Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 13(3), 677 - 688, 2005

EFFECT OF INOCULATION TIMING WITH ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND FLOWERING OF MICROPROPAGATED *Chrysanthemum morifolium*

[44]

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ABSTRACT

A pot experiment was carried out to evaluate the effect of arbuscular mycorrhizal fungi (AMF) inoculation timing on growth and flowering of *Chrysanthemum morifolium* cuttings. AMF inocula were either directly applied to cutting (AMFC), or applied at transplanting stage (AMFT). The data showed: a significant difference in plant growth of AMF treatment compared with non-inoculated treatment at transplanting stage. Rooting rate in AMF treatment was 99% whereas it was 77% in non-mycorrhizal inoculated. The colonization rate was 53.9% in AMF treatment, while no in non-AMF treatment. Tap root length and number of lateral roots in AMF treatments were twice of those recorded for non-AMF treatments. Inoculation of AMF significantly increased shoot and root growth at transplanting stage. After transplantation, chrysanthemum plants in AMFC and AMFT treatments had 76.42 and 64.24% colonization rate, respectively. Plant height, leaf area, root length, fresh and dry weight of shoots, stems and roots in AMF inoculation treatments (AMFC and AMFT) increased significantly than those of control plants. AMF inoculation significantly shortened flowering time compared with non-AMF plants. Fresh weight, width and length of flowers in AMFC and AMFT treatments were generally higher than those in control. However, a significant increase in fresh weight, width and length of flowers was found in AMFC compared with AMFT treatment. A significant increase of macronutrient concentrations in leaves was observed for AMFC treatment compared with control. Mn concentration in AMFC and AMFT was more than double of that in control. In roots, macro and micronutrient concentrations were generally higher in AMFC than AMFT or control treatments.

Key words: Chrysanthemum; Arbuscular mycorrhizal fungi; Colonization; Rooting rate

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(Received April 16, 2005)
(Accepted May16, 2005)

INTRODUCTION

Micropropagated ornamental plants are often grown from cuttings in disinfected soils or growth media to avoid the risk of contamination and to ensure controlled conditions giving homogenous growth. However, such practice could eliminate potential pathogens as well as beneficial microorganisms. Therefore, micropropagated plants should be inoculated with beneficial microorganisms particularly symbiotic associates.

Arbuscular mycorrhizal fungi (AMF) inoculation is known to have tremendous effects on plant growth by enhancing nutrient and water uptake (**Kim *et al* 1998; Badr El-Din and Attia 2003**), inducing changes in root morphology (**Azcon-Aguilar *et al* 1996**) and providing protection to colonized roots against pathogens (**Abdalla and Abdel-Fattah, 2000 and Attia *et al* 2004**). Inoculation of micropropagated plants with AMF at the beginning of the acclimatization period resulted in greater plant height, leaf area and fresh weight of shoots and roots, as well as higher rate of photosynthesis and transpiration than controls (**Yano-Melo *et al* 1999**). Mycorrhizal inoculation at transplanting stage also improved growth and success in transplanting micropropagated plantlets of *Sesbania sesban*. Only 30% of plantlets transferred to soil without AMF survived, whereas all the plantlets inoculated with AMF survived due to greater vigor to successfully withstand transplantation shock (**Subhan *et al* 1998**). **Monticelli *et al* (2000)** found that micropropagated plants of *Prunus* genus inoculated with AMF showed increases in plant height; shoot dry weight, and root diameter compared with control plants.

Moreover, mycorrhizal inoculation at the early weaning phase resulted in better plant growth compared with that at the transplanting phase, 1 month after the start of acclimatization. AMF inoculation of *Malus* sp. before acclimatization, i.e directly at rooting phase of microcuttings did not improve rooting rate. However, microcuttings established with AMF resulted in greater shoot height than non-inoculated plants throughout the growing season (**Uosukainen and Vestberg, 1994**).

The effect of inoculation timing on survival of cuttings, their subsequent growth, and flowering of ornamental plants seemed to be an interesting area of research and not been fully understood. Therefore, this study aimed to evaluate the effects of AMF colonization on rooting growth, flowering and nutrient concentrations of chrysanthemum at rooting and transplanting stage.

