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Rooting and growth response of grapevine nurslings to inoculation with arbuscular mycorrhizal fungi and irrigation intervals

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Abstract

This study was conducted during two successive seasons (2005 and 2006); in the experimental farm of Faculty of Agriculture, Kafr El Sheikh University; with the aim to investigate the influence of arbuscular mycorrhizal fungi (AMF) inoculation and irrigation intervals on growth of grapevine nurslings cv. Ruby King. Two mix mycorrhizal fungi including *Glomus fasiculatum* and *Glomus mosseae* were used for inoculation. The AMF inoculated and non-AMF nurslings were irrigated at 3, 6 and 9 days interval. The results showed that a combined treatment of AMF inoculation and irrigation at 3 days intervals recorded the highest values in terms of length of main root, total root length, root volume, root dry weight (%), top/root ratio, number of fine roots (< 2 mm), number of small roots (2-5 mm), number of leaves and leaf area per nursling. These results are of practical importance, as they highlight the potential of using mycorrhizal fungi inoculation for root development and growth improvement in grapevine nurslings and hence increases its adaptability upon transfer from the nursery to the open field.

Key words: Arbuscular mycorrhizal fungi, grape, irrigation, nurslings

Introduction

Most of the major plant families are able to form mycorrhiza naturally, the arbuscular mycorrhizal (AM) associations being the commonest mycorrhizal type involved in agricultural systems (Berta *et al*., 1993). Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between AM fungi and the roots of terrestrial plants. The fungus biotrophically colonizes the root cortex and develops an extra-matrical mycelium that helps the plant to acquire mineral nutrients from soil (Harley and Smith, 1983). It has been recognized that mycorrhizal symbioses play a key role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress (Barea and Jeffries, 1995).

The primary effect of AM symbiosis is the increase in the supply of mineral nutrients to the plant, particularly those whose ionic forms have a poor mobility rate, or those which are present in low concentration in the soil solution. This mainly concerns phosphate, ammonium, zinc and copper (Barea and Azcon-Aguilar, 1982). Mycorrhizal fungi also enable plants to cope with abiotic stress by means of alleviating nutrient deficiencies, improving drought tolerance, overcoming the detrimental effects of salinity and enhancing tolerance to pollution (Berta *et al*., 1993).

Since AM symbiosis can benefit plant growth and health, there is an increasing interest in ascertaining their effectiveness in particular plant production situations and, consequently, in manipulating them so that they can be incorporated into production practices when feasible. Evidence is accumulating to show that indigenous and/or introduced AM fungi (AMF) are involved in the development of different plant production systems including both field sown and plantation crops and transplantable horticultural crops. Fruit crops are one of the target systems in which AM biotechnology can express its potential. This is due to the characteristics of the components and the plant production practices, which make it necessary and easy to inoculate fruit crop plants with AMF. Considering the high returns from fruit crops, it seems that the application of AM inoculation may represent only a small contribution to the input costs. In the present study, we investigated the influences of mycorrhizal inoculation and irrigation intervals on rooting and growth of grapevine nurslings.

Materials and methods

Plant material: Grapevine nurslings cv. Ruby King were transplanted individually in clay pots (40 cm in diameter) containing clay loamy soil. The nurslings were first pruned to two eyes and were allowed to grow without fertilizers. Then, two shoots were left on the nurslings and the other shoots were removed. These nurslings were used as initial plant material. The experiments werer conducted during two successive years (2005-2006), from February through September of each year. All experiments were carried out at the experimental farm of the Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.

Mycorrhizal fungi and irrigation treatments: Two mix mycorrhizal fungi including *G. fasiculatum* and *G. mosseae* were used to inoculate the nurslings with the same inoculation density according to the method described by Gerdemann and Nicolson (1963) and all mycorrhizal and non-mycorrhizal nurslings were irrigated at the day of AMF inoculation. To investigate the effects of irrigation intervals, irrigation was done at 3, 6 or 9 days interval that gives six treatments as follows: (1) Mycorrhiza inoculation + irrigation at 3 days (AMF + 3 d), (2) No AMF + 3 d, (3) AMF + 6 d, (4) No AMF + 6 d, (5) AMF + 9 d, (6) No AMF + 9 d.

