (1:3) mixture and 50 g mycorrhizal inoculum. Fertilization of the plants was carried out with humus at the rate of 500 g per pot. The plants were grown under 24-28 °C with 16:8 h photoperiod. After thirty days, plants were sprayed with three concentrations of tryptophan (150, 250 and 350 mg/l), in addition to the control plants. The samples were sprayed four times at one month intervals. The pots were arranged in a randomized complete design, with four replicates. The plants were uprooted after 120 days and the root system of each plant was separated from the shoot to measure growth parameters.

Extraction of protein

The shoots and roots parts of the plants were harvested and frozen by liquid nitrogen. 500 mg of the samples were extracted in 0.8 ml of Tris-boric buffer (0.09 M Tris, 0.08 M Boric acid, 0.93 g/l of Na_2EDTA) and 0.8 ml of 40% sucrose. Then, the extract was centrifuged at 13000 g for 10 min. The supernatant was used as protein extract (Mohammadkhani and Heidari, 2008).

Measurement of total protein content

Total protein content of shoots and roots were measured using the method of Bradford and Bovine Serum Albumin (BSA) as a standard (Bradford, 1979).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE gel electrophoresis was performed using 12% running gel and 3% stacking gel. Running gel contained 30% w/v acrylamide, 0.8% w/v methyl bis acrylamide, 10% w/v SDS,

1.5 M Tris - HCl (PH= 8.3) , 9.3 ml deinonized H_2O, 10% w/v ammonium persulfate (APS) solution and 23 µl of Tetra Methylen Diamine (TEMED). Stacking gel contained 30% w/v acrylamide, 0.8% w/v methylene bis acrylamide, 10% w/v SDS, 0.5 M Tris - HCl (PH= 6.8), 10.10 ml of deionized H_2O, 10% w/v APS and 23 µl of TEMED. Running buffer for the tank contained 0.125 M Tris – HCl (PH=8.5), 0.96 M glycine and 0.5% w/v SDS. The sample buffer was used with an equal volume of protein extraction supernatant (Hames and Rickwood, 1990).

Statistical analysis

Data were subjected to analysis of variance and one way ANOVA was applied to comparison of results between different groups using the Duncan and Tukey multiple range test ($P < 0.05$).

Results

In this study, we evaluated the protein content and SDS- PAGE patterns in mycorrhizal *C. roseus* plants treated with some concentrations of tryptophan amino acid. Analysis of total protein content in the shoots of these treatments revealed that this parameter was higher in 350 mg/l tryptophan application compared to other concentrations of tryptophan and this increment was higher in *G. etunicatum* and *G. versiforme* inoculated plants, respectively (Fig. I). The statistical analysis of the results showed that the differences were significant at 5% level between these fungi groups. Also, the evaluation of total protein contents of roots in these samples showed higher contents in *G. versiforme* symbionts plants sprayed with 350 mg/l tryptophan, and the differences with the other groups were significant at 5% level (Fig. II). The evaluations of SDS-PAGE analysis in the shoots and roots of *C. roseus* L. indicated that the accumulation of a peptide with about 45-55 kDa M.W in both shoots and roots gel electrophoresis was in response to mycorrhizal and tryptophan treatments (Figs. III and IV). Finally, the results showed that in two organs, the presence of proteins with these molecular weights were higher in mycorrhizal samples.

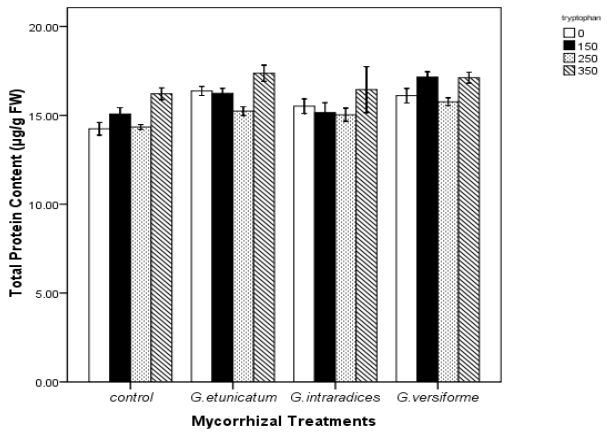

Fig. I. Effect of mycorrhizal fungi and tryptophan on total protein content in shoots of *C. roseus.* Results are shown as mean ± standard error ($P < 0.05$), obtained from four replicates.

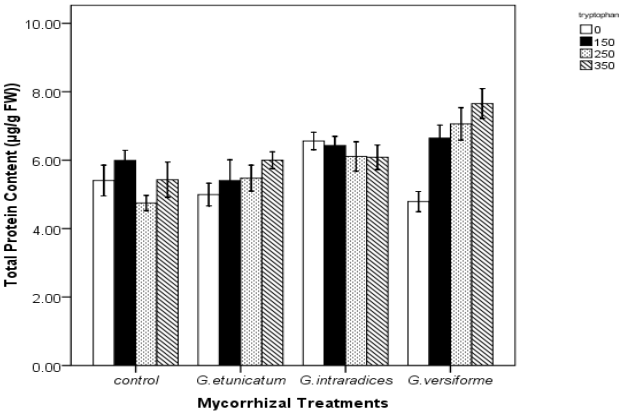

Fig. II. Effect of mycorrhizal fungi and tryptophan on total protein content in roots of *C. roseus.* Results are shown as mean ± standard error ($p < 0.05$), obtained from four replicate.

