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Full Research Article

Symbiotic Effect of Arbuscular Mycorrhizal Fungi on Growth and Flowering of Micropropagated Plants of Chrysanthemum (*Chrysanthemum dendranthemum*)

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Abstract

Field mortality and poor growth of *in vitro* raised plantlets of chrysanthemum is a major hindrance for commercial propagation through tissue culture. In the present investigation four AMF strains namely, *Acaulospora laevis*, *Acaulospora scorbiculata*, *Glomus fasciculatum* and mixed AMF strains (Nutrilink®) were used as bio-hardening agents to improve survival, flower quality and yield of *in vitro* raised chrysanthemum plantlets of cv. Yellow Bangla. Plantlets inoculated with mixed AMF gave the highest survival (94.89%) and root colonization (66.23%). The predominant effect of mixed AM fungi was also evident as increased plant height (32.34 cm), root length (24.62 cm), leaf number (46) and leaf area (10.93 cm^2) of the inoculated plantlets. *Acaulospora laevis and Glomus fasciculatum* were found more effective in improving both shoot and root biomass at 30, 60 and 90 days after inoculation (DAI). All the treatments a improved survival and growth significantly over uninoculated control. The plantlets inoculated with *Glomus fasciculatum and* mixed AMF flowered early, produced higher yield, bigger flowers with increased sugars and carotene content in the flowers than other treatments.

1. Introduction

Among the top three cut flower crops in the International market, chrysanthemum is ranked third next to rose and tulip. Due to its demand in North America and Europe, it is popularly called as dollar earning flower. *In vitro* multiplication of elite plant genotypes is widely used commercial application of plant biotechnology. Tissue culture techniques have been used extensively for micropropagation of many commercially grown chrysanthemum genotypes for rapid multiplication of disease free planting material. Though tissue culture is advantageous, but it suffers from different problems such as poor survival of plants upon field transfer, which delimits its wide spread use. Poor survival after field transplant is attributed to different factors viz., weak root system, unfavourable nutritional and environmental conditions (Schubert et al., 1990), poorly developed cuticle, non-functional stomata, excessive proneness to microbial infection and poor photosynthetic efficiency *etc*. Hardening is considered as the most critical stage in micropropagation as it is the beginning of the biochemical and physiological processes in plants, which are necessary for survival. During hardening, the *in vitro* raised plantlets are subjected to *ex vitro* conditions to develop functional root system resulting in increased absorption of water, mineral, nutrients and photosynthesis etc.

Bio-hardening with Arbuscular Mycorrhizal Fungi (AMF) have proved to be beneficial to tissue culture plants in reducing the impact of *ex vitro* stressful environmental conditions. As the media used for *in vitro* stages are sterilized before use, to eliminate harmful fungi / bacteria, which results in elimination of useful fungi also like mycorrhiza (Sohn et al., 2003). Therefore, if micropropagated plantlets are inoculated with microbial inoculum artificially early in micropropagation process, they can achieve a suitable growth since AMF plays a great role in development of stronger root system (Haripriya and Sriramachandrasekharan, 2002), improved growth (Zandavalli et al., 2004), nutrient uptake (Rajadurai and Beaulah, 2000), increased tolerance of host roots to soil borne pathogens (Nelson and Achar, 2001), increased production of growth hormones such as auxins, gibberellin like substances and cytokinins (Allen et al., 1980) and drought stress (Ruiz Lozano and Azcon, 1995), thereby enhancing plant growth and survival after field transplant. Besides promoting growth of the plants, microbes such as *Frankia*, *Rhizobium*, *Bradyrhizobium*, *Mycorrhiza*, improve aggregation, stability and physical

properties of soil, thereby making it conductive for growth of micro-propagated plants, which generally have fragile root system (Varma and Schuepp, 1994). There is an enormous scope to make use of AMF as bio-hardening agent for tissue culture raised chrysanthemum plantlets. Little information is available about how microbes improve acclimatization and growth of micropropagated plantlets. Hence, the present investigation was carried out to study the effect of selected AM fungi on the enhancement of survival, growth and yield of micropropagated chrysanthemum plantlets.

