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Effect of Arbuscular Mycorrhiza on Growth and Physiological Behavior of PHL-C Rootstock

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Abstract

PHL-C is one of the dwarf sweet cherry rootstocks which is a hybrid between *P. avium* L. × *P. cerasus* L. Direct rooting of sweet cherry rootstocks is difficult which can be solved by using in vitro propagation. Transfer of plantlets from in vitro to ex vitro limit the use of micro propagation, because of weak root systems and low survival rates. This study was conducted in order to select the best biohardening agents in order to improve the growth of PHL-C dwarf rootstocks in Khorasan Razavi Agriculture and Natural Resources Research and Education Center. Three arbuscular mycorrhizal fungi (AMF) strains, *Diversispora epigaea*, *Rhizophagus intraradices* and *Rhizophagus fasciculatus*, were used as in vitro raised PHL-C plantlets. Results showed that plantlets inoculated with *Diversispora epigaea* gave the highest leaf area, root diameter, root surface and phosphor concentration. *Diversispora epigaea* was more effective in improving most of the growth and physiological attributes of inoculated tissue culture raised plantlets of PHL-C. However, the highest total root length (4113 mm) was found in *Rhizophagus fasciculatus* inoculated plantlets

Keywords: Arbuscular mycorrhizal fungi; PHL-C; Rooted plantlets

Introduction

Sweet cherry (*Prunus avium* L.) belongs to the Rosacea family and the Prunoideae sub family (Wunsch and Hormaza 2004). According to the Food and Agriculture Organization in 2013, Iran is the third largest producer (200,000 tons) after USA and Turkey. A sweet cherry tree in a good condition averages a height of 15-20 meters, which poses as a major problem for producer. The best way to control the size of tree varieties is to use dwarf- rootstock. PHL-C is a dwarf rootstock produced from a cross between *P. avium* and *P. cerasus* and released in the Czech Republic. This rootstock is compatible with a variety of cherries. It is tolerant to waterlogging, calcareous soils, *Pseudomonas syringae* and agrobacterium and is recommended to be used for high density cultivation (Sarropoulou *et al.* 2012). Producing of plants without genetic variations has been possible in micro propagation technique. Transfer of plantlets from in vitro to ex vitro limit the use of micro propagation, because of weak root systems and low survival rates (Hazarika 2003). The transfer of raised plantlets in vitro to green house is one of the most important steps in the physiological adaptation during micropropagation. Although some species adapt quickly to the new conditions, but others don't act this way and this often leads to the increase in the use of fertilizers, fungicides and other chemicals (Gaur and Adholeya 1999).

Several techniques have been used in the past to improve the growth and reduce the mortality rates of plantlets during transferring. Biological factors for example mycorrhizal fungi can change their host plant roots (Gamalero *et al.* 2004). It has been shown that the AMF symbiosis with roots of micropropagated plantlets, improved absorption of water and nutrients and increased stress tolerance (Jaizme-Vega *et al*. 1997). Mycorrhizal fungi can interact with the host plant roots to establish a mutual coexistence. The host plant gains inorganic compounds and carbon compounds achieved from photosynthesis (Smith and Read 2008). Mycorrhiza can increase the absorption of non-mobile elements through external hyphae increase (Abdul Jalil *et al.* 2007). Many studies on plants such as apple (M9, M26, Golden)(Branzanti *et al.* 1992), *Prunus avium* L. (Arines and Ballester 1992), *Vitis vinifera* L. (Singh *et al.* 2004), Banana cv. Grade Naine (Rodrı´guez-Romero *et al*. 2005), pomegranate (Singh *et al.* 2012) have utilized AMF to increase the growth rate and nutrient uptake of plantlets.

In order to reduce harvest losses and reduction of plantlets it is necessary to pay special attention to the use of bio-fertilizers, so this study was conducted to determine the best mycorrhizal fungi on growth, physiological and biochemical attributes of in vitro raised plantlets of PHL-C root stock.

