Effects of the co-inoculation with saprobe and mycorrhizal fungi on *Vaccinium corymbosum* growth and some soil enzymatic activities

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Abstract

In this study we evaluated the initial plant growth and some biochemical changes produced in rizhosphere soil of blueberry (*Vaccinium corymbosum* L.) cv. 'Elliot' plantlets obtained from *in vitro* culture and inoculated with six arbuscular mycorrhizal (AM) fungi strains (*Gigaspora rosea*; *Glomus claroideum*; *G. deserticola*; *G. viscosum*; *G. intraradices* and *G. constrictum*) and five saprobe fungi (*Coriolopsis rigida*; *Phanerochaete chrysosporium*; *Trametes versicolor*; *Trichoderma harzianum* and *Penicillium chrysogenum*). Our results showed a significant "like" AM root colonization (25-46%), highlighting the treatment co-inoculated with *G. rosea* + *P chrysosporium* or *C. rigida*; *G. intraradices* + *C. rigida*; *G. viscosum* + *P chrysosporium*, but this root colonization was morphologically atypical, and dominated by interstitial hyphae, without develop of other typical AM structures. Furthermore, significant increase in the shoot and root growth of the plantlets inoculated with *G. viscosum* + *P chrysosporium*, and *G. intraradices* + *C. rigida* were observed. On the other hand, *G. claroideum* + *T. versicolor* showed the high increase in the dehydrogenase enzyme activity, while inoculation with *G. claroideum* alone showed the greatest ß-glucosidase activity. These results suggest that the interaction of AM symbionts together with saprobe fungi could be a biological alternative to promote the growth of micro-propagated blueberry plantlets in their early growth stages.

Keywords: Arbuscular mycorrhizae, biochemical properties, blueberries, plant symbionts, saprophytic fungi.

1. Introduction

Vaccinium corymbosum L. (Ericaceae) is a shrub native from North American, which currently is extensive cropped in Chile. The surface under plantation has grown constantly since the introduction of the species into the country, reaching 12.000 ha. at present. In natural conditions this species grows in acidic soils, usually poor deficient and with high organic matter content. In this conditions, spontaneously forms mutualist symbiotic associations with certain soil fungi belonging mainly to the phylum Ascomycota, called "ericoid mycorrhizae" (Scagel *et al.*, 2005; Smith and Read, 2008). Ericoid mycorrhizae would be specific to species of the families *Ericaceae* and *Epacridaceae*, due to their co-evolution with the native host plants growing in limiting edaphic conditions, allowing the plant to complete its life cycle (Brundrett, 2002). However, some fossil evidence indicates that originally the *Ericales* were able to establish arbuscular mycorrhizae (AM) (Brundrett, 2002), which have been reported in some species of this order, including several *Vaccinium* species (Koske *et al.*, 1990).

Although these reports are scarce, and there is little in-depth studies in this sense (Brundrett, 2002), some authors suggest that the absence of ericoid mycorrhizal fungi inoculum may allows *Ericales* plants to associate with AM-forming fungi (Koske *et al.,* 1990). Arbuscular mycorrhiza symbiosis is the most extended mutualistic association in nature, and unlike the ericoid mycorrhizae, it is not host-specific, being possible its formation between members of the phylum Glomeromycota and most of terrestrial plant species (van der Heijden, 2004; Singh, 2007).

In several micro-propagated plantlets, the application of mycorrhizal inoculants has proved to be effective improving their establishment and growth at transplantation, its resistance to hydric stress, its acquisition of mineral nutrients, and increasing its tolerance to pathogens (Scagel *et al.*, 2005; Binet *et al.* 2007). Furthermore, the lapse for micro-propagation during their acclimatization period can also be reduced in mycorrhizal plants (Salamanca *et al.,* 1992).

On the other hand, the saprobe fungi are important and common components of the rhizosphere. These fungi obtain a greater nutritional benefit when living roots release organic and inorganic compounds with sloughed cells (Dix and Webster, 1995). Additionally they produce plant growth-stimulating substances such as hormones and vitamins (Shetty *et al.,* 1994). The saprobe fungi release energy and nutrients present in complex substrates in forms which can be assimilated by the plants by means the production of extracellular enzymes, and also are capable of degrading potentially phytotoxic substances (Arriagada *et al.,* 2009a). In addition, some of them are able to produce substances that promote the growth of other microorganisms in the rhizosphere, including AM fungi (Fracchia *et al.,* 2000).