Measurements of growth parameters: Total root volume (cm^3) was measured by water displacement according to Kolesnikov (1971). Total root length was determined according to methods of Newman (1966), Marsh (1971) and Tennant (1975). Dry weight was measured after drying the stems, roots and tops for 48 h at 70^{o}C and top/root ratio and dry weight percentage was calculated. Total leaf area was measured using an area meter (LI-3100, Li-Cor, Lincoln, NE) and average leaf area was calculated using 10 individual leaves per nursling.

Experimental design and statistical analysis: The experiments were set up in a randomized complete block design and each treatment was represented by three replicates, each replicate contained 4 pots. Data were subjected to Duncan's Multiple Range Test for mean comparison (Snedecor and Cochran, 1980).

Results and discussion

Effect of AMF inoculation and irrigation regime on root growth, root thickness and root branching: The results presented in Table 1 indicate that AMF inoculation treatments had the greatest root growth in terms of length of main root, total root length, root volume, root dry weight (%) and top/root ratio compared to non-AMF. The best results were obtained when AMF inoculation was combined with irrigation at 3 days interval. Root volume tended to decrease with increasing irrigation intervals in the non-inoculated plants. However, the highest root volume in the AMF inoculated plants was obtained in the 6 days irrigation intervals. Although, there was a gradual increase in top/root ratio with increasing irrigation interval, it was clear that AMF inoculation and 9th day irrigation had a better root system compared to non-AMF and 9th day irrigation (Table 1). It could also be noticed that top/root increased as the irrigation intervals increased.The heighest value in this concern was obtained in the AMF inoculated plants at 9 days irrigation interval.

AMF inoculation and irrigation intervals also influenced root thickness and root branching (Table 2). AMF nurslings had the highest number of fine roots (<2 mm) and number of small roots (2-5 mm) compared to non-AMF nurslings and best results were recorded at 3 days irrigation treatment. However, number of medium roots (5-10 mm) was highest when AMF inoculation treatment was combined with irrigation at 6 days in both seasons and recorded 2.5 and 3.0, respectively.

Arbuscular mycorrhiza (AM) fungi can induce morphological modifications in the host plant root system (Berta *et al.,* 1993; Atkinson *et al.,* 1994). A more branched root system has been observed in mycorrhzal plants of different herbaceous and woody species (Amijee *et al.*, 1989; Berta *et al.*, 1990; Schellenbaum *et al.*, 1991; Tisserant *et al.,* 1992). Different AM fungal species can induce different effects on root system morphology (Berta *et al.* 1995), which could be related to the extent of root colonization (Hooker *et al.,* 1992). Although root system morphology is genetically determined (Harper *et al.,* 1991), many environmental factors can influence root development, including mineral nutrition (Drew and Saker, 1978). In addition, differences between mycorrhizal and non-mycorrhizal plants may decrease at high external phosphorus levels (Hetrick *et al.,* 1998). Vidal *et al.* (1992) reported that the inoculation of plants of Avocado with vescular–arbuscular mycorrhiza fungus improved root growth and increased the top/root ratio. Inoculation of citrus seedling with vescular–arbuscular mycorrhiza fungus increased growth and dry weight (Dixon, 1989; Nawar *et al.*, 1998).

Aguin *et al.* (2004) indicated that inoculation with vescular–arbuscular mycorrhiza fungus (VMF) *G. aggregatum* in rooting beds of grapevine cuttings changed root morphology by increasing branching of first-order lateral roots. When rooted cuttings were transplanted to pots, a significant growth enhancement was found in two of inoculated rootstocks. *G. aggregatum* alone or in synergy with the indigenous AM fungi seemed to have a higher affinity for 161-49 Couderc, the roots of which were more extensively colonized and exhibited a greater positive growth response. Marschner *et al.* (1997) reported that mycorrhizal infection had a little effect on the physiological status compared to the non-mycorrhizal plants. Dell' Amico *et al.* (2002) found that mycorrhizal infection improved physiological activity in non–stressed and stressed plant. This improvement was accompanied by higher root hydraulic conductivity values, indicating enhanced