Materials and Methods

This study was conducted in Khorasan Razavi Natural Resources and Agriculture Research and Education Center, Iran. Plantlets of PHL-C rootstock were used for AMF inoculation. Explants (healthy grown mature plants of cultivar PHL-C) were maintained in MS medium supplemented with 1 mg L^{-1} Banzyl amino pourine. After the proliferation stage, plantlets were rooted in MS medium containing 1 mg L^{-1} IBA. Ex vitro inoculation was used for bio hardening and, therefore, micropropagated plantlets were inoculated with AMF spores after transplanting into pots (Puthur *et al.* 1998). The rbuscular mycorrhizal fungi were provided from Biotech Company Turan, under the protection and supervision of Science and Technology Park of Semnan Province, Iran. The soil based AMF inoculum contained spores, hyphae and root segments of host plants (*Trifolium repens*). The rooted plantlets of PHL-C were transferred to pots that were filled with sterile soil. Soil was sterilized in autoclave at 121 °C and 15 lb/in.^{2} for 20 min. The mycorrhiza preparation specified was spread below the rhizospheres and then the pot was covered with a thin layer of un- sterile Coco peat. Three AMF species, *Diversispora epigaea*, *Rhizophagus intraradices* and *Rhizophagus fasciculatus*, were used along with non-inoculated plantlets as the control. The spore density ranged between 50 and 150/ g soil containing mycorrhizal plant roots and mycorrhizal fungal hyphae. Mycorrhizal treatments consisted of 10, 20 and 30 g inoculum with rhizosphere soil. The inoculated plants and the control plantlet were transferred to a moist chamber in a greenhouse with 70% relative humidity and day-night temperatures about 25 ± 1 °C. After 45 days PHL-C plantlets were separated and various growth, physiological and biochemical factors were measured to study the effects of treatments. Leaf area was measured using leaf area meter. Roots were rinsed with tap water then carefully arranged for the image capture using a

scanner, by the analysis of root system architecture (Rillig *et al.* 2008). With this system, total root length, root surface area, average root diameters of different sizes were analyzed. Mycorrhizal growth response (MGR) was calculated using the total plant dry weight (DW) of mycorrhizal and the mean dry weight of non-mycorrhizal plants as follows (Cavagnaro *et al*. 2003):

$$\text{MGR (\%)} = \frac{\text{DW(M)} - \text{DW(NM)}}{\text{DW(NM)}} \times 100$$

Mycorrhizal phosphorous response (MPR) was calculated similarly. For determination of phosphorus, 0.2 g of grounded dried plant material was ashed at 550 °C followed by dissolution in 3.3% HCl. After the digestion of the plant material, the concentration of P in this solution was determined colorimetrically (Murphy and Riley 1962). The phosphorus content was calculated with the following formula using shoot dry weight (DW) and phosphor concentration:

Phosphorus content= shoot DW × P concentration

The experiment was factorial based on completely randomized design with three replications. The differences among treatment means were compared by the least significant difference (LSD) test. All analyses were done using JMP 8 software.

Results and Discussion

There was a significant difference ($P \leq 0.01$) for leaf area between VAM fungi (Table 1). Greater leaf area was recorded in *Diversispora epigaea* species with 916.185 cm^2/plantlet. Plantlets that were treated with mycorrhizal fungi had greater leaf area as compared to the control (Table 1). Mycorrhizal plant weight and leaf area increases under both irrigation and drought stress; this increase could be attributed to better water absorption resulting by increasing the absorption of phosphorus (Ruiz-Lozano 2003; Roldan *et al*. 2008). These results were consistent with the reports of Dietz and Foyer (1986) that showed an increase in the leaf area in the plants treated with mycorrhizal fungi due to increased absorption of phosphorus. AMF and their concentrations differed significantly in terms of the rootstock root length. Results showed that the root length increased by the use of mycorrhizal fungi (Table 1, Figure 1) and there was a significant difference between different species of fungi. *Rhizophagus fasciculatus* produced greater root length than other species. Although concentrations of AMF species produced greater root length than the control, but no apparent patterns between concentrations of AMF fungi were observed (Table 1). The results showed that the use of mycorrhizal fungi increased root diameter and the maximum diameter of roots was observed in *Diversispora epigaea*. There were significant differences for root diameter among AMF fungi, but no significant difference was observed between the concentrations of fungi (Table 1). AMF and their concentrations (inoculum rate) were significantly different for the root surface area of the rootstock. Results showed that the root surface increased by the use of mycorrhizal fungi and the highest surface was observed in the inoculated plantlets with *Rhizophagus fasciculatus*. Reports show that mycorrhizal fungi lead to changes in the shape and size of the roots, first by changing the nutritional status (Yao *et al.* 2005). AMF helps in the development of root system by increasing the root intensity and surf area (Puthur *et al.* 1998). The branches and the volume of roots have been affected by nutrients, especially phosphorus (Lopez-Bucio *et al.* 2003).