For these reasons, we suggest that co-inoculation with AM and saprobe fungi in the acclimatization stage of micro-propagated blueberry plantlets will have a beneficial influence on their growth, therefore requiring a shorter-time for their establishment in the further field conditions. Thus, the aim of the present study was to evaluate the initial susceptibility of blueberry plantlets to colonization by AM fungi, as well as the effect exercised by saprobe fungi in improving this symbiosis and promoting the growth of blueberry plantlets in the acclimatization phase.

2. Material and methods

2.1 Plant species

After *in-vitro* culture (ten months) *Vaccinium corymbosum* cv. 'Elliot' uniform seedlings were planted in 1 L pots filled with a 1:4 mixture of sterilized sand and soil type Andisol, Serie Freire (pH 5.84; 10% organic matter; 13.7 mg kg^{-1} P-Olsen). The soil was sterilized at 100°C for 1 h for three consecutive days and distributed in 1000 mL pots. Plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps (400 Em^{-2} s^{-1}; 400–700 nm) with a 16/8 h day/night cycle at 25/19 °C and 50% relative humidity.

2.2 Arbuscular Mycorrhizal fungi

The AM fungi used in the experiment were *Gigaspora rosea* Nicolson and Schenck; *Glomus claroideum* (Schenck and Smith) Walker and Vestberg; *Glomus deserticola* (Trappe, Bloss and Menge); *Glomus viscosum* T.H. Nicolson, *Glomus intraradices* Schenck and Smith and *Glomus constrictum* Trappe, obtained from the culture collection at the Laboratorio de Biorremediación at Universidad de La Frontera, Temuco, Chile. The AM inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores and colonized root fragments of *Medicago sativa* L. in amounts of 10 g per pot, which were previously analyzed, showing a high potential to produce significant levels of root colonization. Plants uninoculated with AM fungi were given a filtrate (suspension of 50 g of fresh field soil in 1 l of distilled water for 10 min and filtered through a Whatman no. 1 paper) of the soil inoculum containing the common soil microflora (principally soil bacteria), but free of AM fungal propagules.

2.3 Saprobe fungi

The saprobe fungi *Coriolopsis rigida* (Berk. Et Mont.) Murrill, *Phanerochaete chrysosporium* Burds, *Trametes versicolor* (L.:Fr.) Pillat, *Trichoderma harzianum* Rifai and *Penicillium chrysogenum* Thorn., were obtained from the fungal collection at the Laboratorio de Biorremediación at Universidad de La Frontera, Temuco, Chile. The strains were stored on Malt Extract Agar (MEA) plates at 4 °C and periodically subcultured. To prepare the fungal inoculum, barley seeds were inoculated with a 1 cm^2 disk of MEA withdrawn from 14 day-old fungal cultures grown at 28 °C. Ten barley seeds colonized by the mycelium of the fungi were added to the soil in each pot.

2.4 Experimental design

Four treatments were used: (1) Uninoculated plants (controls), (2) Soil inoculated with each saprobe fungi, (3) Soil inoculated with each AM fungi, and (4) Soil inoculated with each saprobe combined with each AM fungus. Plants were inoculated at the time of transplanting (after 4 weeks of growth). The saprobe fungi were inoculated at the same time as AM fungi. Three replicate per treatment were used.

2.5 Harvests and analyses

Plants were harvested after 14 weeks, dried in a air-forced oven (70°C, 48 h) and shoots and roots biomass were determined. After the harvest, fresh samples of roots were taken from the entire root system at random. The samples were cleared and stained using trypan blue in lactic acid (Phillips and Hayman, 1970) and the percentage of root colonized by mycorrhizal fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980). The presence or ab-

sence of AM colonization at each intercept was used to determine the percentage of colonization and the colonized root length.

