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In vitro Propagation of Cut Flower Variety *Muscari armeniacum* Leichtl. ex Bak. Through Direct Bulblet Proliferation Pathways

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Abstract

Muscari armeniacum is one of the important ornamental cut flower in floriculture industry which native to Southern Europe, North Africa, Western Asia and Asia Minor. In this study, bulb explants (basal plate of bulb having meristem), bulb scales and leaf segments from in vitro derived bulblets were culture in Murashige and Skoog (MS) medium with different plant growth regulator concentrations and combinations to assess growth regulators effect on different bulblet organogenesis pathways in vitro. The results demonstrated that cytokinin in combination with auxin is required for both axillary and adventitious bulblet regeneration. Benzyladenine (BA) - α-naphthalene acetic acid (NAA) combination, showed significant effects compared to other growth regulator combinations tested. 4.0 µM BA + 2.0 µM NAA was the found to the best for axillary bulblet formation from bulb explants. Likewise, bulb and leaf segments showed the best response in adventitious bulblet organogenesis when they were cultured in BA-NAA combinations. Out of several concentrations of BA with NAA, 4.0 µM BA + 1.0 µM NAA was optimum for adventitious bulblet regeneration. Bulblets, properly isolated from both axillary and adventitious proliferation systems, showed maximum percentage of rooting on half strength MS medium containing 2.0 µM Indole-3-butyric acid (IBA). However, the higher concentration of all the auxins showed either callus formation at the base of shoots or malformation of roots. All the *in vitro* regenerated plantlets were successfully acclimatized under *ex vitro* environment in the garden soil with 60% survival rate.

Keywords: Direct bulblet proliferation, Micropropagation, Muscari armeniacum, Ornamental plant.

INTRODUCTION

Agro-climatic conditions of Bangladesh are found to be suitable for flower cultivation, and thus floriculture industry has been established countrywide. Currently, some common flowers used in floriculture sector are rose, tuberose, gladiolus, gerbera, marigold and beli, and these are sold countrywide as a cut flower in both retail market and wholesale market. Due to the high recent demand of cut flowers, commercial floriculture sector is gradually expanding day by day signifying the importance of introducing new flower varieties. Hence, we focused our interest in studying with some foreign ornamental flower varieties having high aesthetic values.

Muscari armeniacum, a member of the family Hyacinthaceae, is generally known as grape hyacinths, and characterized with cobalt blue flowers of a cup-shaped crown with a white rim (Wang *et al*., 2013; Yucesan *et al*., 2014). It is one of the important ornamental plants grown both for its aesthetic and commercial values worldwide. It has a good potential market value as cut flowers and potted plants. Traditional propagation methods of *Muscari* species are rather slow, since the bulblet production from the mother bulbs is extremely low (Uzun *et al*., 2014). Herein, we attempted tissue culture techniques with *M. armeniacum*, which offer a possible alternative for rapid multiplication, conservation (Jevremovic *et al*., 2009) and commercial propagation (Ozel *et al.,* 2015). The objective of this study was therefore to develop *in vitro* propagation techniques for producing large number of *M. armeniacum* plantlets using different type of explants and employing different regeneration system. This study could be beneficial for producing large number of plantlets of *M. armeniacum* within a short period of time with high acclimatization potential.

MATERIALS AND METHODS

Plant material and surface sterilization

The bubs were collected from Japan. They were initially washed with savlon (Chlorhexidine gluconate 0.3% w/v + cetrimide 3% w/v solution) containing water for 15 min and then cleaned carefully with running tap water for 20 min. These clean explants were transferred to laminar air flow cabinet and surface sterilized with 0.1% $HgCl_2$ for 10 min and washed with sterile distilled water thoroughly 3 to 4 times and soaked with sterile blotting paper. After successful sterilization, the bulbs were excised (5 - 7 mm in width and 8 - 10 mm in length) to prepare desired explants which were used in *in vitro* cultures for subsequent experiments.