Mycorrhizal inoculation increased root and shoot dry weight, which is similar to the results obtained by Graham and Timmer (1984). The increase in dry weight of banana plantlets inoculated with mycorrhizal fungi has also been reported (Declerck *et al*. 2002).

The mycorrhizal fungi improve the development and water uptake of plant. The main way to measure the effectiveness of the fungus is to determine the mycorrhizal growth response (MGR) (Janos 2007). Responses to mycorrhiza directly linked with the growth rate of the host and phosphorus application (Koide 1991). Our results showed that *Diversispora epigaea* achieved the highest growth response among other species of AMF. There was a significant difference between the inoculum densities and the maximum length of root was observed for the density of 20 g and the lowest was observed in the control (Table 1).

Table 1. Comparison of the effects of various arbuscular mycorrhizal fungi (AMF) on growth of PHL-C rooted plantlets

Treatments	Total root length (mm)	Root diameter (mm)	Root surface area (mm^2)	Root dry weight g/plant	Mycorrhizal growth response	Leaf area (cm^2)
AMF						
Diversispora epigaea	3064.6b	0.7138a	2298.3b	0.1914a	69.39a	185.9a
Rhizophagus intraradices	3302.6b	0.6903b	2386.0b	0.1598ab	41.44ab	144.5b
Rhizophagus fasciculatus	4113.4a	0.6227b	3260.2a	0.1357b	20.13b	124.5b
Concentration (20 g)						
0	2464.2b	0.6172a	1927.0b	0.1133b	0.29b	122.0 b
10	3637.4a	0.7027a	2709.1a	0.1733a	53.39a	175.2a
20	4250.9a	0.6741a	3145.0a	0.1822a	61.25a	151.4ab
30	3621.6a	0.7084a	2811.6a	0.1804a	59.68a	157.9a

Means followed by the same letter in each column and for each factor do not differ at $P \leq 0.05$ by the least significant difference test

P uptake among plant species and cultivars is different (Brown *et al*. 1977). Mycorrhizal fungi can increase absorption of non-mobile elements through external hypha (Abdul Jaleel *et al*. 2007). According to our results the use of mycorrhizal fungi increased the amount of phosphorus in shoots and the effects of different species of fungi, concentrations and their interactions were significant. *Diversispora epigaea* with the concentration of 20 g results in the highest phosphorus (Figure 2). Increased external hyphae in the soil increase the uptake of non-mobile elements (Azevedo-Neto *et al*. 2006). Ortas (2008) reported that the amount of phosphorus and zinc in several plant species that were inoculated with mycorrhizal fungi was higher than the control. Ortas *et al.* (2002) showed that mycorrhiza significantly increased the uptake of phosphorus in citrus. The overall uptake of phosphorus and zinc in the soil treated with inoculated of mycorrhizal was reported to be more than the uptake in the control treatment (Yano-Melo *et al*. 1999; Munkvold *et al*. 2004). Mycorrhizal fungi using external hypha changed root morphology, increased root absorption and transport of nutrients

to roots (Kung'u *et al.* 2008). In this regard, the fungi produce and secrete an insoluble and soluble form of phosphate phosphatase (Song 2005).

Results showed that the use of mycorrhizal fungi increases the phosphorus content and there was a significant difference among different fungal species and concentrations. *Diversispora epigaea* at the concentration of 20 grams resulted in the highest phosphorus content.

Plantlets transferring from the laboratory to the environment limits the use of micropropagation, due to weak root systems and high seedling mortality (Hazarika 2003). Plantlets that have been obtained from in vitro culture have poor cuticular development and will be dry because of excess transpiration and transition to new environmental conditions (Wang *et al.* 1993). It is necessary to pay special attention to environmental biofertilizers, in order to reduce the problems of dwarf seedling harvest and to reduce the losses during transmission of the ex vitro plantlets. In our study, the effects of all AMF were significantly superior over the non-mycorrhizal control with regard to physical, physiological and biochemical attributes. Among all mycorrhizal strains *Diversispora epigaea* was found superior suggesting its high suitability as biohardening agents for tissue culture raised PHL-C plantlets.

