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Effects of Mycorrhizal Colonization on Growth Parameters of Onion under Different Irrigation and Soil Conditions

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Abstract: The effects of three Arbuscular Mycorrhizal Fungi (AMF), *Glomus versiforme*, *G. intraradices* and *G. etonicatum* and three irrigation intervals (7, 9 and 11 days) on growth of onion (*Allium cepa* L.) cv. Red Azar Shahr were studied under two soil conditions (sterilized and non-sterilized). The results indicated that, AMF colonization improved plant height, Leaf Area Index (LAI), total biomass, bulb dry mass and diameter, Harvest Index (HI) and chlorophyll content ($p < 0.001$). Bulbing occurred 10-15 days earlier in mycorrhizal plants. Irrigation interval decreased biomass, LAI, Leaf Area Ratio (LAR), bulb diameter and dry mass and chlorophyll content (b and total) at 11 day irrigation interval. In term of interaction, *G. versiforme* at 9 day and non-mycorrhizal plants at 11 day produced the greatest and the lowest LAI (8.56 vs. 1.57), respectively. Mycorrhizal onions in contrary to non-mycorrhizal ones produced more LAI and biomass in sterilized soil and inoculation with *G. etonicatum* and the non-mycorrhizal onions in sterilized soil had the highest and the lowest biomass, respectively.

Key words: Onion, arbuscular mycorrhizal fungi, growth indices, bulbing, dry mass

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) have been shown to promote plant growth and nutrient uptake and are particularly important for phosphorus nutrition. One of their effects is to increase the surface of root system for nutrient absorption (Sanders and Tinker, 1973). Mycorrhizal plants have greater capacity to overcome adverse environmental conditions and thus produce higher yield (Fortunato *et al.*, 2005). It has been shown repeatedly in pot studies, both sterilized and non-sterilized soil, the inoculation with AMF can cause spectacular increase in dry weight and bulb yield of onion (*Allium cepa* L.) (Stribley, 1990; Charron *et al.*, 2001a).

Onion is one of the most important horticulture crops at North West of Iran. It is cultivated as an irrigated (every 7 day) crop and it is regarded as fairly large water consumer. Transpiration, photosynthesis and growth rates of onion plants were lowed by a mild degree of water stress and they are more sensitive to water deficit stress than most other crops (Brewster, 1994). Nelsen and Safir (1982) reported that mycorrhizal onion was more tolerant to water deficit stress than non-mycorrhizal plants.

AMF are endophytes and obligate symbionts and their agricultural use as inoculants has remained limited

due to large quantities of inoculum required (Waterer and Coltman, 1988). Employing preinoculated seedlings could be a very promising solution to this problem, especially for the crops that are grown in nursery for the latter out planting (Sasa *et al.*, 1987). Preinoculated seedlings become more tolerant to transplant shock than non-mycorrhizal plants (Waterer and Coltman, 1988).

The objective of this study was to evaluate the effect of mycorrhizal colonization on growth indices and yield of onion under three irrigation intervals both in sterilized and non-sterilized soils.

MATERIALS AND METHODS

A glasshouse pot experiment was conducted during 2005-2006 in the Agricultural Research Station of Tabriz University, Iran. The experiment was a factorial complete randomized block design with 3 factors and 4 replications. The factors were irrigation intervals (7, 9 and 11 days), soil sterility (sterile and non-sterile soil) and Arbuscular Mycorrhizal Fungi (AMF) species, (*Glomus versiforme*, *G. intraradices*, *G. etonicatum*) and Non-mycorrhizal (NM) plants as control. Onion (*Allium cepa* L. cv. Red Azar Shahr) seeds were disinfected for 15 min in 1% sodium hypochlorite and sown in sandy loam autoclaved

soil. Fifteen grams of inoculum (spore, hyphae, AM root fragment and soil) were mixed to one kg of the medium. The control received the same amount of sterilized inoculum. After seedling emergence, three plants from each treatment were sampled randomly every week for 50 days (emergence to transplanting). Their roots were washed, cut into about 1 cm long pieces and mixed thoroughly. The fragments then were cleared with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactoglycerol (Phillips and Hayman, 1970). Percentage of mycorrhizal colonization was determined by gridline intersect method (Furlan and Fortin, 1973). At transplanting (9 weeks after sowing) root colonization in seedlings had reached above 60%.