MATERIAL AND METHODS

Chrysanthemum cuttings

Cuttings of chrysanthemum (*Chrysanthemum morifolium*) were obtained from 2-years-old stock plants grown in pots under greenhouse conditions at El Kata village, Giza Governorate. Cuttings consisted of a lateral shoot with three leaves giving a length of 6 cm and 0.30 cm diameter.

Arbuscular mycorrhizal fungi (AMF) inoculum

The AMF inoculum was obtained from a pot culture of sorghum that was grown for 5 months in a mixture of peat: vermiculite: perlite mix 1:1:1 by volume

and inoculated with *Glomus* spp. (**Badr El-Din *et al* 1999**). The inoculum was a mixture of colonized roots, hyphae, and spores (50 spores per gram).

Experimental technique

To evaluate the effects of AMF inoculation timing on growth of cuttings, two treatments and one control were made. A 150 out of 450 cuttings were directly inoculated with AMF by placing them into holes contained 2 g of inoculum in a sterile medium (vermiculite : sand = 1:1, v/v) on a nursery bed (0.5ml x 0.5mW x 0.1 mH). The other 300 cuttings were raised in the bed without AMF inoculation. Each nursery bed received 50 cuttings at spacing of 6 cm and then placed on a bench in a greenhouse with a completely randomized design. The cuttings were grown for 1 month in 14 h light at 30 °C and 10 h dark at 20 °C and fed with a half strength of Hoagland's solution (**Hoagland and Arnon, 1938**). After 1 month (transplanted stage), 100 plantlets with medium size of those directly inoculated with AMF were transplanted in to a tray (3.45 ml x 0.66 mW x 0.15 mH) filled with a non-sterile PP medium (perlite : peat 1:1, v/v) (AMFC). Two hundred plantlets with medium size out of the uninoculated 300 plantlets were selected and transplanted into the PP medium. One hundred out from 200 plantlets were inoculated with 2 g of AMF inoculum (AMFT); while, 100 plantlets did not receive AMF inoculum (control). Each tray had 20 plantlets at spacing of 30 cm placed on a greenhouse bench in a completely randomized design. The medium used was enriched by adding superphosphate (15.5% P_2O_5), ammonium nitrate (33.5% N) plus potassium sulphate (48% K_2O) by the rate of 50, 300 and 150 g /1200 L, respectively.

After transplanting, plantlets of chrysanthemum were grown for 16 weeks in 14 h light at 28 °C for and in 10 h dark at 20°C. The plantlets were fed with a nutrient solution (KNO_3 2mM, $NH_4H_2PO_4$ 0.33 mM, $MgSO_4.7H_2O$ 0.5 mM, Fe-EDTA 0.022 mM, $Ca(NO_3)_2.4H_2O$ 1 mM, $MnSO_4.5H_2O$ 0.33 mM, H_3PO_3 2.36 mM, $ZnSO_4.4H_2O.7H_2O$ 0.035 mM, $CuSO_4.5H_2O$ 0.02 mM, $(NH_4)_6Mo_7O_{24}$ 0.0013 mM) for 4 weeks, and 1.5 strength of the nutrient solution for the rest of growing period. At transplanting stage, rooting rate and early growth response of plantlets from AMF inoculation and non-AMF inoculation treatments were measured. After transplanting plant growth, colonization rate and flowering characteristics were examined.

Plant analysis

Mycorrhizal colonization of chrysanthemum roots was determined by the magnified intersect method described by **McGonigle *et al* (1990)**. The roots of chrysanthemum plants were collected at transplanting stage (1 month) and after transplanting plant growth (100 days after transplanting). Fresh roots were thourghly washed with water and then cut into 1 cm segments. Roots were placed into 20 ml vials containing 10% KOH solution. The vials with root samples were kept for 1 h at 90°C and then washed with water and stained with Chlorazol black E and held at 50°C overnight. Roots were then washed with water and glycerin until clearing stage. Root colonization percentage was determined by dividing the num-

ber of colonized roots by the total number of examined root segments **McGonigle *et al* (1990)**.