Figure 1. Effect of AMF inoculation on root length of in vitro raised PHL-C plantlets. A) Control B) *Diversispora epigaea*

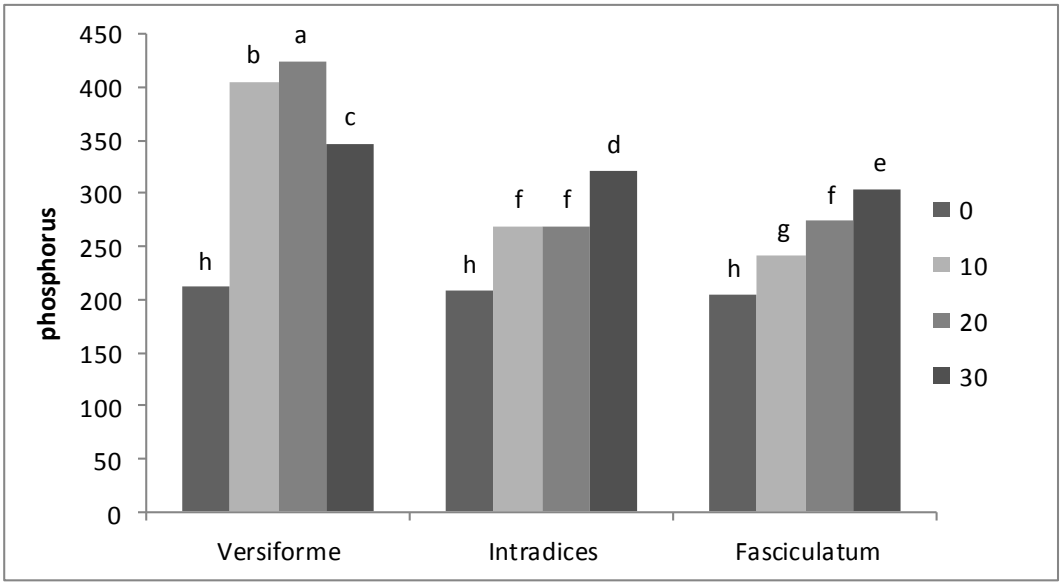

Figure 2. Effect of arbuscular mycorrhizal fungi on P concentration of the shoots of the PHL-C rooted plantlets

References

Abdul Jaleel C, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R and Panneerselvam R, 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany 73 (2): 190–195.

Arines J and Ballester A, 1992. Mycorrhization of micropropagated Prunus avium L. plantlets. In: Micropropagation, Root Regeneration, and Mycorrhizas. P 45. Joint Meeting Between COST 87 and COST 8.10, Dijon, France.

Azevedo-Neto AD, Prisco JT, Enis-Filho J, Braga de Abreu CE and Gomes-Filho E, 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation maize genotypes. Environmental and Experimental Botany 56 (1): 87–94.

Branzanti B, Gianinazzi-Pearson V and Gianinazzi S, 1992. Influence of phosphate fertilization on the growth and nutrient status of micropropagated apple infected with endomycorrhizal fungi during the weaning stage. Agronomie 12 (10): 841–845.

Brown JC, Clark RB and Jones WE, 1977. Efficient and inefficient use of phosphorus by sorghum. Soil Science Society of America Journal 41 (4): 747-750.

Cavagnaro TR, Smith FA, Ayling SM and Smith SE, 2003. Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. New Phytologist 157: 127–134.

Declerck S, Risede, JM and Delvaux B, 2002. Greenhouse response of micropropagated bananas inoculated with in vitro monoxenically produced arbuscular mycorrhizal fungi. Scientia Horticulturae 93 (3-4): 301–309.

Dietz KJ and Foyer C, 1986. The relationship between phosphate and photosynthesis in leaves: reversibility of the effects of phosphate deficiency on photosynthesis. Planta 167: 376–81.

Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG and Berta G, 2004. Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. Mycorrhiza 14: 185–192.

Gaur A and Adholeya V, 1999. Mycorrhizal effects on the acclimatization, survival, growth and chlorophyll of micro propagated Syngonium and Draceana inoculated at weaning and hardening stages. Mycorrhiza 9: 215-219.

Graham JH and Timmer LW, 1984. Vesicular-arbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: effect of phosphorus source. Journal of the American Society for Horticultural Science 109: 118–121.