2. Materials and Methods

2.1. Study sites

The experiment was carried out at the Division of Floriculture and Landscaping, Indian Agricultural Research Institute (IARI), New Delhi-110012 along with the facilities availed from the Division of Fruits and Horticultural Technology, IARI, Central Tissue culture Laboratory, NRCPB, IARI and Division of Microbiology, IARI, New Delhi.

2.2. Method of data collection

In vitro raised rooted plantlets of cv. Yellow Bangla (30 days after root initiation) were taken as the planting material. The experiment was laid out in randomized block design comprising of five treatments and five replications. Plantlets inoculated with different microbial inoculants viz., T_o (Control), T_1 (*Acaulospora laevis)*, T_2 (*Acaulospora scorbiculata*), T_3 (*Glomus fasciculatum*) and T_4 (mixed AMF strains (Nutrilink®)). *In vitro* raised chrysanthemum plantlets were acclimatized by washing the roots with sterile distilled water and then potted into mixture consisting of sterile soil, sand and FYM (2:2:1) in 6 inch plastic pots. Different soil based microbial inocula were used by laying 20 g culture below the roots of *in vitro* raised plantlets. The potted plants were watered with distilled water and transferred to ployhouse conditions. The plantlets were maintained in the polyhouse. The percentage of root colonization was determined after cleaning the root bits with 10% KOH and staining with trypan blue (Phillips and Hayman, 1970) at different post-inoculation periods viz., 30, 60 and 90 days after inoculation. To assess per cent root colonization, root segments (1 cm long) were selected at random from a stained sample and mounted on microscopic slides. Ten such slides were observed. On each slide, ten root segments were observed under an inverted binocular (Nikon Japan) (125×magnification). Extent of root colonization was assessed in ten slides which was averaged and expressed as per cent root length. Survival of inoculated plants was also observed at different post-inoculation periods. Various growth parameters like plant height, root length, number of leaves, leaf area, stem diameter, fresh and dry weights of shoot and roots was recorded at 30, 60 and 90 days after inoculation. Flowering parameters like, days taken for initiation of flowering, number of flowers per plant, flower size, weight of the flower was recorded. Concentration of reducing, non reducing sugars in the flowers and carotenoid content of the flowers was determined by a modified method as described by Ranganna (1986).

3. Results and Discussion

3.1. Root colonization

Among the different microbial strains applied, maximum colonization (66.23%) was found in T_4 inoculated plantlets. Irrespective of treatment, maximum colonization was found at 90 DAI while minimum was observed at 30 DAI. The two-way interaction between treatment and duration showed that T_4 gave the maximum colonization (Table 1).

The superiority of mixed cultures, in present investigation, can be attributed to that of AMF communities. When plants were colonized by more than one AMF isolates, performance of host for specific isolates of the community was pertinent (Johnson et al., 2004). Highest plantlet survival was observed in T_4, i.e. mixed AMF inoculated plantlets. The two-way interaction between treatment and duration revealed that plantlets inoculated with T_4 at 30 DAI showed maximum survival, while minimum survival was observed in T_0 (Table 1). These results were in conformity with Sohn et al. (2003).

3.1.1. Growth characters

To know the effect of AMF on physical characters like, plant height, root length, leaf number, leaf area, stem diameter, shoot and root biomass samples were collected randomly at different durations from all the treatments. Irrespective of duration and treatment, maximum plant height was observed in plantlets inoculated with T_4 (32.34 cm) which was at par with T_1 (29.70 cm) and T_3 (28.10 cm). The shortest plants were observed in control (18.88 cm), followed by T_2 (23.74 cm). In the present study, plant height increased significantly as a result of microbial inoculation. Maximum height was recorded in plantlets inoculated with mixed AMF strain, followed by *Acaulospora laevis* (Table 2). The results were in agreement with the earlier findings of Gnanadevi and Haripriya (1999) and Sohn et al. (2003). The root length of tissue-cultured plantlets was found to be significantly influenced by microbial inoculation. Mean value of root length was maximum in T_4 (24.62 cm) followed by T_1 (21.57 cm) while minimum was in T_2 (16.91 cm) inoculated plantlets irrespective of duration. The lowest root length was recorded in control (10.79 cm). These results were in agreement with earlier observations of Puthur et al. (1998) and Sohn et al. (2003). Inoculation with AMF resulted in increased root length in all the treatments. Irrespective of treatment and duration, mixed AMF strain was found to be the best with regard to increase in root length