2.6 Biochemical determinations

To evaluate the biochemical changes in the rhizosphere, the enzymatic ß-glucosidase and dehydrogenase activities were determined. Rhizosphere soil samples were collected after harvest at direct proximity of plant roots. The β-glucosidase was determined by measuring *p*-nitrophenol (PNP) released from *p*-nitrophenyl-β-D-glucopyranoside (PNG) according to Eivazi and Tabatabai (1990). This assay is also based on the release and detection of PNP. One unit of enzyme activity was defined as 1 μmol of product released per min (IU). The amount of PNP was determined in a spectrophotometer at 398 nm. Dehydrogenase activity was determined to the method described by Trevors (1984). One gram of fresh soil was mixed with 1 ml of buffered triphenyl tetrazolium chloride (TTC) solution and after 24 h incubation at 30°C, red coloured triphenyl formazan (TPF) formed by reduction of TTC was colorimetrically determined at 546 nm.

2.7 Statistical analysis

The percentage values were arcsine transformed for statistical analyses. Statistical procedures were carried out with the SPSS software, version 11.0 (SPSS Inc., 1989-2001). The data were analyzed by means of an analysis of variance (ANOVA) and the means were compared using orthogonal contrast test. Statistical significance was determined at $P < 0.05$.

3. Results and discussion

3.1 Root colonization

Results showed variations in intraradical AM colonization in *V. corymbosum* roots among the different AM fungal strains used, measured principally as appressoria, intracellular hyphal coils, arbuscules as intracellular arbusculate coils like branches and intraradical hyphae (Smith and Read, 2008) (Figure 1). In all the AM fungal strains studied, morphologically the fungus seems to begin the symbiotic growth, but in no case were clearly distinguished the typical symbiotic structures (arbuscules and vesicles). Some studies as Vega *et al.* (2009) found high levels of AM colonization in *Vaccinium* plants cropped with the native populations of AM endophytes. In the present study the structures visualized in *V. corymbosum* roots resembled to dark septate endophytes (DSE), characterized by the presence of microsclerotial structures on the epidermal root cells according to described by Jumpponen (2001). In this case, the DSE colonization can reduce the AM colonization (Jumpponen and Trappe, 1998) or produce a negative effect on the extramatrical phase of the AM fungi (Scervino *et al.,* 2009). Further studies using molecular tools are needed to corroborate the presence and extension of AM colonization in *V. corymbosum* roots, either in the acclimatization phase as well as in field conditions.
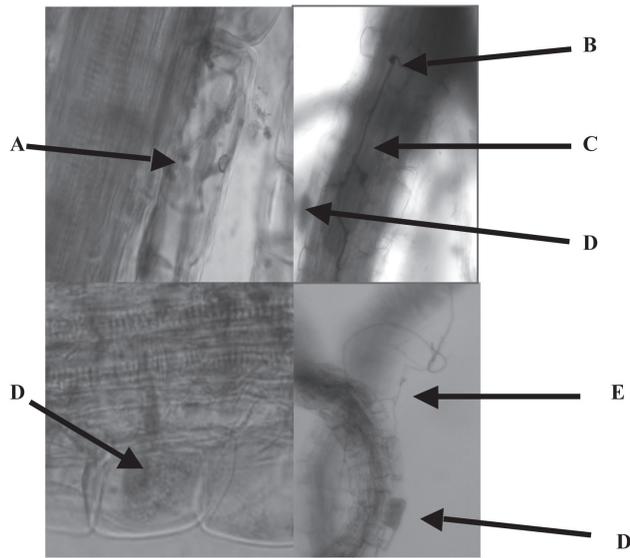
Figure 1. Arbuscular mycorrhizal fungi in cleared roots stained with trypan blue in *Vaccinium corymbosum* cv. '*Elliot'* inoculated with *G. viscosum*. A: Coils in cortical cells. B: Apresoria. C: Intercellular hyphae. D: Arbuscule. E: Infective hyphae.

