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Effect of Auxins and Bioagents on Concurrent *ex vitro* Rooting and Hardening (CEVRH) of Micro-shoots in Chrysanthemum (*Chrysanthemum morifolium*)

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ABSTRACT: The experiment was carried out at Dr. YSRHU- Horticultural Research Station, Kovvur during 2021-2022 in factorial completely randomized design with 18 treatment combinations replicated twice. After *in vitro* shoot multiplication, micro-shoots of cv. New Man and Urban Red were treated with 3 auxins (Distilled water, NAA 100 ppm and IBA (100 ppm) and 3 bioagents (Arbuscular mycorrhizal fungi (AMF) 2g/micro-shoot, Phosphate-solubilizing bacteria (PSB) 2g/micro-shoot and *Pseudomonas fluorescens* 2 ml/micro-shoot) for simultaneous rooting and hardening shade net. Per cent survival, shoot and root characters were recorded after hardening and subjected to data analysis. Among the different treatment combinations evaluated, Urban Red micro-shoots treated with IBA and AMF recorded maximum per cent survival, shoot thickness, number of leaves, leaf area, number of roots per shoot and root thickness. However, micro-shoots of cv. New Man treated with IBA and AMF recorded highest shoot height and lengthiest roots over other treatments. From the present experimentation it can be concluded that, among two cultivars, response of Urban Red is high for CEVRH when compared to New Man. Among auxins, IBA @ 100 ppm had shown significant difference with respect to shoot and root characters. Among bioagents, Arbuscular mycorrhizal fungi (AMF) had shown significant difference with respect to shoot and root characters.

Keywords: micro-propagation, mycorrhiza, auxins, survival, growth, rooting.

INTRODUCTION

Chrysanthemum is an important ornamental crop mainly grown for production of cut flowers, loose flowers and pot plants. The word chrysanthemum comes from two Greek words '*Chrysos'* meaning golden and '*anthemon'* meaning flower. Chrysanthemum (*Chrysanthemum morifolium*) is commonly called as autumn queen, Queen of the East. It belongs to family Asteraceae (Sharma, 2015) and native to China. It is a herbaceous perennial plant extensively grown all over the world for its beautiful charming flowers with varied uses like cut flower, loose flower, exhibition type, pot mums (Sheela, 2008). It is highly popular in flower industry owing to diversity in flower shape, size, colour, form, growth habit, foliage and excellent vase life to fulfil the diverse requirements of flower users (Mao *et al*., 2012). Each and every year several chrysanthemum varieties are being released by several private and public institutes but the availability of quality, disease free planting material is a major hindrance for its commercial cultivation.

Micro-propagation is order of the day for the large-scale production of several clonally propagated plants. Micro-propagated chrysanthemum plantlets are highly desirable for commercial cultivation as they can be produced throughout the year, uniform in size, pest & disease free and best owed with high productivity. A robust *in vitro* protocol for different chrysanthemum genotypes was standardized at Dr. YSRHU-Horticultural Research Station, Kovvur by using ray florets, leaves, axillary buds and shoot tips as explants. However, higher production cost of tissue culture micro-propagated plants was the major impediment for its commercial production. The production cost of tissue culture plants can be reduced by eliminating one of the most expensive step i.e *in vitro* rooting. Hence to reduce the expenditure and speed up the production process of tissue culture plants, an experiment was performed on the effect of auxins and bioagents on concurrent *ex vitro* rooting and hardening (CEVRH) of two chrysanthemum cultivars.

MATERIAL AND METHODS

The research work was carried out at Horticultural Research Station, Kovvur supported by, Dr. YSRHU, College of Horticulture, Venkataramannagudem, West

Godavari district, Andhra Pradesh. The design of the experiment is factorial completely randomized design with 3 factors. The first factor is cultivars which has 2 levels, C_1: New Man (White) and C_2: Urban Red (Red). The second factor is auxins which have 3 levels namely, A_0: Distilled water, A_1: IBA 100 ppm @ 5 min. and A_2: NAA 100 ppm @ 5 min. The third factor is bioagents which has 3 levels namely, B_1: Arbuscular mycorrhizal fungi (AMF) 2 g/micro-shoot, B_2: Phosphate-solubilizing bacteria (PSB) 2 g/ micro-shoot and B_3: *Pseudomonas fluorescens* 2 ml/ micro-shoot. It has 18 treatment combinations with 2 replications.