Culture medium and growth conditions

The disinfected explants were implanted on to the Murashige and Skoog's (1962) agar-gelled medium augmented with various concentrations/combinations of growth regulators. The media were prepared with 3% (w/v) sucrose and 0.8% (w/v) agar (Sigma Chemical Co. USA). The pH of the medium was adjusted 5.7 ± 0.1 before autoclaving at 121ºC for 20 minutes at 1.2 kg/cm^2 pressure. All the cultures were maintained at 25 ± 2ºC under a 16h light and 8h dark cycle with the light intensity of 2000 - 3000 lux provided by cool-white fluorescent tubes (36W).

Axillary bulblet proliferation

In order to determine the optimal explant source for axillary bulblet proliferation, explants containing meristematic zones were cultured into 125 ml conical flux containing MS medium supplemented with 2.0, 4.0 and 6.0 µM BAP or Kn alone or in combination with 1.0, 2.0 µM NAA or IBA. After 4 weeks of culture, pro-axillary bulblets were isolated and sub cultured at weekly intervals on the same medium for another 4 weeks for the induction of mature bulblets.

Adventitious bulblet proliferation

The explants (bulb-scales and leafs which were separated from axilliary proliferation

systems) was cut into small pieces and subsequently sub-cultured on the MS medium supplemented with 2.0, 4.0 and 6.0 µM BAP or Kn in combination with 1.0, 2.0 µM NAA or IBA for the proliferation of adventitious bulblets.

In vitro rooting

Regenerated bulblets (3.0 - 4.5 cm) were excised from the *in vitro* grown culture and transferred to half strength MS medium supplemented with either IBA or NAA in concentration of 0.5, 1.0, 2.0, 3.0 and 4.0 µM. The cultures were maintained under a 16h photoperiod at 25 ºC (±2 ºC) and observed after six weeks of incubation. Observation was recorded after six weeks of incubation.

Hardening and acclimatization

In vitro regenerated plantlets were taken out from the culture tubes and washed thoroughly under running tap water. Small plastic pots were kept ready filled with garden soil and compost (1:1). Taking special care not to damage the roots, plantlets were then planted one in each pot and the potted plantlets were transferred to culture room condition under artificial light and temperature (25 ± 2°C) and 70 - 80% relative humidity. The polythene cover was removed periodically in order to gradual acclimatization of plantlets. After three weeks of indoor acclimatization potted plantlets were transferred to outdoor laboratory conditions.

Culture observation, data collection, and data analysis

Five cultures were used per treatment and each experiment was repeated thrice times. The data on axillary/adventitious bulblets proliferation were recorded after 8 weeks of culture whereas the data on adventitious rooting were recorded after 6 weeks of culture. The effect of different treatments was determined with respect to number and length of bulblets and roots. Data were statistically analyzed by the Duncan's multiple Range Test at the $P < 0.05$ level of significance using a statistical software- SPSS.

RESULTS

Axillary bulblet proliferation

In the present investigation, attempts were taken to produce large number of bulblets through axillary proliferation from the bulb explants containing pre-formed meristem. Explants were cultured on MS medium supplemented with different concentrations and combinations of cytokinin (BA and Kn) and auxin (NAA and IBA). Concentrations of cytokinins were usually 2.0, 4.0, 6.0 µM, whereas auxins were used in 1.0, 2.0 µM. Cultured explants were started to form bulblets at axil of the bulbs after 1 weeks of incubation (Fig. 1 A), and the proliferated bulblets attained maturity within 8 weeks (Fig 1B.). Callus was seen on the basal portion of the explants when the cytokinin and auxin were used in approximately equal concentration, and hence the numbers of bulblets were found to be reduced.

When the explants were cultured on 4.0 µM BA + 2.0 µM NAA containing medium, 5.6 ± 0.40 bulblets with the maximum height (5.2 ± 0.13 cm) were observed per culture (Table 1). No callus was formed in this growth regulator combination. During this experiment, bulb explants were primarily cultured on only BA or Kn containing medium, but no significant shoot proliferation was observed. In addition, NAA was found to be the best compared to IBA for axillary proliferation in combination with cytokinin. On the other hand, BA exhibited better proliferation than Kn. These findings clearly indicated the synergistic effect of cytokinin-auxin growth regulators on bulblet proliferation in *M. armeniacum*.