As Figure 2 shows, plants co-inoculated with any saprobe fungi did not showed an manifest affect on the AM colonization (25 and 46% for any interaction). In the Figure 2A and 2F the dual inoculation with *C. rigida* increases the colonization of *Gigaspora rosea* or *G. intraradices* (34 and 35% respectively). The same values was observed in the Figure 2A to the dual inoculation with *P. chrysosporium* and *G. rosea* (34%); however, the dual inoculation with *P. chrysosporium* and *G. viscosum* produced a significant increase in the percentage of root coloni-

zation (46%; Figure 2D). These results suggest that *P. chrysosporium* inoculation can produce a synergistic effect on the root colonization by *G. viscosum*, which could be used as a biotechnological tool for improving the adaptation of *Vaccinium* plants at acclimatization phase. Similar effect was observed with *C. rigida* in interaction with *G rosea* or *G. intraradices*. The specific mechanisms implicated in the beneficial effect of saprophytic fungi exudates on AM fungal colonization of roots are very complex and still poorly understood (Aranda *et al.,* 2009).

Figure 2. Percentage of mycorrhizal root colonization of *V. corymbosum* plants inoculated or not with the AM fungi (A: *Gigaspora rosea*; B: *G. deserticola*; C: *G. claroideum*; D: *G. viscosum*; E: *G. constrictum*; F: *G. intraradices*) and inoculated or not with the saprobe fungi (*C. rigida, P. chrysosporium, T. versicolor, T. harzianum* and *P. chrysogenum*). The data are the means ± standard error (n=3).
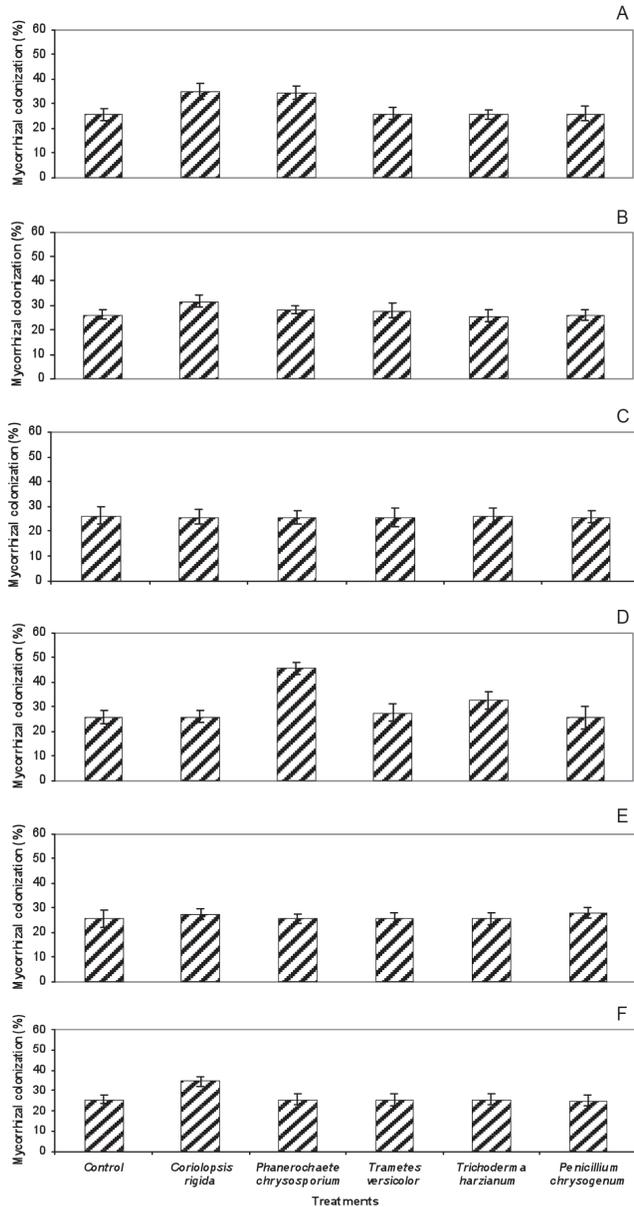
3.2 Plant growth parameters

Only few inoculation treatments produced a significant increase in the shoot and root biomass production compared to the control treatment (Table 1 and 2). Among the treatments including AM fungal inoculation, such corresponding to *G. claroideum* and *G. intraradices* produced the higher shoot and root biomass. Therefore, although it was not possible to demonstrate clearly the AM colonization, these results suggest that these AM fungi had a beneficial influence on plantlet growth.