Table 1: Root colonization (%) and survival of mycorrhizae inoculated plantlets of chrysanthemum during acclimatization

Treatment	Root colonization (%)				Survival (%)			
	Duration (days)				Duration (days)			
	30	60	90	Mean	30	60	90	Mean
T_0	2.20 (8.53)*	6.17 (14.39)*	7.69 (16.11)*	5.35 (13.38)*	68.00 (55.58)*	56.00 (48.47)*	55.00 (47.89)*	59.67 (50.60)*
T_1	27.73 (31.79)	54.83 (47.80)	83.97 (66.43)	55.51 (48.19)	89.33 (70.97)	88.67 (70.37)	88.00 (69.77)	88.67 (70.37)
T_2	15.50 (23.20)	34.89 (36.22)	66.41 (54.61)	38.93 (38.62)	72.00 (58.08)	64.00 (53.16)	63.00 (52.56)	66.33 (54.56)
T_3	29.33 (32.81)	58.33 (49.82)	81.95 (64.89)	56.51 (48.77)	86.67 (68.62)	81.33 (64.43)	81.33 (64.43)	83.11 (65.77)
T_4	36.63 (37.26)	66.91 (54.91)	86.14 (68.16)	66.23 (54.50)	98.67 (83.42)	93.33 (75.07)	92.67 (74.33)	94.89 (76.97)
Mean	22.28 (28.18)	44.21 (41.70)	65.23 (53.90)	-	82.93 (65.63)	76.66 (61.14)	76.00 (60.10)	-
*$p<0.05$								
Treatment			4.223				2.879	
Duration			3.271				2.230	
T×D			7.314				4.987	

*Transformed data

(Table 2). Earlier, Bagyaraj and Powell (1985) suggested the positive effect of AMF on growth of marigold and nutrient uptake depends on the plant species as well as strains used. This effect was mainly due to profuse branching of lateral roots or elongation of main roots. In the present study also, the increased plant height and growth of chrysanthemum could be attributed to increased root length as a result of microbial inoculation.

Improved root growth with the inoculation of AM fungi can be attributed to growth hormone production and increased nutrient uptake (Azcon-Aguilar and Barea, 1996). Mycorrhizal inoculation also increased the plant phosphorous (P) content. Phosphorus a constituent of phosphonucleotides which tend to increase cell division, would have increased the root and shoot growth. Among different AMF treatments applied, leaf number and leaf area was observed to be maximum in T_4 i.e. mixed AMF inoculated plantlets. Significant increase in leaf area and number of leaves in microbial inoculated plantlets was observed. Results showed that irrespective of treatment and duration, maximum numbers of leaves were significantly superior over control (Table 2). These results were in conformity with findings of Bierman and Linderman (1983a) and Sohn et al. (2003). This increased leaf area may be attributed to an increase in photosynthetic rate of inoculated plantlets Allen et al. (1981).

The results of fresh weight and dry weight of both shoots and roots as influenced by microbial inoculation were significant at all the stages, i.e. 30, 60, 90 days after inoculation. Irrespective of treatment and duration, maximum fresh and dry weight of both shoots and roots was observed in plantlets inoculated with *Acaulospora laevis* inoculated plantlets followed by mixed AMF inoculated strains. Control plants showed minimum fresh and dry weights (Table 3). Similar observations have been reported earlier for other ornamental crops viz., China aster (Hemla Naik et al.,1995), Geranium (Biermann and Linderman, 1983a), Chrysanthemum (Sohn et al., 2003) and Araucaria (Zandavalli et al., 2004). The improved biomass production could be attributed to improved nutrient uptake and enhanced photosynthetic rate (Mathur and Vyas, 1999).