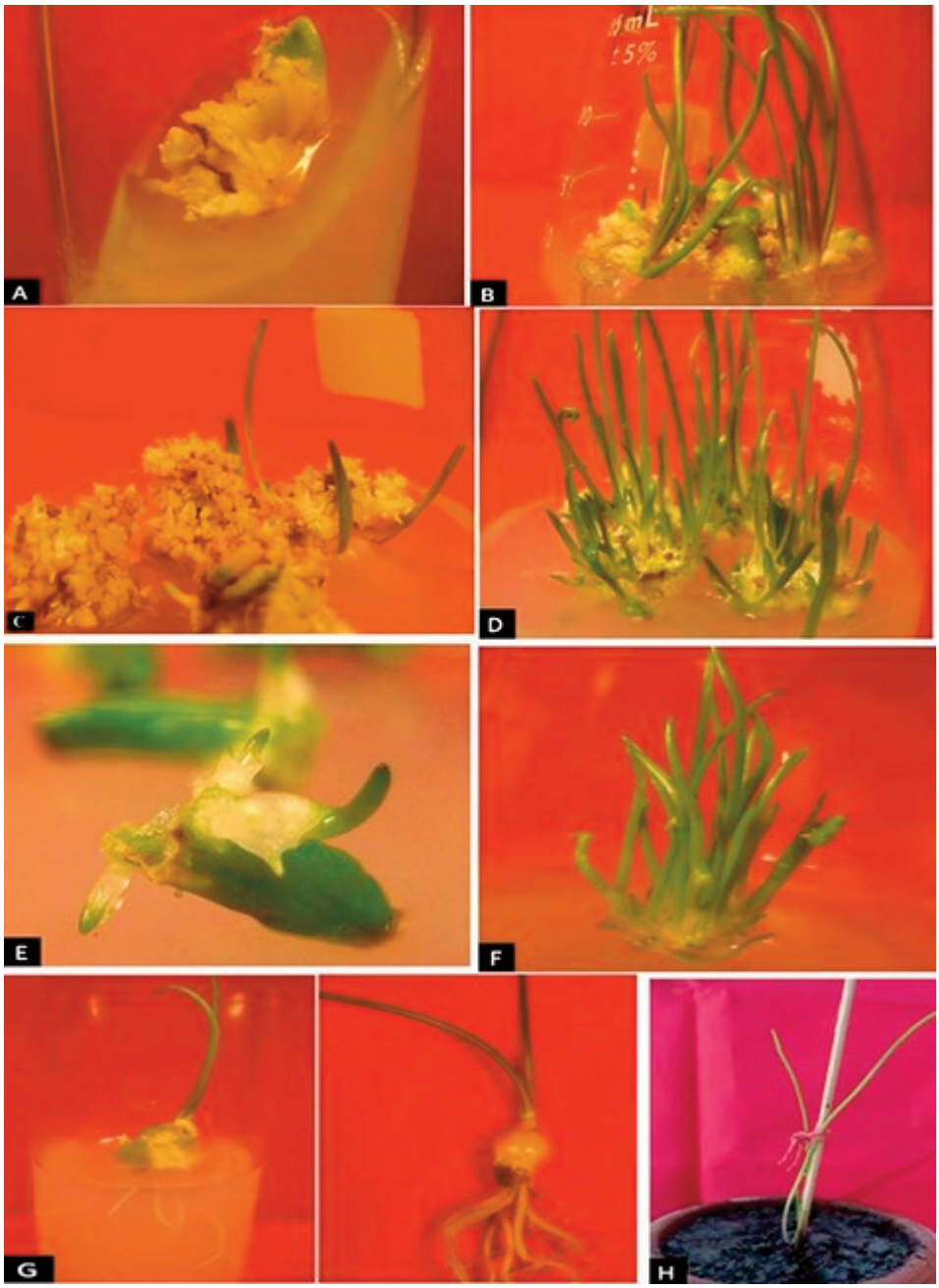

Fig. 1. *In vitro* bulblet regeneration of *M. armeniacum* from different types of explants. (A-B) Axillary bulblets developed from bulb explant after 1 week and after 8 weeks of culture on MS + 4.0 µM BA + 2.0 µM NAA; (C-D) *In vitro* adventitious bulblets from bulb explant after 2 weeks and after 8 weeks of culture on MS + 4.0 µM BA + 1.0 µM NAA; (E-F) Adventitious bulblets from leaf explant after 2 weeks and after 8 weeks of culture on MS + 4.0 µM BA + 1.0 µM NAA. (G) Rooted plantlets ready for transplantation; (H) Acclimatized plantlets after 7 days of transplantation onto soil mix.