For the present experiment, ray florets were used as explants for *in vitro* culture establishment. Multiple shoots were produced by using Murashige and Skoog (1962) basal media supplemented with standardized growth regulator combinations. After 8 cycles of shoot proliferation, *in vitro* grown micro-shoots of chrysanthemum were collected and used as experimental material in the present experiment. The present study was performed using, plant growth hormones (IBA and NAA) which were procured from Hi-Media Laboratories Pvt. Ltd., India. The bio-agents used in present study were Arbuscular mycorrhizal fungi (AMF), Phosphate solubilising bacteria (PSB), *Pseudomonas fluorescens* which were procured from ANGRAU-Amaravati.

After employing the treatment with auxin and bioagent, the micro-shoots were planted in disposable paper cups filled with a mixture of sterilized sand and coco peat (1:1) under polythene sheet covered tunnel presented in 50% shade net house for maintaining optimum humidity. Fifty micro-shoots were tested for each replication. Daily watering with 19:19:19 (0.5 g/l) was applied through spray application. After 20 days after treatment, survived plant lets were transferred to shade net house conditions with natural ventilation and all the recommended cultural practices were followed with weekly spraying of liquid fertilizers (N: P: K = 19:19:19 and 13:0:45). Data on percent survival root and shoot characters were recorded 30 days after treatment.

Factorial completely randomized design (FCRD) was followed and the data were analysed using analysis of variance (ANOVA) with OPSTAT statistical package. Treatment means were compared using SE, 95% confidence intervals. Significant differences between means were assessed by Least significant difference (LSD) at P = 0.05 for FCRD.

RESULTS AND DISCUSSION

Per cent survival (%). The success of CEVRH is highly depends on survival of the micro-shoots to the given auxin and bioagent treatment combination. It is clearly evident from data presented in Table 1 that, significant differences were observed among the cultivars (C), auxins (A), bioagents (B), interaction between cultivars and auxins (C × A), cultivars and bioagents (C × B), auxins and bioagents (A × B), and interaction between cultivars, auxins and bioagents (C × A × B). Among the two cultivars tested, Urban Red (C_2) was found to exhibit highest survival percentage (36.28 %) as compared to New Man (C_1) (27.94 %). Among auxins (A), IBA (A_1) was found significantly superior (40.58 %) over NAA (A_2) (30.08 %). The lowest survival percentage (25.67 %) was noticed in distilled water (A_0). Among bioagents (B), AMF (B_1) was found to be statistically significant with respect to per cent survival (41.25 %) whereas the lowest per was noticed in PSB (B_2) (25.08 %).

Among the interactions between cultivars and auxins (C × A), *in vitro* micro-shoots of Urban Red treated with IBA (C_2A_1) recorded the highest per cent survival (43.50 %) followed by New Man + IBA (C_1A_1) (37.67 %). The lowest per cent survival (20 %) was observed in New Man + distilled water (C_1A_0). Interactions between cultivars and bioagents (C × B) showed that highest survival percentage (49.17 %) was observed in Urban Red + AMF (C_2B_1) followed by New Man + AMF (C_1B_1) (33.33 %). Among the interactions between auxins and bioagents (A × B), IBA + AMF (A_1B_1) recorded the highest survival percentage (49.50 %) followed by IBA + *P. fluorescens* (A_1B_3) (41.50 %). However, the lowest survival percentage (19.25 %) was observed in distilled water + *P. fluorescens* (A_0B_3).