3.1.1.1. Flowering and quality analysis

In this study flowering parameters were also recorded to know the effect of different microbial strains. The plantlets inoculated with T_3 flowered fifteen days earlier than control. T_4 is also on par with T_3. Maximum numbers of flowers were obtained from mixed AMF inoculated plantlets (43.00). Bigger flowers were recorded in T_4 (6.03 cm) followed by T_1 (6.00 cm). Flower diameter was found minimum in uninoculated plantlets (7.37 cm). Higher flower weight was observed in plantlets inoculated with T_3 strain (4.88 g) followed by T_4 (3.90 g).

Longest flower stalk length was recorded in T_4 (4.73 cm) followed by T_3 (4.26 cm). T_4 inoculated plants produced flowers having maximum floret number (277.33 cm) followed by T_3 (261.33 cm). All the treatments showed significantly higher value over control in terms of earliness in flowering, flowers per plant, size of the flower, weight of the flower, flower stalk length and floret number (Table 4). Maximum total carotene (153.15 mg 100 g^{-1}) content was recorded in flowers of T_3 inoculated plantlets followed by T_1. Maximum ß-carotene value was recorded in flowers of T_3 inoculated plantlets (26.63 mg 100 g^{-1}) followed by T_4 (23.86 mg 100 g^{-1}). In case of reducing sugars alone, T_4 (2.03 mg g^{-1}) and T_1 (1.956 mg g^{-1}) plantlets showed marked variation over the control. Other treatments were found non-significant with control. Among all the treatments T_1 plantlets showed maximum total sugars (6.54 mg g^{-1}) followed by T_2 (5.84 mg g^{-1}). All the treatment

Table 2: Effect of amf on plant height (cm), root length (cm), leaf number and leaf area (cm^2) of tissue culture raised chrysanthemum plantlets during hardening

Treatment	Plant height (cm)				Root length (cm)				Leaf number				Leaf area (cm^2)			
	Duration (days)				Duration (days)				Duration (days)				Duration (days)			
	30	60	90	Mean	30	60	90	Mean	30	60	90	Mean	30	60	90	Mean
T_0	9.33	17.13	30.17	18.88	5.90	12.57	13.90	10.79	13.00	21.33	29.33	21.22	3.78	5.34	6.62	5.25
T_1	16.33	30.50	42.27	29.70	10.87	20.93	32.90	21.57	22.67	43.00	55.33	40.33	7.14	8.85	14.17	10.05
T_2	14.33	21.40	35.50	23.74	9.97	18.43	22.33	16.91	18.00	28.67	36.33	27.67	6.22	6.47	8.21	6.97
T_3	17.67	24.83	41.80	28.10	10.47	18.20	33.37	20.68	20.67	38.67	53.67	37.67	6.89	8.36	12.07	9.11
T_4	17.00	32.43	44.60	32.34	17.57	20.30	36.00	24.62	22.33	48.67	67.00	46.00	7.40	10.09	15.29	10.93
Mean	14.93	25.26	38.87	-	10.95	18.09	27.70	-	19.33	36.07	48.33	-	6.28	7.82	11.27	-
$^*p<0.05$																
Treatment			1.820				1.648				3.014				0.895	
Duration			1.410				1.277				2.335				0.694	
T×D			3.150				2.855				5.221				0.551	

Table 3: Effect of AMF on shoot fresh weight (g), shoot dry weight (g), root fresh weight (g) and root dry weight (g) of tissue culture raised chrysanthemum plantlets during hardening