Hazarika BN, 2003. Acclimatization of tissue-cultured plants. Current Science 85 (12): 12–25.
Jaizme-Vega MC, Tenoury P, Pinochet J and Jaumot M, 1997. Interactions between the root-knot nematode Meloidogyne incognita and Glomus mosseae in banana. Plant and Soil 196 91): 27–35.
Janos DP, 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza 17: 75–91.
Koide RT, 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytologist 117: 365-386.
Kung'u JB, Lasco RD, Dela Cruz LU, Dela Cruz RE and Husain, T, 2008. Effect of vesicular arboscular mycorrhiza (VAM) fungi inoculation on coppicing ability and drought resistance of Senna spectabilis. Pakistan Journal of Botany 40: 2217-2224.
Lopez-Bucio J, Cruz-Ramirez A and Herrera-Estrella L, 2003. The role of nutrient availability in regulating root architecture. Current Opinion in Plant Biology 6: 280–287.
Munkvold L, Kjoller R, Vestberg M, Rosendahl S and Jakobsen I, 2004. High functional diversity within species of arbuscular mycorrhizal fungi. New Phytologist 164: 357–364.
Murphy J and Riley JP, 1962. A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta 27: 31-36.
Ortas I, 2008. Field trials on mycorrhizal inoculation in the Eastern Mediterranean horticultural region. In: Feldmann F, Kapulnik Y and Baar J (Eds). Mycorrhiza Works: Proceedings of the International Symposium "Mycorrhiza for Plant Vitality" and the Joint Meeting for Working Groups 1-4 of COST Action 870, 3-5 October 2007, Hannover, Germany. Pp. 56-77.
Ortas I, 2012. Mycorrhiza in Citrus: Growth and Nutrition. In: Srivastava A (Ed). Advances in Citrus Nutrition. Springer, Dordrecht.
Puthur JT, Prasad KVSK, Sharmila P and Pardha Saradhi P, 1998. Vesicular arbuscular mycorrhizal fungi improves establishment of micropropagated Leucaena leucocephala plantlets. Plant Cell, Tissue and Organ Culture 53: 41–47.
Rillig MC, Ramsey PW, Gannon JE, Mummey DL, Gadkar V and Kapulnik Y, 2008. Suitability of mycorrhiza-defective mutant/wildtype plant pairs (Solanum lycopersicum L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. Plant and Soil 308 (1–2): 267–275.
Rodrı´guez-Romero AS, Pin˜ero-Guerra MS and Jaizme-Vega MC, 2005. Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. Agronomy for Sustainable Development 25: 395–399.
Roldan A, Diaz-Vivancos P, Hernandez JA, Carrasco L and Caravaca F, 2008. Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil. Journal of Plant Physiology 165 (7): 715–722.
Ruiz-Lozano JM, 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13: 309–17.
Sarropoulou V, Dimassi-Theriou K, Therios I and Koukourikou-Petridou M, 2012. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (Prunus avium × Prunus cerasus). Plant Physiology and Biochemistry 61: 162-168.
Singh SK, Minakshi G, Khawale RN, Patel VB, Krishna H and Saxena AK, 2004. Mycorrhization as an aid for biohardening of in vitro raised Grape (Vitis vinifera L.) plantlets. Acta Horticulturae 662: 289-295.
Sigh NV, Sigh SK, Sigh AK, 2012. Meshram DT, Suroshe SS and Mishra DC, 2012. Arbuscular mycorrhizal fungi (AMF) induced hardening of micropropagated pomegranate (Punica granatum L.) plantlets. Scientia Horticulturae 136: 122-127.
Smith SE and Read DJ, 2008. Mycorrhiza Symbiosis. 3rd edition. Academic Press, San Diego, CA. 787 pages.
Song H, 2005. Effects of VAM on host plant in the condition of drought stress and its mechanisms. Electronic Journal of Biology 1: 44-48.
Wang H, Parent S, Gosselin A and Desjardins Y, 1993. Study of vesicular-arbuscular mycorrhizal peat-based substrates on symbiosis establishment, acclimatization and growth of three micropropagated species. Journal of the American Society for Horticultural Science 118: 896–901.
Wunsch A and Hormaza JI, 2004. Molecular evaluation of genetic diversity and Sallele composition of local Spanish sweet cherry (Prunus avium L.) cultivars. Genetic Resources and Crop Evolution 51 (6): 635–641.

Yano-Melo AM, Maia LC, Saggin Jr. OJ, Lima-Filho, JM and Melo NF, 1999. Effect of arbuscular mycorrhizal fungi on the acclimatization of micro propagated banana plantlets. Mycorrhiza 9: 119-123.

Yao Q, Zhu HH and Chen JZ, 2005. Growth responses and endogenous IAA and iPAs changes of litchi (Litchi chinensis Sonn.) seedlings induced by arbuscular mycorrhizal fungal inoculation. Scientia Horticulturae 105 (1): 145–151.