Among the three way interactions (C × A × B), Urban Red + IBA + AMF ($C_2A_1B_1$) recorded highest survival percentage (56.50 %) whereas the lowest survival percentage (15.50 %) was recorded in New Man + distilled water + PSB ($C_1A_0B_2$).

AMF strains can be used as bio hardening agent for micro-propagated chrysanthemum plants by enhancing survival rate and reducing field mortality (Singh *et al*., 2008). This report was in close conformity with the result in the present study. IBA is regarded as best rooting hormone and it increases the survival per cent in plants by initiating the roots. Similar findings were reported by Ranpise *et al*. (2004) in chrysanthemum, Bharmal *et al*. (2005) in chrysanthemum, Hirapara *et al*. (2007) in jasmine and Parmar *et al*. (2010) in bougainvillea.

Shoot height (cm). Among the two cultivars tested, New Man (C_1) was found to exhibit highest shoot height (5.62 cm) as compared to Urban Red (C_2) (4.90 cm) (Table 2). Among auxins (A), NAA (A_2) was found to be significantly superior with respect to shoot height (5.60 cm) followed by IBA (A_1) (5.39 cm). The lowest shoot height was noticed in distilled water (A_0) (4.78 cm). Among bioagents (B), AMF (B_1) was found significantly superior with respect to shoot height (5.80 cm) whereas the lowest shoot height was noticed in *P. fluorescens* (B_3) (4.94 cm).

Among the three way interactions (C × A × B), New Man micro-shoots treated with IBA and AMF ($C_1A_1B_1$) recorded highest shoot height (6.50 cm) which was statistically at par with NAA and AMF in same genotype ($C_1A_2B_1$) (6.35 cm). The lowest shoot height (3.85) was recorded in Urban red + distilled water + PSB ($C_2A_0B_2$).

AMF helps in converting the unavailable form of phosphorus (P) into available form in soil condition hence better nutrient uptake might have taken by plants which in turn stimulate the vegetative growth and yield attributing traits. The mycorrhizal association improves the plant root biomass which in turn increases the absorption capacity of the crops, enables them to utilize 'P' fertilizer more efficiently and achieve optimum

growth even at curtailed doses of P fertilizer.Our results are in tantamount to Kumar *et al*. (2015) in chrysanthemum with respect to enhanced macro and micro nutrient uptake of tissue cultured chrysanthemum plants bio hardened with AMF.

Shoot thickness (mm). As evident from the Fig. 1 with respect to shoot thickness, Urban Red (C_2) was found to exhibit highest shoot thickness (1.68 mm) as compared to New Man (C_1) (1.65 mm). Among auxins (A), NAA (A_2) was found significantly superior with respect to shoot thickness (1.87 mm) followed by IBA (A_1) (1.79 mm). The lowest shoot thickness (1.34 mm) was noticed in distilled water (A_0). Among bioagents (B), AMF (B_1) was found to be statistically superior with respect to shoot thickness (1.86 mm) whereas the lowest was noticed in PSB (B_2) (1.48 mm).

Association of tissue culture plants with AMF facilitates the plants to grow more vigorously by mediating a series of complex communication events between each other leading to increased photosynthetic rate and other gas exchange-related traits. The enhanced water uptake and photosynthesis might have assisted for developing strong and thick stems in the present study.

Leaf area (cm^2). As evident from the Table 3, the differences were statistically significant with respect to leaf area among the treatments. Among the two cultivars tested, Urban Red (C_2) was found to exhibit highest leaf area (3.56 cm^2) as compared to New Man (C_1) (3.06 cm^2).

Among auxins (A), NAA (A_2) was found significantly superior with respect to leaf area (3.73 cm^2) followed by IBA (A_1) (3.53 cm^2). The lowest leaf area (2.67 cm^2) was noticed in distilled water (A_0). Among bioagents (B), AMF (B_1) was found to be statistically superior with respect to leaf area (3.57 cm^2) whereas the lowest was noticed in PSB (B_2) (3.04 cm^2). Among the three way interactions (C × A × B), Urban Red + IBA + AMF ($C_2A_1B_1$) recorded highest leaf area (4.30 cm^2) whereas the lowest (2.35 cm^2) was recorded in New Man + distilled water + PSB ($C_1A_0B_2$).