Table 1. Shoot dry weight (g per plant) of *V. corymbosum* inoculated or not with the AM fungi (*Gigaspora rosea, G. claroideum, G. deserticola, G. viscosum, G. intraradices* and *G. constrictum*) or with saprobe fungi (*C. rigida, P. chrysosporium, T. versicolor, T. harzianum* and *P. chrysogenum*).

	Saprobe fungi					
Mycorrhiza	**Control**	*C. rigida*	*P. chrysosporium*	*T. versicolor*	*T. harzianum*	*P. chrysogenum*
Control	0.23	0.29	0.39	0.86**	0.65*	0.60*
G. rosea	0.48	0.73**	0.73**	0.43	0.46	0.45
G. deserticola	0.35	0.61	0.41	0.38	0.49	0.41
G. viscosum	0.41	0.43	1.33***	0.42	0.74**	0.37
G. constrictum	0.35	0.67*	0.43	0.49	0.38	0.61*
G. claroideum	0.69*	0.22	0.39	0.41	0.51	0.33
G. intraradices	0.47	0.71**	0.37	0.53	0.97**	0.41

Significance conventions: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2. Root dry weight (g per plant) of *V. corymbosum* inoculated or not with the AM fungi (*Gigaspora rosea; G. claroideum; G. deserticola; G. viscosum; G. intraradices* and *G. constrictum*) or with saprobe fungi (*C. rigida, P. chrysosporium, T. versicolor, T. harzianum* and *P. chrysogenum*).

	Saprobe fungi					
Mycorrhiza	**Control**	*C. rigida*	*P. chrysosporium*	*T. versicolor*	*T. harzianum*	*P. chrysogenum*
Control	0.35	0.43	0.61	0.56	0.87*	0.53
G. rosea	0.23	0.35	0.39	0.29	0.37	0.34
G. deserticola	0.45	0.63	0.26	0.29	0.38	0.27
G. viscosum	0.45	0.52	0.99**	0.45	0.64	0.36
G. constrictum	0.49	0.25	0.17	0.30	0.33	0.33
G. claroideum	0.47	0.21	0.28	0.24	0.37	0.63
G. intraradices	1.28***	1.09**	0.89	0.76*	0.67	0.95**

Significance conventions: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

On the other hand, the inoculation with the saprobe fungus *T. harzianum* resulted in a significant increase of shoot and root biomass. The inoculation with saprobe fungi *P. chrysogenum* and *T. versicolor* produced the highest increase in shoot production, but this trend was not observed at root level. The improvement in the growth of plants inoculated with saprobe fungi has been reported for several plant species, and has been attributed to the production of plant growth promoting substances and antimicrobial compounds (Arriagada *et al.,* 2009b; Arriagada *et al.,* 2010). In addition to, several strains of the genera *Penicillium* fungi have been described as efficient P-dissolving fungi (Silva Filho *et al.,* 2002).

The shoot and root biomass of plantlets co-inoculated with both mycorrhizal and saprobe fungi showed that certain particular combinations also can stimulate plant growth significantly (Table 1 and 2). This suggests a synergetic interaction between both types of fungi, resulting in a beneficial effect on plant growth (Arriagada *et al.,* 2010). Thus inoculation with *G. viscosum + P chrysosporium*, and *G. intraradices + C. rigida* resulted in a significant increase in the shoot and root biomass. Furthermore, the interaction between *G. viscosum + T. harzianum*; *G. rosea + C. rigida; G. rosea + P chrysosporium; G. intraradices + T. harzianum; G. constrictum + P chrysogenum* and *G. constrictum + C. rigida* resulted in a significant increase only in the shoot.

The root dry weight of *V. corymbosum* was improved by *G. intraradices* inoculated alone or together with *C. rigida*, *T. versicolor* or *P. chrysogenum* (Table 2). In this sense, some studies have shown that the transfer of carbohydrate to the AM fungi and colonized roots produce a re-distribution of photosynthates in comparison with non-mycorrhizal ones, being in this case a stronger carbon sink (Bago *et al.,* 2000). Furthermore, the increase in root dry weight may be related with an increase in phytohormone production by the AM fungal colonization, which stimulates the development of the root system (Román García, 2003).