Treatment	Shoot fresh weight (g)				Shoot dry weight (g)				Root fresh weight (g)				Root dry weight (g)			
	Duration (days)				Duration (days)				Duration (days)				Duration (days)			
	30	60	90	Mean	30	60	90	Mean	30	60	90	Mean	30	60	90	Mean
T_0	1.17	7.81	18.46	9.15	0.12	1.50	2.70	1.44	0.75	2.30	6.92	3.32	0.13	0.35	1.45	0.64
T_1	3.57	23.23	61.44	29.41	0.45	5.21	8.98	4.88	0.78	14.09	22.17	12.35	0.09	3.91	4.15	2.72
T_2	2.98	10.76	25.95	13.23	0.30	2.87	3.68	2.28	1.25	4.96	11.00	5.74	0.28	0.73	1.96	0.99
T_3	6.38	19.87	52.67	26.31	0.89	3.64	7.96	4.16	2.49	7.76	19.54	9.93	0.39	1.18	5.57	2.38
T_4	3.95	17.50	52.34	24.60	0.53	3.19	7.76	3.83	0.79	8.03	19.25	9.36	0.21	1.72	4.14	2.02
Mean	3.61	15.83	42.17	-	0.46	3.28	6.21	-	1.21	7.43	15.78	-	0.22	1.58	3.45	-
$^*p<0.05$																
Treatment			3.900				0.703				2.147				0.559	
Duration			3.021				0.560				1.663				0.433	
T×D			6.754				1.252				3.718				0.969	

Table 4: Effect of AMF on flowering parameters of tissue culture raised chrysanthemum plantlets

Treatment	Days to 1st flower emergence	Number of flowersplant^{-1}	Flower diameter (cm)	Flower weight (g)	Flower stalk length (cm)	No. of florets flower^{-1}	Total carotene (mg 100g^{-1})	ß-carotene (mg 100g^{-1})	Reducing sugars (mg g^{-1})	Non reducing sugars (mg g^{-1})	Total sugars (mg g^{-1})
T_0	81.33	19.33	3.73	2.30	3.43	185.66	125.25	20.40	1.56	2.11	3.67
T_1	76.00	32.00	6.00	3.75	4.10	235.00	132.11	21.18	1.96	4.58	6.54
T_2	78.00	25.33	5.40	3.00	3.63	222.00	121.88	19.71	1.45	3.09	4.34
T_3	66.66	33.33	5.57	4.88	4.26	261.33	153.16	26.63	1.68	3.81	5.48
T_4	71.33	43.00	6.03	3.90	4.73	277.33	130.15	23.59	2.02	3.81	5.83
$^*p<0.05$	5.474	7.025	0.563	1.61	0.75	35.884	10.074	2.124	0.373	1.097	1.13

showed significantly higher value over control (Table 4). It is evident from the present investigation that *Glomus fasiculatum* associated plantlets commence early flowering compared to all other treatments. However, microbial inoculated treatments showed significantly early flowering compared to control plants. Maximum number of flowers per plant, Flower diameter and length of flower stalk was shown by mixed AMF inoculated plantlets, control plants showed the least values. Total carotene and β-carotene contents were also reported maximum in *Glomus fasiculatum* inoculated plants. Among the sugars,

reducing sugars was maximum in mixed AMF inoculated plantlets, however *Acaulospora laevis* inoculated plants showed maximum non-reducing and total sugar concentrations. In case of control plants the values were minimal. These results were in confirmity with those reported by Bagyaraj and Powell (1985); Hemla Naik et al. (1995), Gnanadevi and Haripriya (1999), Haripriya and Sreeramachandrasekharan (2002) and Shan et al. (2003).Increased carotene contents in AMF associated plant also reported in carrots and tomatoes (Regvar et al., 2003).The increased flower production and shortening flower duration may be attributed to increased root and shoot biomass, enhanced uptake of macro and micronutrients, increased photosynthesis and sugar content in inoculated plants compared to uninoculated control plants.

4. Conclusion

The unquestionable benefits of the mycorrhizal associations to the *in vitro* raised chrysanthemum plantlets cannot be ignored in hardening process and plantlets must be precolonized by suitable mycorrhizal fungi for better establishment, survival, improved growth, nutrient uptake and yield of plants, which translates to higher profits for farmers. Results in the two year investigation showed that, chrysanthemum plantlets inoculated with mixed AMF strains (Nutrilink®) had shown better field establishment, growth and flowering compared to other treatments.

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