Suitable type and concentration of auxin (IBA) along with the mycorrhizal association in tissue culture plants might have helped for accelerating the concurrent *ex vitro* rooting and hardening (CEVRH) in the present study. Improvement in leaf area may be attributed to optimum level of moisture, nutrient availability to plant, increased photosynthetic rate of plants inoculated with AMF. AMF facilitates uptake of P and micro elements present in soil. These findings are in line with Kumar *et al*. (2014) in chrysanthemum.

Number of roots per shoot. Among the two cultivars tested, Urban Red (C_2) was found to exhibit highest number of roots per shoot (13.84) as compared to New Man (C_1) (12.56). Among auxins (A), IBA (A_1) was found significantly superior (15.67) over NAA (A_2) (14.67). The lowest number of roots per shoot (9.25) was noticed in distilled water (A_0). Among bioagents (B), AMF (B_1) was found to be statistically superior with respect to number of roots per shoot (15.09) whereas the lowest was noticed in PSB (B_2) (11.09) (Table 4).

Among the interactions between cultivars and auxins (C × A), *in vitro* micro-shoots of Urban Red treated with IBA (C_2A_1) recorded the highest number of roots per shoot (17.17) followed by Urban Red + NAA (C_2A_2) (15.84). However, the lowest number of roots per shoot (8.51) was observed in Urban Red + distilled water (C_2A_0). Among the interactions between auxins and bioagents (A × B), IBA + AMF (A_1B_1) recorded the highest number of roots per shoot (17.51) followed by NAA + AMF (A_2B_1) (16.75). However, the lowest (7.51) was observed in distilled water + PSB (A_0B_2).

Among the three way interactions (C × A × B), Urban Red + IBA + AMF ($C_2A_1B_1$) recorded highest number of roots per shoot (18.51) whereas the lowest (6.51) was recorded in Urban Red + distilled water + PSB ($C_1A_0B_2$).

Grewal *et al.* (2005) found that chrysanthemum cuttings treated with IBA @ 400 ppm performed well after transplanting thus resulting in improved root growth and development. This might be due to the fact that auxin group of hormones (IBA and NAA) facilitated the process of adventitious root formation and also control growth and development of roots including lateral root initiation and root gravity response that depends upon auxin transport. Similar findings were reported by Sharma *et al.* (2014) in marigold and Renuka and Sekhar (2014) in carnation. Further, association of endophytic mycorrhizal fungi with the plant roots facilitates the development of stronger root system (Azcon Aguilar and Barea 1996, Kumar *et al*., 2014), improved growth (Zandavalli *et al*. 2004), enhancing nutrient and water uptake (Kim and Kim 1998), increased tolerance of host roots to soil borne pathogens (Nelson and Achar 2001) and drought stress (Ruiz Lozano and Azcon 1995), thereby enhancing plant growth and survival after field transplant.

Length of the longest root (cm). The data pertaining to length of the longest root revealed significant differences (Fig. 2). Among the two cultivars tested, New Man (C_1) was found to exhibit highest length of the longest root (4.39 cm) as compared to Urban Red (C_2) (3.52 cm). Among auxins (A), IBA (A_1) was found significantly superior with respect to length of the longest root (4.36 cm) followed by NAA (A_2) (4.14 cm). The lowest (3.37 cm) was noticed in distilled water (A_0). Among bioagents (B), AMF (B_1) was found to be statistically superior with respect to length of the longest root (4.29 cm) whereas the lowest was noticed in PSB (B_2) (3.64 cm).