3.3 Soil enzyme activity

Certain soil enzyme activities were determined to check the effect of inoculation with AM and saprobe fungi on the biochemical and biological properties in the *V. corymbosum* rhizosphere. In general, dehydrogenase enzyme activity is considered as an index of the total microbial activity of the soil (García-Izquierdo *et al.,* 2003; Arriagada *et al.,* 2009a), then a greater activity would imply a higher biological oxidation of the organic matter and the concomitant higher solubilization and availability of mineral nutrients for the plants. Dehydrogenase activity in the rhizosphere of *V. corymbosum* was increased by the inoculation with all AM and saprobe fungi, but the interaction between both type of fungi not showed a remarkable difference (Table 3). In this case, among the many factors which may affect the biological activity of the soil (e.g. pH, availability of nutrients, soil humidity etc.), some studies showed that a soil pH < 5 has a negative effect on the dehydrogenase activity levels (Ramos and Zúñiga, 2008). In fact, the soil pH here was 5.84. This could explain the response of some combined treatments which presented a similar dehydrogenase activity to the uninoculated control.

Table 3. Dehydrogenase (μmoles TPF g-1 h-1) activity in the rhizosphere soil of V. corymbosum plants inoculated or not with the AM fungi (*Gigaspora rosea*; *G. claroideum*; *G. deserticola*; *G. viscosum*; *G. intraradices* and *G. constrictum*) or with saprobe fungi (*C. rigida*, *P. chrysosporium*, *T. versicolor*, *T. harzianum* and *P. chrysogenum*). The data are the means ± standard error (SE: n=3).

Mycorrhiza	Control	SE	Saprobe fungi									
			C. rigida	SE	*P. chrysosporium*	SE	*T. versicolor*	SE	*T. harzianum*	SE	*P. chrysogenum*	SE
Control	2.67 ±	0.12	3.68 ±	0.18	3.2 ±	0.32	3.4 ±	0.36	3.4 ±	0.31	3.3 ±	0.26
G. rosea	3.54 ±	0.31	5.26 ±	0.54	4.6 ±	0.38	4.6 ±	0.26	5.3 ±	0.61	3.7 ±	0.76
G. deserticola	3.37 ±	0.61	3.31 ±	0.58	3.2 ±	0.31	3.1 ±	0.52	3.1 ±	0.31	3.6 ±	0.32
G. viscosum	4.78 ±	0.64	3.56 ±	0.66	3.5 ±	0.38	3.8 ±	0.36	3.7 ±	0.31	3.4 ±	0.58
G. constrictum	4.55 ±	0.31	3.63 ±	0.58	3.6 ±	0.38	4.1 ±	0.36	8.6 ±	0.89	5.1 ±	0.68
G. claroideum	4.21 ±	0.31	4.13 ±	0.61	6.2 ±	0.78	9.1 ±	0.88	4.6 ±	0.58	3.2 ±	0.58
G. intraradices	4.65 ±	0.31	4.16 ±	0.26	3.9 ±	0.31	3.6 ±	0.36	4.1 ±	0.31	4.4 ±	0.26

Table 4. ß–glucosidase (μmoles p-nitrophenol g-1 h⁻¹) activity in the rhizosphere soil of *V. corymbosum* plants inoculated or not with the AM fungi (*Gigaspora rosea*; *G. claroideum*; *G. deserticola*; *G. viscosum*; *G. intraradices* and *G. constrictum*) or with saprobe fungi (*C. rigida*, *P. chrysosporium*, *T. versicolor*, *T. harzianum* and *P. chrysogenum*). The data are the means ± standard error (SE: n=3).