Table 1. Effect of mycorrhiza inoculation and irrigation intervals on length of main root, total root length, root volume (cm^3), root dry weight (%) and top /root ratio of grape nurslings during 2005 and 2006 seasons

Treatment	Length of main root		Total root length		Root volume ($cm\ L^{-3}$)		Root dry weight (%)		Top / Root ratio	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
AMF+ 3 d	72.33a	80.50a	1400.00a	1341.67a	315.00c	520.00a	96.07b	52.38a	0.67c	0.60d
No AMF+ 3 d	58.33c	62.50b	898.33b	1048.33b	430.00a	530.00a	50.85a	48.21c	0.74c	0.75cd
AMF+ 6 d	58.50bc	59.83b	765.00b	858.33c	525.00a	448.00b	47.77ab	52.41a	0.80c	0.83bc
No AMF+ 6 d	55.83c	58.50bc	181.67c	781.67c	337.33b	410.67b	50.67a	46.71d	0.85c	0.67d
AMF+ 9 d	60.67b	34.50d	276.67c	461.67d	271.67d	310.00c	44.24c	50.18b	1.00b	0.94b
No AMF+ 9 d	44.67b	50.17c	131.67c	34.67e	261.67c	230.00d	43.46c	47.64cd	1.34a	1.28a

Table 2. Effect of mycorrhiza inoculation and irrigation intervals on root thickness and number of roots in grape nurslings during 2005 and 2006 seasons

Treatment	Root thickness						Number of roots			
	Number of medium roots (5-10 mm)		Number of small roots (2-5mm)		Number of fine roots (< 2mm)		2nd order		3rd order	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
AMF+ 3 d	1.17b	2.50ab	14.50a	18.50a	17.33a	18.83a	34.00a	33.67a	34.33a	48.67a
No AMF+ 3 d	1.50b	1.17c	9.50c	11.50b	14.33ab	18.00a	34.33a	28.67b	18.33c	38.00b
AMF+ 6 d	2.50b	3.00a	12.50b	10.50bc	9.50cd	9.00b	32.00a	24.00c	22.17b	23.67c
No AMF+ 6 d	1.17b	1.67bc	8.17d	9.50c	8.50cd	6.50c	19.33c	19.67d	20.00bc	19.50d
AMF+ 9 d	1.67ab	1.17c	5.17e	10.00bc	11.17bc	9.00b	24.67b	19.50d	19.67bc	18.00d
No AMF+ 9 d	1.67ab	2.50ab	5.50e	5.50d	6.00d	8.00bc	12.50d	16.00e	13.33d	14.00e

Table 3. Effect of mycorrhiza inoculation and irrigation intervals on leaf number/nursling, leaf area (cm^2), total leaf area /nursling, stem dry weight (%) of grape vine nurslings during 2005 and 2006 seasons

Treatments/nursling	Leaf number /nursling		Leaf area (cm^2)		Total leaf area		Stem dry weight (%)	
	2005	2006	2005	2006	2005	2006	2005	2006
AMF+3 d	95.33a	115.00a	83.20a	95.44a	8706.53a	9748.14a	54.97cd	54.12b
No AMF+3 d	87.33b	69.67c	72.34b	83.71c	6546.11b	5758.57b	60.23b	57.46a
AMF+6 d	76.33d	81.33b	80.37a	90.35b	5776.74b	5612.68b	56.46c	53.80b
No AMF+6 d	72.67d	53.33d	68.31c	74.83d	5301.14bc	4268.97c	62.22ab	50.48c
AMF+9 d	81.33c	42.00e	55.76d	72.61d	4326.77c	3040.60d	64.47a	54.10b
No AMF+9 d	48.67e	33.00f	50.50e	63.67e	1868.43d	1738.41e	53.48d	53.30b

water uptake in drought conditions. The beneficial effect of the mycorrhizal symbiosis on water status of tomato plants stimulated plant growth. On the other hand, Caravaca *et al.* (2005) also reported that the mycorrhizal inoculation and the irrigation of plants had a strong effect on the growth parameters.