The increase in root length after application of IBA and NAA has been reported by Janakiram *et al.* (2006). The increase in length of the roots might be due to enhanced hydrolysis of carbohydrates, accumulation of metabolites at the site of application of auxins, synthesis of new proteins, cell enlargement and cell division induced by auxins (Strydem and Hartman 1960). These results were in close conformity with the research findings of Parmar *et al*. (2010) in bougainvillea, Ullah *et al*. (2013) in marigold, Renuka and Sekhar (2014) in carnation.

Table 1: Effect of auxins and bioagents on survival (%) of CEVRH chrysanthemum micro-propagated plants.

Cultivars (C)				
Auxins (A)	**Bioagents (B)**	New Man (C_1)	Urban Red (C_2)	**Mean**
Distilled water (A_0)	AMF (B_1)	25.50 (30.29)*	44.50 (41.83)	**35.00 (36.06)**
	PSB (B_2)	15.50 (23.11)	30.00 (33.19)	**22.75 (28.15)**
	P. fluorescens (B_3)	19.00 (25.82)	19.50 (26.15)	**19.25 (25.99)**
Mean		***20.00 (26.41)***	**31.33 (33.72)**	**25.67 (30.07)**
IBA@ 100 ppm (A_1)	AMF (B_1)	42.50 (40.67)	56.50 (48.72)	**49.50 (44.69)**
	PSB (B_2)	29.50 (32.88)	32.00 (34.43)	**30.75 (33.66)**
	P. fluorescens (B_3)	41.00 (39.80)	42.00 (40.38)	**41.50 (40.09)**
Mean		***37.67 (37.78)***	**43.50 (41.18)**	**40.58 (39.48)**
NAA@ 100 ppm (A_2)	AMF (B_1)	32.00 (34.44)	46.50 (42.98)	**39.25 (38.71)**
	PSB (B_2)	22.00 (27.96)	21.50 (27.61)	**21.75 (27.78)**
	P. fluorescens (B_3)	24.50 (29.65)	34.00 (35.65)	**29.25 (32.65)**
Mean		***26.17 (30.68)***	**34.00 (35.41)**	**30.08 (33.05)**
For comparing means of Cultivars (C) × Bioagents (B)				
Bioagents (B)	AMF (B_1)	***33.33 (35.13)***	**49.17 (44.51)**	**41.25 (39.82)**
	PSB (B_2)	***22.33 (27.98)***	**27.83 (31.74)**	**25.08 (29.86)**
	P. fluorescens (B_3)	***28.17 (31.76)***	**31.83 (34.06)**	**30.00 (32.91)**
Mean		**27.94 (31.62)**	**36.28 (36.77)**	**32.11 (34.20)**

Factors	**SE(m)**	**CD at 5%**
Cultivars (C)	0.34	1.01
Auxins (A)	0.42	1.24
Bioagents (B)	0.42	1.24
Cultivars × Auxins	0.59	1.75
Cultivars × Bioagents	0.59	1.75
Auxinsx Bioagents	0.72	2.14
Cultivars × Auxins × Bioagents	1.02	3.03

*Figures in parenthesis are angular transformed values

Table 2: Effect of auxins and bioagents on shoot height (cm) of CEVRH chrysanthemum micro- propagated plants.

Cultivars (C)				
Auxins (A)	**Bioagents (B)**	**New Man (C_1)**	**Urban Red (C_2)**	**Mean**
Distilled water (A_0)	AMF (B_1)	5.54	4.90	**5.22**
	PSB (B_2)	5.30	3.85	**4.58**
	P. fluorescens (B_3)	5.15	3.95	**4.55**
Mean		***5.33***	***4.23***	**4.78**
IBA @ 100 ppm (A_1)	AMF (B_1)	6.50	5.80	**6.15**
	PSB (B_2)	5.45	4.25	**4.85**
	P. fluorescens (B_3)	5.20	5.15	**5.18**
Mean		***5.72***	***5.07***	**5.39**
NAA @ 100 ppm (A_2)	AMF (B_1)	6.35	5.67	**6.01**
	PSB (B_2)	6.15	5.20	**5.68**
	P. fluorescens (B_3)	4.90	5.30	**5.10**
Mean		***5.80***	***5.39***	**5.60**
For comparing means of Cultivars (C) × Bioagents (B)				
Bioagents (B)	AMF (B_1)	***6.13***	***5.46***	**5.80**
	PSB (B_2)	***5.63***	***4.43***	**5.03**
	P. fluorescens (B_3)	***5.08***	***4.80***	**4.94**
Mean		**5.62**	**4.90**	**5.26**