Onion seedlings were transplanted at a density of three plants per pot; each pot was 22 cm in diameter and 21 cm in deep and contained 5.6 kg soil. Main characteristics of the soil were: pH 8.5; EC 2.2 dS m⁻¹; 1.6% organic carbon; 15.5% clay; 13.1% silt; 71.4% sand; 18.5 µg g⁻¹ available P (Olsen method); 797.5 µg g⁻¹ K; 6.63% CaCO₃; Db 1.152 g cm⁻³; D_s 2.5 g cm⁻³. Water content of all pots was maintained at field capacity (29.7% (w/w) equivalent to the 10 kPa moisture content) during the sowing period. The irrigation interval treatments were started after the transplanting; water was added to the pots every 7, 9 or 11 days in order to keep the moisture content to the Field Capacity (FC). For better establishment of seedlings, all pots were irrigated every three days for 9 days before employing the aforementioned irrigation intervals. Plants received 58 mg kg⁻¹ N as urea based on the soil standard test and were kept in a greenhouse under a 16 h photoperiod, 24±4/18±3°C day/night temperatures and 40-60% relative humidity.

At bulb formation stage, leaf chlorophyll (a, b and total) content was determined by spectrophotometer (Hach DR/2000) by extraction in 80% acetone (Arnon, 1949). At this time one plant per pot was harvested and its height, bulb neck and bulb diameter were measured and Bulbing Ratio (BR) was calculated. BR is the maximum bulb diameter divided by the minimum pseudo-stem (neck) diameter (Brewster, 1994). The plant then was separated to leaf, pseudo-stem and bulb. Leaf

area was measured with a leaf area meter (LI 3100C area meter, LI-COR, USA). Dry weight of each part was determined after drying at 72°C until constant weight. Leaf Area Index (LAI) and Leaf Area Ratio (LAR) were calculated according to Hunt (1982). After bulb maturity, bulb fresh weight and diameter, bulb dry weight and final biomass (without root) were determined after drying at 72°C and Harvest Index (HI) was calculated.

Data were statistically analyzed by analysis of variance with the MSTATC PROGRAM (Mich. University, East Lansing, Mich., USA). Significant differences at p = 0.05 were tested using Duncan's multiple range tests.

RESULTS

AMF colonization improved Total Biomass (TB), Bulbing Ratio (BR), Plant Height (PH), Leaf Area Index (LAI) and leaf chlorophyll content (chlorophyll a, b and total (a + b)) significantly (Table 1). In the case of TB and LAI, *G. versiforme* was more effective than *G. ineraradices* and *G. etonicatum*. Leaf Area Ratio (LAR) decreased by AMF colonization significantly (p<0.001) (Table 1).

TB, LAR, LAI and chlorophyll (chlorophyll b and total) content were similar at 7 and 9 days irrigation intervals, but they all decreased at 11 days interval due to water deficit. Chlorophyll a content was not affected by water deficit (Table 2).

Interaction between AMF species and irrigation intervals was significant only for LAI (Fig. 1). Greatest LAI was produced by *G. versiforme* at 9 and 7 days irrigation intervals. LAI was the lowest at 11 day interval in the control plants. Mycorrhizal onions produced more LAI at water deficit condition (11 day) than control ones under well-watered condition (7 day).

Soil sterilization increased LAI and TB significantly (p<0.01). However the interaction between AMF and soil sterility on LAI and TB were also significant. Mycorrhizal onions in contrary to non-mycorrhizal ones produced more LAI and TB in sterilized soil; *G. etonicatum* and control plants in sterile soil produced the greatest and the lowest LAI and TB, respectively (Fig. 2).

Table 1: Effect of AMF colonization on onion Total Biomass¹ (TB), Bulbing Ratio (BR), Plant Height (PH), Leaf Area Index (LAI), Leaf Area Ratio (LAR), chlorophyll content (a, b and total (a+b), Bulb Dry Weight (BDW), Bulb Diameter (BD) and Harvest Index (HI)