Fresh and dry weight of leaf, stem and root were recorded. A 0.5 g finely ground shoot was placed in a 100 ml microkjeldahl flask with 10 ml concentrated H_2SO_4. A 0.5 ml H_2O_2 was added to the sample every 10 min for 90 min (total 4.5 ml). After cooling, the solution was filtered through Whatman no. 6 filter paper into 100 ml flasks. Macro and micronutrient concentrations were assayed by the method of **Jones *et al* (1991)**. Rooting rate was calculated by dividing the number of survival cuttings by the total number of cuttings planted. Flowers were counted in each treatment for 100 days after transplanting. For flower characteristics, total flower fresh weight, number of flower, flower length, and width were examined.

Statistical analysis

Analysis of variance was performed using the SAS version 6.08 (**SAS Institute, 1990**). The least significant difference (LSD) among mean values was calculated at P = 0:05 or 0.01 confidence level.

RESULTS

Colonization of arbuscular mycorrhizal fungi

Before transplanting, cuttings inoculation with mycorrhizal fungi were well colonized. Colonization root of AMF rate was 53.9% in AMF treatment, while no colonization was observed in non-AMF treatments. After transplanting, AMFC treatments, chrysanthemum plants showed 76.42% colonization rate, while mycorrhizal plants in AMFT scored 64.24% colonization rate (Table, 1). There was a 12.18% increase in colonization rate in AMFC compared to AMFT. Hyphal colonization was consistently quite high, and considerable numbers of vesicles formed in all roots. Arbuscular colonization was very high in AMFT and low in AMFC.

Plant growth as influenced by AMF inoculation at cutting stage

Data presented in Table (2) showed that, at transplanting stage, AMF treatment had significant affect on growth characters of the cuttings compared with control treatment. The inoculated cuttings produced greater length plants than of the control treatments. Rooting rate was 77% in non-mycorrhizal treatment, whereas it reached to 99% in AMF treatment (Table 2). Tap root length and number of lateral roots in AMF treatment increased to more than twice of those in non-AMF treatment. Fresh weight of plants showed insignificant differences among both inoculated and uninoculated treatments. However, fresh and dry weight of root significantly increased in response to AMF inoculated treatments.

Plant growth by AMF inoculation after transplanting

Data presented in Table (3) show that, AMF had a statistical significant effect on plant height, leaf area, while root length did not affected significantly by inoculation with AMFC. Number of leaves also increased with inoculated with AMFC by about 20.37% over the control plants. Data also showed significant increase on fresh and dry weight of shoots, stems and roots in AMFC and AMFT inoculation treatments compared with control plants

Table 1. AMF colonization of chrysanthemum root as affected by the different timing of AMF inoculation

Inoculation timing	Colonization rate (%)			
	Vesicle	Hyphae	Arbuscule	Total
	Before transplanting			
Inoculated	0	32.44	21.46	53.9
	After transplanting			
Control	0.24	2.70	3.12	6.08
AMFT	2.12 **	35.18**	26.94 **	64.24 **
AMFC	5.34 **	48.06 **	23.02 **	76.42 **
LSD (P = 0:05)	1.06	3.47	9.02	9.21
LSD (P = 0:01)	1.48	4.87	12.64	12.92

AMFT= AMF inoculation at transplanting stage; AMFC= direct AMF inoculation to cuttings

** Significant at 1%.

Table 2. Effect of AMF inoculation at transplanting stage on growth characteristics of chrysanthemum cuttings

Treatments	Plant height (cm/ plant)	No. of lateral roots (plant)	Tap root length (cm)	Rooting rate (%)	Fresh weight (g/plant)		Dry weight (g/plant)	
					Shoot	Root	Shoot	Root
Control	4.7	26.8	4.7	77	1.03	0.20	0.16	0.02
Inoculated	6.6 *	79.8 *	10.6 **	99 **	1.28	0.56**	0.22	0.07*

* Significant at 5%. ** Significant at 1%.