Mycorrhiza	Control	SE	Saprobe fungi									
			C. rigida	SE	*P. chrysosporium*	SE	*T. versicolor*	SE	*T. harzianum*	SE	*P. chrysogenum*	SE
Control	77.2 ±	1.6	108.4 ±	6.7	108.6	2.7	88.1 ±	0.5	105.4 ±	8.5	108.1 ±	8.4
G. rosea	82.2 ±	2.1	66.7 ±	8.9	83.2	2.8	84.8 ±	3.1	68.1 ±	2.5	69.8 ±	4.6
G. deserticola	84.1 ±	1.3	83.8 ±	4.1	74.3	4.9	78.1 ±	5.3	95.9 ±	1.5	63.2 ±	2.6
G. viscosum	105.7 ±	8.6	82.9 ±	5.7	105.4	1.9	86.5 ±	4.2	94.9 ±	0.7	64.3 ±	3.9
G. constrictum	87.6 ±	4.9	83.3 ±	2.8	85.9	7.3	83.5 ±	0.8	64.1 ±	5.7	38.4 ±	2.1
G. claroideum	112.4 ±	4.7	105.9 ±	7.2	105.7	11.6	78.4 ±	4.3	101.6 ±	8.7	65.1 ±	5.9
G. intraradices	104.9 ±	7.1	100.5 ±	9.8	103.2	4.9	103.8 ±	7.8	104.3 ±	1.5	84.1 ±	4.5

The ß-glucosidase activity in the rhizosphere of *V. corymbosum* was slightly increased by the inoculation with all AM fungi, but this effect was more evident in treatments with *G. claroideum*; *G. viscosum* and *G. intraradices* (Table 4). In addition to, the inoculation with any saprobe fungi resulted in

a similar increase of this activity in the rhizosphere. Therefore, although there were not significant differences evidence an increase of ß-glucosidase activity, it may be suggested that the AM and saprobe fungi generate a higher demand and competition for nutrients (Arriagada *et al.,* 2009a; Richardson *et al.,* 2009).

4. Conclusion

Directed inoculation with specific AM fungi and saprobe fungi strains to the rhizosphere of *V. corymbosum* cv. 'Elliot' favored significantly the growth and development of the plantlets, thus demonstrating the beneficial effect produced by the incorporation of these plant growth promoting microorganisms during an early growth stage in the greenhouse, and allowing to reduce the acclimatization time. In this order, further studies will be focused to understand the nature of the mechanisms involved in the interactions between AM fungi, saprobe fungi and *Ericaceae* plants, to design strategies to improve responses in the co-inoculation with both type of fungi.

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References

Aranda, E., Sanpedro, I., Díaz, R., García-Sánchez, M., Arriagada, C.A., Ocampo, J.A., García-Romera, I. 2009. The effects of the arbuscular mycorrhizal fungus *Glomus deserticola* on growth of tomato plants grown in the presence of olive mill residues modified by treatment with saprophytic fungi. Symbiosis. 47, 133-140.

Arriagada, C., Sampedro, I., Garcia-Romera, I*.,* Ocampo, J.A. 2009a. Improvement of growth of *Eucalyptus globulus* and soil biological parameters by amendment with sewage sludge and inoculation with arbuscular mycorrhizal and saprobe fungi. Sci. Total Environ. 407, 4799-4806.

Arriagada, C., Aranda, E., Sampedro, I., Garcia-Romera, I*.,* Ocampo, J.A. 2009b. Contribution of the saprobic fungi *Trametes versicolor* and *Trichoderma harzianum* and the arbuscular mycorrhizal fungi *Glomus deserticola* and *G. claroideum to* arsenic tolerance of *Eucalyptus globulus.* Biores. Technol. 100, 6250-6257.

Arriagada, C., Pereira, G., Garcia-Romera, I*.,* Ocampo, J.A. 2010. Improved zinc tolerance in *Eucalyptus globulus* inoculated with *Glomus deserticola* and *Trametes versicolor* or *Coriolopsis rigida.* Soil Biol. Biochem. 42, 118-124.

Bago, B., Pfeffer, P.E., Shachar-Hill, Y. 2000. Carbon Metabolism and Transport in Arbuscular Mycorrhizas. Plant Physiol. 124*,* 949-957.

Binet, M.N., Lemoine, M.C., Martin, C., Chambon, C., Gianinazzi, S. 2007. Micropropagation of olive (*Olea europaea* L.) and application of mycorrhiza to improve plantlet establishment. In vitro Cell Dev Biol Plant. 43, 473-478.