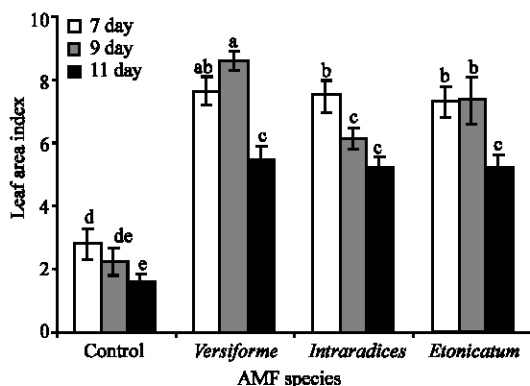
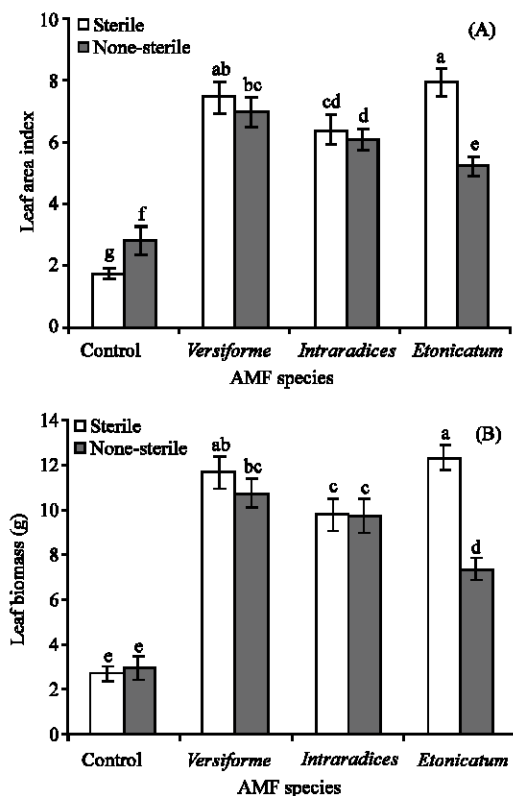
AMF	TB (g)	BR	PH (cm)	LAI	LAR (cm ² g ⁻¹)	Chl* a (mg g ⁻¹)	Chl. b (mg g ⁻¹)	Total Chl (mg g ⁻¹)	BDW (g pot ⁻¹)	BD (mm)	HI
Control	2.82c**	1.55b	59.8b	1.97c	99.70a	0.236b	0.075b	0.311b	6.96c*	29.3b	0.528c
<i>G. versiforme</i>	11.16a	2.09a	77.82a	6.52a	81.68b	0.293a	0.102a	0.395a	20.21a	47.8a	0.593b
<i>G. intraradices</i>	9.70b	2.1a	77.55a	4.46b	82.86b	0.301a	0.120a	0.420a	16.03b	47.0a	0.683a
<i>G. etonicatum</i>	9.82b	2.34a	80.88a	5.03b	87.36b	0.292a	0.110a	0.402a	16.26b	46.2a	0.621b
LSD 5%	1.02	0.46	6.60	0.56	5.50	0.023	0.016	0.034	3.14	0.37	0.060

¹Without root, *Chlorophyll, **Within each column, means followed by the same letter(s) are not significantly different (p<0.05) using Duncan's multiple range test (n = 24)

Table 2: Effect of irrigation intervals on onion Total Biomass¹ (TB), Leaf Area Index (LAI), Leaf Area Ratio (LAR), chlorophyll content (a, b and total (a+b), Bulb Dry Weight (BDW), Bulb Diameter (BD)

Irrigation interval (Days)	TB (g plant ⁻¹)	LAI	LAR (cm ² g ⁻¹)	Chl. a (mg g ⁻¹)	Chl. b (mg g ⁻¹)	Total Chl. (mg g ⁻¹)	BDW (g pot ⁻¹)	BD (mm)
7	8.71a**	5.04a	95.84a	0.289a	0.113a	0.402a	15.18ab*	42.0ab
9	9.25a	5.04a	88.24ab	0.282a	0.102ab	0.384b	16.58a	45.0a
11	7.15b	3.42b	79.62b	0.270a	0.090b	0.360b	12.83b	40.8b
LSD5%	0.88	0.49	4.77	0.018	0.014	0.030	2.72	3.16

¹Without root, *Chlorophyll, **Within each column, means followed by the same letter(s) are not significantly different ($p < 0.05$) using Duncan's multiple range test ($n = 32$)

Fig. 1: Interaction of AMF species and irrigation intervals on onion leaf area index. Dissimilar letters indicate significant difference ($p < 0.05$)Fig. 2: Interaction of AMF species and soil sterility on onion leaf area index (A) and total biomass (B). Dissimilar letters indicate significant difference ($p < 0.05$)

At bulb maturity, dry weight and diameter and Harvest Index (HI) of bulb were substantially affected, by AMF colonization (Table 1). *G. versiforme* increased bulb dry weight about 2.9 fold in comparison with non-mycorrhizal onion (20.2 g pot⁻¹ vs. 6.96 g pot⁻¹). In mycorrhizal onions bulb diameter were significantly ($p < 0.01$) higher than the control ones. HI increased by AMF colonization and *G. intraradices* produced a highest one. At 11 day irrigation interval bulb dry weight and diameter were decreased significantly in comparison with 7 and 9 day intervals (Table 2).