Factors	**SE(m)**	**CD at 5%**
Cultivars (C)	0.04	0.12
Auxins (A)	0.05	0.15
Bioagents (B)	0.05	0.15
Cultivars × Auxins	0.07	0.21
Cultivars × Bioagents	0.07	0.21
Auxins× Bioagents	0.09	0.26
Cultivars × Auxins × Bioagents	0.12	0.37

Table 3: Effect of auxins and bioagents on leaf area (cm^2) of CEVRH chrysanthemum micro- propagated plants.

Cultivars (C)				
Auxins (A)	**Bioagents (B)**	**New Man (C_1)**	**Urban Red (C_2)**	**Mean**
Distilled water (A_0)	AMF (B_1)	2.60	3.00	**2.80**
	PSB (B_2)	2.35	2.65	**2.50**
	P. fluorescens (B_3)	2.50	2.90	**2.70**
Mean		***2.48***	***2.85***	**2.67**
IBA@ 100 ppm (A_1)	AMF (B_1)	3.85	4.30	**4.08**
	PSB (B_2)	2.90	3.20	**3.05**
	P. fluorescens (B_3)	3.10	3.85	**3.48**
Mean		***3.28***	***3.78***	**3.53**
NAA@ 100 ppm (A_2)	AMF (B_1)	3.50	4.18	**3.84**
	PSB (B_2)	3.26	3.90	**3.58**
	P. fluorescens (B_3)	3.48	4.05	**3.77**
Mean		***3.41***	***4.04***	**3.73**
For comparing means of Cultivars (C) × Bioagents (B)				
Bioagents (B)	AMF (B_1)	**3.32**	**3.83**	**3.57**
	PSB (B_2)	**2.84**	**3.25**	**3.04**
	P. fluorescens (B_3)	**3.03**	**3.60**	**3.31**
Mean		**3.06**	**3.56**	**3.31**

Factors	**SE(m)**	**CD at 5%**
Cultivars (C)	0.02	0.05
Auxins (A)	0.02	0.06
Bioagents (B)	0.02	0.06
Cultivars ×Auxins	0.03	0.09
Cultivars × Bioagents	0.03	0.09
Auxins ×Bioagents	0.04	0.11
Cultivars × Auxins × Bioagents	0.05	0.15

Table 4: Effect of auxins and bioagents on induction of number of roots per shoot in CEVRH chrysanthemum micro-propagated plants.

Cultivars (C)				
Auxins (A)	**Bioagents (B)**	**New Man (C_1)**	**Urban Red (C_2)**	**Mean**
Distilled water (A_0)	AMF (B_1)	11.51	10.51	***11.01***
	PSB (B_2)	8.51	6.51	***7.51***
	P. fluorescens (B_3)	10.00	8.51	***9.25***
Mean		***10.00***	***8.51***	**9.25**
IBA@ 100 ppm (A_1)	AMF (B_1)	16.51	18.51	***17.51***
	PSB (B_2)	12.51	15.51	***14.01***
	P. fluorescens (B_3)	13.51	17.51	***15.51***
Mean		***14.17***	***17.17***	**15.67**
NAA@ 100 ppm (A_2)	AMF (B_1)	15.51	18.00	***16.75***
	PSB (B_2)	10.51	13.00	***11.75***
	P. fluorescens (B_3)	14.51	16.51	***15.51***
Mean		***13.51***	***15.84***	**14.67**
For comparing means of Cultivars (C) × Bioagents (B)				
Bioagents (B)	AMF (B_1)	***14.51***	***15.67***	**15.09**
	PSB (B_2)	***10.51***	***11.67***	**11.09**
	P. fluorescens (B_3)	***12.67***	***14.17***	**13.42**
Mean		**12.56**	**13.84**	**13.20**