Effect of AMF inoculation and irrigation intervals on shoot growth: Table 3 shows that both AMF inoculation and irrigation treatments significantly influenced shoot growth of grapevine nurslings. The greatest number of leaves, leaf area and total leaf area per nursling were obtained in both seasons when nurslings were inoculated with AMF and irrigated at 3 days interval. The lowest values were recorded with AMF+9 days treatment. The percentage of stem dry weight recorded the highest value (64.47) in both seasons when nurslings were inoculated with AMF and irrigated at 9 days interval. Al-Karaki (1998) reported that shoot and root dry matters were higher for water stressed AM wheat than those for corresponding non-AM plants. Effect of AM fungi on shoot behaviors was not often closely linked to the extent of AM colonization of roots (Fitter and Merryweather, 1992; Smith and Read, 1997).

The results obtained strongly indicate that inoculation of grapevine nurslings with mycorrhizal fungi increased root growth of young nurslings. The improved growth could be due to direct effects of mycorrhizal fungi on plant water and nutrient uptake and also indirect effects via mycorrhizal-induced changes in the bacterial community composition. These results are of practical importance, as they highlight the potential of using mycorrhizal fungi inoculation for root development and growth improvement in grapevine nurslings and hence increase its adaptability upon transfer from the nursery to the open field.

References

Aguin, O., J.P. Mansilla, A.Vilarino and M.J. Sainz, 2004. Effects of mycorrhizal inoculation on root morphology and nursery production of three grapevine rootstock. *American Journal of Enology and Viticulture,* 55(1): 108-111.

Al-Karaki, G.N. 1998. Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress. *Mycorrhiza,* 8: 41-45.

Amijee, F., P.B.Tinker and D.P. Stribey, 1989. Effects of phosphorous on morphology of VA mycorrhizal root system of leek (*Allium porrum*). *Plant and Soil,* 119: 334 - 336.

Atkinson, D., G. Berta and J.E. Hookeer, 1994. Impact of mycorrhizal colonization on root architecture, root longevity and the formation of growth regulators. In: *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems* (Gianinazzi, S.and Schuepp, H., Eds). Birkhauser Verlag, Basel, pp89-99.

Berea, J.M. and C. Azcon-Aguilar, 1982. Production of plant growth regulating substances by vesicular arabuscular mycorrhiza *Glomus mosseae*. *Appl. Environ. Microbiol*., 43:810-813.

Barea, J.M. and P.J. Jeffries, 1995. Arbuscular mycorrhizas in sustainable soil-plant systems. In: *Mycorrhiza*. A. Varma and B. Hock, eds. Springer-Verlag, Berlin. pp 521-560.

Berta, G., A. Fusconi, A.Trotta and S. Scnnerini, 1990. Morphogenic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum*. *New Phytologist*, 114: 207-215.

Berta, G., A. Fusconi and A.Trotta, 1993. VA mycorrhizal infection and the morphology and function of root systems. *Environmental and Experimental Botany*, 33(1): 159-173.

Berta, G., A.Trotta, A. Fusconi, J.E. Hooker, M. Munro, D. Atkinson, M.G. Iovannetti, S. Morini, P. Fortuna, B. Tisserant and V. Giovaninazzi-Pearson, 1995. Arbuscular mycorrhizas induces changes to plant growth and root system morphology in *Prunus cerasifera* L. *Tree physiology*, 15: 281-293.

Caravaca, F., M.M. Algucil, G. Diaz, P. Marin and A. Roldan, 2005. Nutrient acquisition and nitrate reductase activity of mycorrhizal *Retama sphaerocapa* L.seedling afforested in an amended semiarid soil under two water regimes. *Soil Use Mangement,* 21(1): 10-16.