DISCUSSION

Arbuscular mycorrhizal fungi improved onion growth and development in comparison with non-mycorrhizal ones. This improvement resulted from increasing LA, plant height and leaf chlorophyll content, which led to greater leaf area per unit soil surface (LAI) and probably photosynthesis capacity both leading to greater dry mass and larger bulb (Table 1). Increasing plant size and yield (Charron *et al.*, 2001a; Aguilera-Gomez *et al.*, 1999) and enhancing chlorophyll content (Morte *et al.*, 2000) by AMF colonization have been already reported.

G. versiforme was more effective in enhancing TB, LAI and bulb dry mass than *G. intraradices* and *G. etioncatum* (Table 1). It is likely that *G. versiforme* spreaded faster through root system than *G. intraradices* and supplies host plant more nutrients such as P and K (Charron *et al.*, 2001b).

Mycorrhizal onions had greater bulbing ratio than control plants at first harvest, implying that bulb initiation and bulbing process occurred earlier and produced faster in mycorrhizal plants than non-mycorrhizal ones (Table 1). According to Brewster (1994) in onion plants, when BR index exceeds to 2, bulbing was considered to be complete. In control plants, bulbing occurred 10-15 days latter. Our results agree with findings of Charron *et al.* (2001a), who reported that mycorrhizal onion reached to marketable size 2-3 weeks earlier than non-mycorrhizal onion.

LAR was decreased by AMF colonization and control plants had significantly greater LAR. This is contrary to the finding of Mc-Arthur and Knowles (1992)

for potato. It is possible that the higher LAR in control plants was due to delay in bulbing and allocation of photo-assimilates to the leaves.

Harvest index (bulb to aerial biomass ratio) increased by AMF colonization and *G. interaradices* was more effective than *G. versiforme* and *G. etomicatum* (Table 2). This corroborates with results of Al-Karaki *et al.* (2004) on field grown mycorrhizal wheat under well-watered condition.

By increasing irrigation intervals LAI was decreased in control onion (Fig. 1), substantially other growth parameters decreased also. In mycorrhizal plants especially in *G. versiforme* and *G. etonicatom*, LAI and dry biomass at 9 day increased or were constant in comparison with 7 day irrigation interval (Fig. 1 and Table 2). Our data agree with the finding of Subramanian *et al.* (2006), who reported that in mycorrhizal tomato, dry weight and fruit yield did not decreased under moderate water deficit, but these factor were decreased under sever condition.

Chlorophyll (a) content was not affected at 11 day irrigation interval but (b and total) content decreased (Table 2) presumably due to limited water and nutrient minerals. Our data is in agreement with finding of Alberte and Thornber (1977), they reported that water deficit decreased the accumulation of chlorophyll b and elevated chlorophyll a/b ratio. Chlorophyll loss is a negative consequence of water stress and it has also been considered as an adaptive feature in plants grown under such conditions. Chlorophyll loss reduces the amount of light intercepted by leaves and at the same time reduces the possibility of further damage of the photosynthetic machinery by oxidant molecules (Munne-Bosch and Alerge, 2001).

Soil sterilization decreased non-mycorrhizal onion growth contrary to mycorrhizal ones. Stunted growth of control plants grown in sterilized soils without fungal inoculation has been reported (Sasa *et al.*, 1987; Charron *et al.*, 2001b). It is possible the ecological equilibrium of microflora could not be re-established in the experiment time. Smith and Smith (1981) mentioned that, despite the addition of a microbial filtrate from a natural soil to a sterilized soil, the microbial flora of broccoli was not re-established in a 28 or 40 day period.

In conclusion, AMF colonization effectively improved onion growth indices such as LAI and hastened bulbing nearly 2 weeks leading to significantly greater bulb yield. Irrigate at 9 days interval in inoculated plants produced greater biomass and edible yield compared to 7 and 11 day interval.

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