Table 3. Effect of AMF inoculation on growth parameters of chrysanthemum after transplanting

Inoculation stage	Plant height (cm/plant)	No. of leaves	Root length (cm/plant)	Fresh weight (g/plant)			Dry weight (g/plant)		
				Leaf	Stem	Root	Leaf	Stem	Root
Control	37.2	84.32	20.5	19.9	11.6	8.2	5.4	3.3	2.2
AMFT	48.5**	96.34	29.0*	28.6	26.2*	24.0*	7.8	7.6*	9.4*
AMFC	52.0**	101.50*	27.3	35.9*	27.0*	26.6*	9.6*	8.0*	7.2
LSD (P = 0:05)	6.3	13.5	7.9	11.2	13.8	19.6	3.1	4.1	5.4
LSD (P = 0:01)	9.6	18.5	12.0	16.9	20.9	29.7	4.8	6.2	8.2

AMFT= AMF inoculation at transplanting stage; AMFC= direct AMF inoculation to cuttings.
* Significant at 5%. **Significant at 1%.

(Table, 3). However, AMFC treatments gave insignificantly greater fresh weight of leaves, stem and root than those obtained by AMFT. The highest increase in plant growth was observed in AMFC treatment. Dry weight of leaves and stems were higher for cutting of AMFC treatments while cutting of AMDA treatments showed significant effect on root dry weight.

Flowering characteristics

Plants inoculated with AMF flowered earlier than the uninoculated control (Fig. 1). Chrysanthemum in AMFC treatment flowered after 98 days of transplanting, whereas those grown in AMFT and control treatments needed more 6 and 14 days for flowring, respectively. Fresh weight, width and height of flowers in AMFC and AMFT treatments were generally higher than those in control treatment (Table, 4). However, significant increase in fresh weight, width and height of flowers were recorded in AMFC treatment.

Nutrient concentrations

Significant macronutrient concentrations (P, K, Mg, and Ca) into leaves was observed for AMFC treatments compared with control plants. On the other hand, micronutrient concentrations (Mn, Cu, and Zn) was considerably increased in AMFC and AMFT treatments compared with control where Mn concentration in AMFC and AMFT treatment was more than double of that in the control. In roots, macro and micronutrient concentrations were generally higher in AMFC treatments (Table, 5). Both treatments, AMFT and AMFC, stimulated the concentrations of mineral nutrients in both leaves and roots of dry plantlet. Data clearly show that there were no significant differences between the AMFT and control plants regarding P, N, K, M and Ca concentrations, while, AMFT treatments showed significant effect on Fe, Mn, and Cu concentration.

DISCUSSION

The first objective of this study was to determine how much of the chrysanthemum plant roots could be colonized at cutting and transplanting stage of growth. At transplanting stage, colonization rate in plantlets inoculated with AMF was 53.9%, whereas control plants did not show any colonization, as expected. Increased plant growth was observed in the plants inoculated with AMF. At transplanting stage, inoculation with AMF resulted in higher records of height, number of lateral roots, tap root length, and fresh as will as dry weight of shoots and roots compared with non-AMF treatment (Table, 2). After that, the highest colonization rate was observed in AMFC (76.42%) followed by AMFT (64.24%), and then control treatment (6.08%) where plant growth positively affected as colonization rate increased (Tables, 1 and 3). These results were similar to the finding of **Kim *et al* (1998)**, who reported that plant growth, fresh and dry weight of tomato colonized with 54.4% was significantly increased compared with 28.9% colonization rate. This result could be associated with the increase of nutrient concentrations by mycorrhizal roots. As the colonization rate of roots increases, a mycorrhizal root system can explore more volume of soil with more easily uptake of nutrients from less soluble