Brundrett, M. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154, 275-304.

Dix, N.J., Webster, J. 1995. Fungal Ecology. Chapman and Hall, England, 549 p.

Eivazi, F., Tabatabai, M.A. 1990. Factors affecting glucosidase and galactosidase activities in soils. Soil Biol. Biochem. 22891-897.

Fracchia, S., García-Romera, I., Godeas, A., Ocampo, J.A. 2000. Effect of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials. Plant Soil. 223, 175-184.

García-Izquierdo, C., Gil-Sotres, F., Hernández-Fernández, T., Trasar-Cepeda, C. 2003. Técnicas de Análisis Bioquímicos en suelos: Medida de Actividades Enzimáticas y Biomasa Microbiana, Mundi-Prensa Madrid, 371 p.

Giovannetti, M., Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489-500.

Jumpponen, A., Trappe, J. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol. 140, 295-310.

Jumpponen, A. 2001. Dark septate endophytes – are they mycorrhizal?. Mycorrhiza. 11, 207-11.

Karagiannidis, N., Bletsos, F., Stavropoulos, N. 2002. Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization growth and nutrient uptake in tomato and eggplant seedlings. Sci hortic. 94, 145–156.

Koske, R.E., Gemma, J.N., Englander, L. 1990. Vesicular–arbuscular mycorrhizae in Hawaiian Ericales. Am. J. Bot. 77, 64-68.

Phillips, J., Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit. Mycol. Soc. 55, 158-161.

Ramos, E., Zúñiga, D. 2008. Efecto de la humedad temperatura y ph del suelo en la actividad microbiana a nivel de laboratorio. Ecol Apl. 7, 123-130.

Richardson, A.E., Barea, J., Mcneill, A.M., Prigent-Combare, C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 321, 305-339.

Román-García, F. 2003. Concentración de reguladores del desarrollo vegetal inducida por hongos endomicorrízicos en dos cultivares de Chile (*Capsicum annuum* L.). Dissertation, Universidad de Colima.

Salamanca, C.P., Herrera, M.A., Barea, J. 1992. Mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified Mediterranean ecosystems. Agronomie. 12, 869-872.

Scagel, C.F., Wagner, A., Winiarski, P. 2005. Inoculation with Ericoid Mycorrhizal Fungi Alters Root Colonization and Growth in Nursery Production of Blueberry Plants from Tissue Culture and Cuttings. Small fruits review. 4, 113-135.

Scervino, J.M., Gottlieb, A., Silvani, V.A., Pérgola, M., Fernández, L., Godeas, A. 2009. Exudates of dark septate endophyte (DSE) modulate the development of the arbuscular mycorrhizal fungus (AMF) *Gigaspora rosea*. Soil Biol. Biochem. 41, 1753-1756.

Shetty, K.G., Hetrick, B.A., Figge, D.A., Schwab, A.P. 1994. Effects of mycorrhizae and other soil microbes on revegetation of heavy-metal contaminated mine spoil. Environ. Poll. 86, 181-188.

Silva Filho, G.N., Narloch, C., Scharf, R. 2002. Solubilização de fosfatos naturais por microrganismos isolados de cultivos de Pinus e Eucalyptus de Santa Catarina. Pesq. Agropec. Bras. 37, 847-854.

Singh, A. 2007. Molecular basis of plant-symbiotic fungi interaction: an overview. Scientific World. 5, 115-131.

Smith, S.E., Read, D.J. 2008. Mycorrhizal symbiosis. Academic Press. Third Edition. 800 p.

Trevors, J. 1984. Dehydrogenase activity in soil. A comparison between the INT and TTC assay. Soil Biol. Biochem. 16, 673-674.

van der Heijden, M.G.A. 2004. Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. Ecol Lett. 7, 293-303.

Vega, A.R., Garciga M., Rodríguez A., Prat L., Mella J. 2009. Blueberries mycorrhizal symbiosis outside of the boundaries of natural dispersion for ericaceous plants in Chile. Acta Hort. (ISHS) 810, 665-672.