Table 1. Effects of plant growth regulators on axillary bulblets proliferation from bulb explants of *M. armeniacum*.

Plant growth regulators(µM)				Number of bulblets(mean ± SE)	Length of bulblets (cm)(mean ± SE)	Callus response
BA		Kn				
0		0		0.4 ± 0.24^{k}	0.5 ± 0.33^{j}	No callus
2		-		0.8 ± 0.20^{k}	1.4 ± 0.38^{hkij}	No callus
4		-		1.4 ± 0.24^{gh}	2.3 ± 0.16^{efgh}	No callus
6		-		0.9 ± 0.44^{k}	1.1 ± 0.47^{ij}	Light yellow nodular callus
-		2		0.4 ± 0.24^{k}	1.2 ± 0.84^{kij}	No callus
-		4		0.8 ± 0.20^{k}	1.7 ± 0.45^{ghki}	No callus
-		6		0.6 ± 0.24^{k}	1.1 ± 0.44^{kij}	Light yellow nodular callus
BA	Kn	NAA	IBA			
2.0	-	1.0	-	1.2 ± 0.44^{hk}	2.5 ± 0.11^{efgh}	No callus
2.0	-	2.0	-	0.6 ± 0.24^{k}	1.0 ± 0.43^{ij}	Light yellow nodular callus
4.0	-	1.0	-	4.9 ± 0.10^{ab}	4.5 ± 0.13^{abc}	No callus
4.0	-	2.0	-	5.6 ± 0.40^{a}	5.2 ± 0.13^{a}	No callus
6.0	-	1.0	-	4.2 ± 0.37^{bcd}	3.7 ± 0.15^{cd}	Light yellow nodular callus
6.0	-	2.0	-	2.5 ± 0.22^{f}	3.1 ± 0.07^{def}	Light yellow nodular callus
2.0	-	-	1.0	1.4 ± 0.24^{gh}	2.1 ± 0.20^{fghk}	No callus
2.0	-	-	2.0	0.5 ± 0.24^{k}	1.1 ± 0.51^{kij}	Yellow nodular callus
4.0	-	-	1.0	3.4 ± 0.24^{de}	3.2 ± 0.17^{de}	No callus
4.0	-	-	2.0	4.5 ± 0.22^{bcd}	3.8 ± 0.08^{cd}	No callus
6.0	-	-	1.0	4.1 ± 0.10^{bcb}	2.4 ± 0.12^{efgh}	Yellow nodular callus
6.0	-	-	2.0	2.1 ± 0.10^{fg}	2.3 ± 0.08^{efgh}	Brown compact callus
-	2.0	1.0	-	1.2 ± 0.20^{hk}	2.4 ± 0.13^{efgh}	No callus
-	2.0	2.0	-	0.4 ± 0.24^{k}	0.6 ± 0.44^{j}	Light yellow nodular callus
-	4.0	1.0	-	4.2 ± 0.37^{bcd}	4.2 ± 0.1^{bc}	No callus
-	4.0	2.0	-	4.5 ± 0.22^{bc}	4.9 ± 0.08^{ab}	Light yellow nodular callus
	6.0	1.0	-	3.8 ± 0.20^{cd}	3.6 ± 0.14^{cd}	Light yellow nodular callus
-	6.0	2.0	-	2.1 ± 0.33^{fg}	2.9 ± 0.03^{def}	Light brown compact callus
-	2.0	-	1.0	1.1 ± 0.10^{hi}	1.6 ± 0.18^{ghki}	No callus
-	2.0	-	2.0	0.6 ± 0.24^{i}	1.0 ± 0.41^{ij}	Light brown compact callus
-	4.0	-	1.0	2.8 ± 0.37^{ef}	2.9 ± 0.27^{def}	No callus
-	4.0	-	2.0	4.2 ± 0.37^{bcd}	3.6 ± 0.08^{cd}	No callus
-	6.0	-	1.0	3.8 ± 0.37^{cd}	2.3 ± 0.17^{efgh}	Light yellow nodular callus
	6.0	-	2.0	2.4 ± 0.24^{f}	2.5 ± 0.08^{efg}	Light brown compact callus

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the Duncan's test.