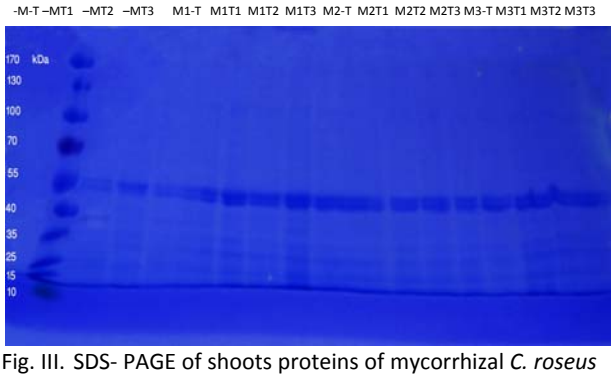

Fig. III. SDS- PAGE of shoots proteins of mycorrhizal *C. roseus* in response to some concentrations of tryptophan amino acid. -M, M1, M2, and M3 indicate the Control, *G. etunicatum, G. intraradices* and *G. versiforme* inoculated samples, respectively. Also, -T, T1, T2 and T3 revealed 0, 150, 250 and 350 mg/l applied tryptophan, respectively.

Discussion

The study of the mycorrhizal species and some concentrations of tryptophan treatments in *C. roseus* plants revealed that the total protein content in the shoots and roots was higher in *G. etunicatum* and *G. versiforme* mycorrhizal plants treated with 350 mg/l tryptophan, and this enhancement was significant at 5% statistical level. The beneficial effect of mycorrhizal fungi on total protein content has been reported on *Ocimum basilicum* inoculated with *G. moseae* and *G. intraradices* (Enteshari et al., 2012). Regardless of AMF inoculation, trypthophan resulted in higher protein content. Similar results have earlier been reported by Talaat et al. (2005) on *C. roseus* and Abdel Aziz and Balbaa (2007) on *Salvia farinaceae*. High level of shoots protein content compared to roots may indicate that the foliar application of tryptophan on the shoots had been more efficient.

On the other hands, this study evaluated the SDS-PAGE of extracted proteins and enzyme present in the treatments. The results showed that the application of mycorrhizas and tryptophan caused significant accumulation of a 50-55 kDa M.W protein in shoots and roots which probably could be attributed to TDC enzyme. Jacobs (2005) argues that the TDC enzyme with 49.5 kDa could be present in the band of molecular weight which was also observed in this study. Also, the findings showed that the treatment of plants with tryptophan and inoculation with *G. versiforme* resulted in higher amount of this enzyme.

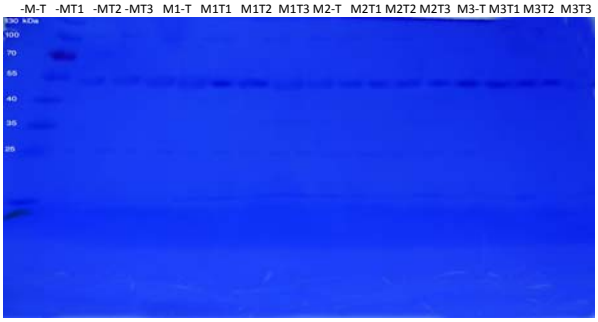

Fig. IV. SDS- PAGE of roots proteins of mycorrhizal *C.roseuse* in response to some concentrations of tryptophan amino acid. -M, M1, M2 and M3 indicate the Control, *G.etunicatum, G.intraradices* and *G.versiforme* inoculated samples, respectively. Also, -T, T1, T2 and T3 revealed 0, 150, 250 and 350 mg/l applied tryptophan , respectively.

The findings of the present study suggest that the feeding of *C. roseus* L. plants by tryptophan precursor led to the higher expression of TDC enzyme which catalyze conversion of tryptophan to tryptamine and consequently, could result in increasing of TIAs in the treatments. Because TDC contributes in both TIAs and auxin biosynthesis, it could be concluded that the growth improvement of the plants treated by mycorrhizas and tryptophan could be due to the increment of auxin biosynthesis in these samples. Witmer et al. (2002) reported that the feeding of *C. roseus* L. by tryptophan precursor in combination with another precursor (tryptamine and loganine) caused accumulation of a high level of TIAs compared to controls. Therefore, it could be concluded that the mycorrhizal fungi played the role of an elicitor in this study. Finally, it is suggested that the feeding of plants with tryptophan as a precursor, should be involved in enhancement of TIAs accumulation and the improvement of protein content in this medicinally important plant. Also, it may be concluded that mycorrhizal fungi probably could enhance the uptake of tryptophan through signal transduction pathways and therefore, could enhance the plant condition.

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