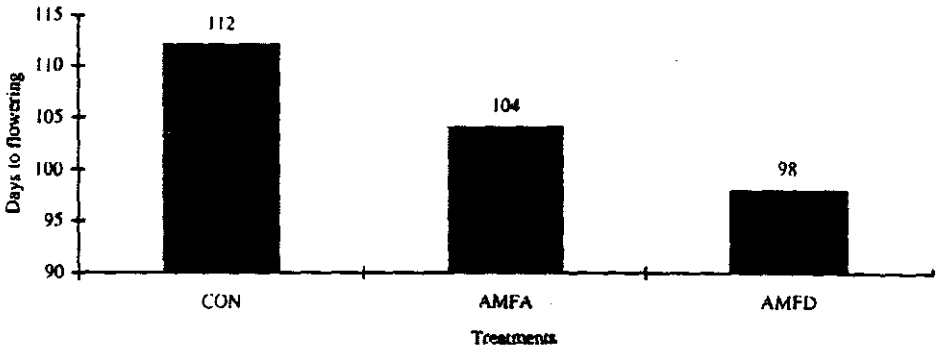

Fig. 1. Time of chrysanthemum flowering as affected by the different timing of AMF inoculation after transplanting.
CON= control; AMFT= AMF inoculation at transplanting stage; AMFC= direct AMF inoculation to cuttings.

Table 4. Comparison of flowering characteristics of chrysanthemum as affected by the timing of AMF inoculation (after transplanting)

Inoculation stage	No. of floweret	Total flower fresh weight (g/plant)	Flower width (cm)	Flower height (mm)	Flower stalk (mm)
Control	37.4	14.94	4.90	13:83	4.63
AMFT	37.8	21.17**	5.27	14.70	4.83
AMFC	41.2	22.82**	5.93**	15.13*	5.63
LSD (P = 0:05)	5.9	3.80	0.38	0.96	1.91
LSD (P = 0:01)	8.9	5.75	0.57	1.45	2.90

AMFT= AMF inoculation at transplanting stage; AMFC= direct AMF inoculation to cuttings.
*Significant at 5%. ** Significant at 1%.

Table 5. Mineral nutrient concentration of the chrysanthemum as affected by the different timing of AMF inoculation (after transplanting)

Items	Inoculation stage	P (mg/g)	K (mg /g)	Mg (mg /g)	Ca (mg/g)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Leaf	Control	3.20	4.08	1.61	5.07	92.50	30.8	3.1	54.2
	AMFT(1)	3.28	4.15	1.83	6.05	104.80*	62.4**	3.9**	57.1
	AMFC(2)	3.84**	4.40*	2.27**	6.13*	108.00*	70.0 **	4.6**	58.3
	LSD (P = 0:05)	0.41	0.31	0.24	0.77	10.43	17.53	0.38	6.28
	LSD (P = 0:01)	0.62	0.46	0.36	1.16	15.81	26.56	0.57	9.51
Root	Control	2.78	2.65	0.99	1.77	114.20	13.1	4.1	56.7
	AMFT	2.96	2.66	0.99	2.17	141.00	44.6 *	5.6	63.7
	AMFC	3.38	3.15**	1.02	2.31*	185.4*	70.2**	9.1**	104.4**
	LSD (P = 0:05)	0.65	0.74	0.23	0.44	59.17	25.48	1.98	26.41
	LSD (P = 0:01)	0.99	1.12	0.36	0.66	89.63	38.60	3.00	40.00

(1) AMFT= AMF inoculation at transplanting stage; (2) AMFC= direct AMF inoculation to cuttings.
* Significant at 5% ** Significant at 1%.

sources (**Attia, 1999**). As shown in Table (5), significant macronutrient concentration in leaves was observed in AMFC treatment compared with control plants. On the other hand, micronutrient concentration was considerably increased in AMFC and AMFT treatments compared with control. We found that rooting rate was 77% in non-mycorrhizal treatment, whereas it was 99% in AMF treatment at transplanting stage (Table, 2). Plant growth in AMF treatment increased by more than twice than those in non-AMF treatment. After transplanting, the fresh and dry root weights were greatest in AMFC treatment followed by AMFT treatment, and then control treatment (Table, 3). This result clearly indicate that early AMF inoculation (directly to cuttings) was most advantageous for the growth of chrysanthemum. **Attia *et al* (2004)** reported that early infection by VAM fungi was very important for growth and yield response of mycorrhizal plants, especially in short-duration crops. Colonization rate was 76.42% when AMF inoculation was performed directly to cuttings and 64.24% when AMF inoculation was done at transplanting. As shown in Fig. (1) and Table, (4), AMFC treatment significantly shortened flowering time and increased flower quality. This suggests that early colonization of young plant roots improved plant root surface absorbing hence nutrients more efficiently, thereby resulting in better plant performance.