Factors	**SE(m)**	**CD at 5%**
Cultivars (C)	0.05	0.14
Auxins (A)	0.06	0.17
Bioagents (B)	0.06	0.17
Cultivars × Auxins	0.08	0.23
Cultivars × Bioagents	-	NS
Auxinsx Bioagents	0.10	0.29
Cultivars × Auxins × Bioagents	0.14	0.41

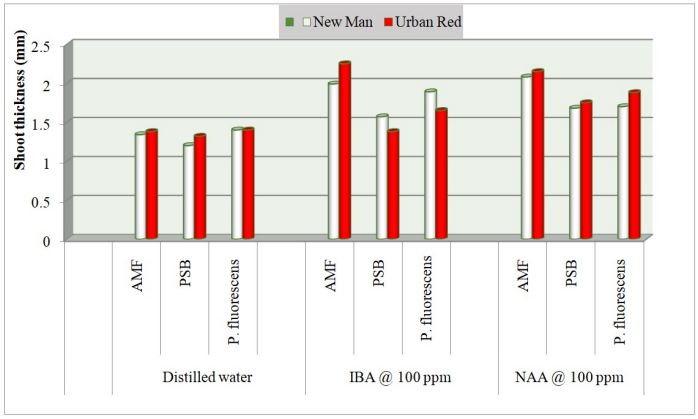

Fig. 1. Effect of auxins and bioagents on shoot thickness (mm) in CEVRH chrysanthemum micro-propagated plants.

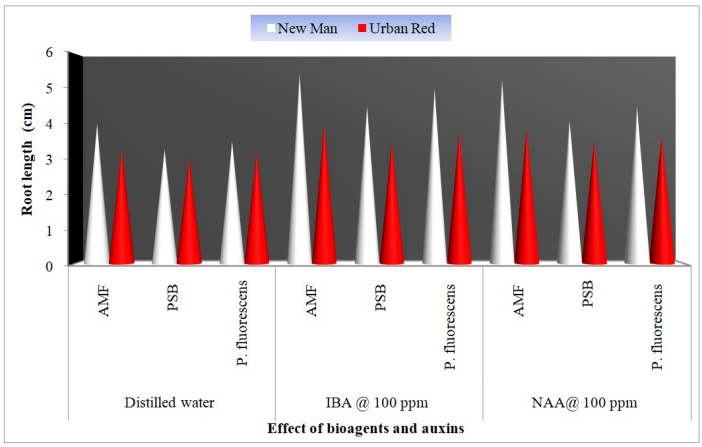

Fig. 2. Effect of auxins and bioagents on length of root (cm) in CEVRH chrysanthemum micro-propagated plants.

CONCLUSION

Among different treatment combinations evaluated, *in vitro* originated Urban Red micro-shoots treated with IBA 100 ppm and Arbuscular mycorrhizal fungi (2 g/shoot) recorded highest per cent survival, shoot thickness, number of leaves, leaf area, number of roots per shoot and root thickness. However, maximum shoot height and lengthiest roots were found in New Man cultivar treated with IBA 100 ppm and Arbuscular mycorrhizal fungi. It can be concluded from the present study that, micro-shoots treated with IBA and biohardened with AMF is proved to be highly suitable for CEVRH of chrysanthemum.

FUTURE SCOPE

1. Different auxins, their concentrations and combinations may be further tested during different seasons in a year to increase rooting and survival of micro-shoots in concurrent *ex vitro* rooting and hardening (CEVRH).
2. Other genotypes of chrysanthemum may also be tested for concurrent *ex vitro* rooting and hardening (CEVRH) to reduce the production cost of planting material.

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Conflict of Interest. None.

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