Dell'Amico, J., A. Torrecillas, P. Rodriguez, A. Morte and M.J. Sanchez-Blanco, 2002. Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Gloumus clarum* during drought and recovery. *J. Agric. Sci*., 138: 387-393.

Dixon, R.K. 1989. Cytokinin activity in citrus seedling colonized by mycorrhizal fungi. *Ann. Sci*., 46 Suppl., 734-736.

Drew, M.C. and L.R. Saker, 1978. Nutrient supply and the growth of seminal root system in barley. III. Compensatory increase in growth of lateral root system and in rates of phosphate uptake, in response to a localized supply of phosphate. *Jouranl Expermintal Botany*, 299: 435-451.

Fitter, A.H. and J.W. Merryweather, 1992. Why are some plants more mycorrhizal than others ? An ecological enquiry. In: *Mycorrhizas in ecosytems*, D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander eds.. CAB International, Wallingford, UK. pp 26-36.

Gerdeman, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal *Endogone* sp. extracted from soil by wet-sieving and decanting. *Trans. Brit. Mycol*., 46: 235-244.

Harley, J.L and S.E. Smith, 1983. *Mycorrhizal symbiosis*. London: Academic Press, p 483.

Harper, J.L., M. Jones and N.R. Hamilton, 1991. The evaluation of roots and the problems of analyzing their behaviour. In: *Plant root growth. An ecological perspective* (Atkinson D.Ed.). Blackwell Scientific Publications, Oxford, 2-24.

Hetrick, B.A.D., J.F. Lesli, G. Thompsom Wilson and D. Gerschefske Kitt, 1998. Physical and topological assessment of effects of a vesicular–arbuscular mycorrhizal fungus on root architecture of big bluestem. *New Phytologist*, 110: 85-96.

Hooker, J.E., M. Muno and D. Atkinson, 1992. Vasicular-arbuscular mycorrhizal fungi induced alteration in popular root system morphology. *Plant Soil*, 148: 207-209.

Kolesnikov, V.A. 1971. *The root system of fruit plants*. Moscow, USSR, Mir Publihers, p. 269.

Marschner, P., D.E. Crowley and R.M. Higashi, 1997. Root exudation and physiological status of a root colonizing fluorescent pseudomonad in mycorrhizal and non- mycorrhizal pepper (*Capsicum annuum* L.). *Plant Soil*, 189(1): 11-20.

Marsh, B.B. 1971. Measurement of length in random arrangments of lines. *J. Appl. Ecol*., 8: 256-267.

Nawar, A.M., H.A. El-Shamy and K. Fawaz, 1998. Growth, leaf chlorophyll and carbohydrate metabolism of mycorrhizal sour orange seedlings. *J. Agric. Res. Tanta Univ.,* 14(2): 1064-1073.

Newman, E.I. 1966. A method of estimating the total length of root length in a sample. *J. Appl Ecol.,* 3: 139-145.

Schellenbaum, L., G. Berta, F. Ravolaniriaa, B. Tisserant, S. Giannazzi and A.H. Fitter, 1991. Influnce of endomycorrhizal woody plant species (*Vitis venifera* L.). *Annals Botany*, 68: 135-141.

Smith, S.E. and D.J. Read, 1997. *Mycorrhizal symbiosis.* 2nd Edn. Academic Press, London, UK. p. 605.

Snedecor, G.W. and W.G. Cochran, 1980. *Statistical methods*. 7th Edn. Iowa State, Univ. Press. Ames. Iowa, U.S.A.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. *J. Ecol*., 63: 995-1001.

Tisserant, B., l. Schellenbaum, V. Gianinazzi-Pearson, S. Gianinazzi and G. Berta, 1992. Influnce of infection by endomycorrhizal fungus on root development and architecture in *Plantanus acerifolia*. *Allionia*, 30: 171-182.

Vidal, M.T., C. Azcon-Aguilar, C. Berea and F.P. Liegs-Alfaro, 1992. Mycorrhizal inoculation enhances growth and development of micropropagated plants of avocado. *HortScience*, 27(7): 785-787.