Plants inoculated with AMF flowered earlier than the uninoculated controls. Chrysanthemum in AMFC and AMFT treatments flowered 98 and 104 days after transplanting, respectively, whereas plants in control treatments took 112 days to flower. Similar results have been reported in number of ornamental plants. **Gaur and Adholeya (2000)** reported that inoculated *Callistephus chinensis* flowered 27 days after transplanting, whereas the uninoculated plants took 22 days longer. Also, *Impatiens balsamia* flowered at 37 days, which was 16 days earlier than uninoculated plants and those of *Petunia hybrida* flowered after 29 days, 12 days ahead of the uninoculated plants.

Fresh weight, width and height of flowers in AMFC and AMFT treatments were generally higher than those in control treatment. **Gaur and Adholeya (2000)** reported that mycorrhizal inoculation of seedlings led to marked improvement in both reproductive (number of flowers) and vegetative (dry matter) phase of the ornamental plants. The highest fresh weight, width and height of flowers in AMFC treatment confirmed the result of **Monticelli *et al* (2000)** that early AMF inoculation significantly increased plant growth compared with applying mycorrhizal inoculation at the transplanting stage.

As shown in our results, AMF inoculation is a vital factor for enhancing plant growth; however, the timing of inoculation should be considered. With better nutrient concentrations and early flowering resulting from directly inoculated cuttings, the expenses of fertilizers could be reduced and time of production will be saved, which translates to higher profits for farmers.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١٣(٣) ، ٦٧٧ - ٦٨٨ ، ٢٠٠٥

تأثير توقيت التلقيح بفطريات الميكوريزا على النمو وتزهيرة الأرولا موريفوليم

[٤٤]

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أجريت تجربة أصص لدراسة تأثير التلقيح بفطريات الميكوريزا فى المراحل المختلفة لنمو زهرة الأرولا على تكوين الجذور وصفات الأزهار لتحقيق ذلك تم التلقيح المباشر للعقل بفطريات الميكوريزا عند الزراعة وكذلك بعد تكوين الشتلات وقد أظهرت النتائج الاستجابة الكبيرة للشتلات للتلقيح بفطريات الميكوريزا بالمقارنة بغير الملقحة. حيث وصلت نسبة التجذير فى الشتلات الملقحة إلى ٩٩% بالمقارنة ٧٧% فى الشتلات غير الملقحة. وقد وصلت أطول الفروع الرئيسية للجذور والشعيرات الجذرية فى الشتلات الملقحة إلى ضعفي غير الملقحة.

وصلت نسبة مستعمرات الميكوريزا إلى ٧٦,٤٢% فى الشتلات الناتجة من العقل الملقحة بينما وصلت إلى ٦٤,٢٤% فى الشتلات الملقحة بعد الشتل. أدى التلقيح بفطريات الميكوريزا بصفة عامة إلى زيادة معنوية فى كل الصفات الخضرية للشتلة مع تقليل فترة التزهير بالمقارنة بغير الملقحة، وقد اظهر التلقيح المباشر للعقل زيادة معنوية عالية فى الصفات الخضرية والمظهرية للشتلات مقارنا بغير الملقحة.كذلك ظهرت زيادة معنوية فى تركيز العناصر الكبرى فى أوراق النباتات الملقحة مقارنا بغير الملقحة، وقد وصل تركيز المنجنيز إلى أكثر من ضعفي محتواه فى الشتلات غير الملقحة، وفى الجذور كانت تركيزات العناصر الكبرى والصغرى بصفة عامة عاليا بالمقارنة بالشتلات غير الملقحة.

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