Direct adventitious bulblet induction and proliferation

Two different explants- bulb scale and *in vitro* derived leaf sheath segments, which don't contain any pre-formed meristems, were used in this experiment with the same cytokinin-auxin combinations used in previous experiment to explore their potentiality for direct adventitious bulblet organogenesis without intermediate callus formation.

Effect of auxins and cytokinins on direct adventitious bulblet proliferation from the bulb-scale explants

In this experiment bulb-scale explants were cultured on MS medium supplemented with cytokinin and auxin with different concentrations. Adventitious bulblets were formed in most of

the treatment within 2 weeks of culture (Fig. 1 C), however, significant difference was observed in the number and length of bulblets. In MS medium containing 4.0 µM BA + 1.0 µM NAA the cultured explants showed best results for all the parameters evaluated. In this medium formulation, bulb-scale explants produced the highest number of bulblets (8.4 ± 0.24) as well as the longest bulblets (5.2 ± 0.12 cm) after 8 weeks of culture (Fig. 1D). The media containing BA always showed better performance enhancing the proliferation than that of containing Kn, whereas NAA was observed as a potent auxin compared to IBA (Table 2). During this study, 4.0 µM Kn + 1.0 µM NAA exhibited the second highest performance regarding direct bulblets induction in bulb-scale explants when it produces 6.8 ± 0.37 bulblets with 4.5 ± 0.14 cm length. A higher concentration of BA or Kn with NAA or IBA did not result in any improved effect on adventitious bulblets induction. It was remarkable to note that there was no response on the adventitious bulblets initiation on the medium supplemented with BA or Kn, even at significantly higher concentrations of both.

Effect of auxins and cytokinins on direct adventitious bulblet proliferation from the leaf explants

Leaf explants were taken from the *in vitro* proliferated shoots and they were cultured on MS medium supplemented with different cytokinin-auxin combinations for direct adventitious shoot organogenesis. Direct adventitious shoot induction was occurred in different extent in all the treatments (Table 2). The number of adventitious bulblets per explant was recorded after 8 weeks. During this experiment, no calluses were formed in MS medium augmented with 4 µM BA and 1µM NAA. The highest number of bulblets/explant (10.8 ± 0.58) and the highest mean length (4.2 ± 0.14) of bulblets/explant were observed in the medium containing 4 µM BA and 1µM NAA (Fig. 1 E & F) followed by 4 µM BA and 2µM NAA containing medium. When BA was increased gradually, explants produced fewer bulblets with fewer lengths. Like axillary proliferation, BA was also found to be a potent cytokinin for direct bulblets induction, however BA showed the best performance when it was combined with NAA.

Rooting of *in vitro* proliferated bullblets

Bulblets regenerated from bulb-scale and leaf explants need roots to acclimatize and establish them in the *ex vitro* condition. Investigations were also carried out on adventitious rooting, and transplantation of rooted bulblets on to the soil and acclimatize them successfully under the field condition. In this experiment, no rooting was observed from the base of the bulblets before 7 days of culture. Rooting frequency increased gradually with the incubation period, and it was reached to 100 % after 15 days of culture on rooting medium. Percentages of root induction, number of root per shoots were greatly controlled by the concentrations and type of the auxin. IBA was found to be more effective in root induction of *M. armeniacum* bulblets. The maximum percentage of culture that regenerated roots was 100 % when the bulblets were cultured on ½ MS media having 2.0 µM IBA (Fig. 1 G). Media containing NAA was not good as that contained IBA. Only 40-80 % of rooting was observed on the media supplemented with 0.5-4.0 µM NAA. The highest numbers and maximum length of the longest roots per bulblets 4.6 ± 0.68 and 4.2 ± 0.38 cm, respectively on the ½ MS medium containing 2.0 µM IBA (Fig. 2).

Hardening and acclimatization

Plantlets obtained from different rooting media, which had well developed root system i.e. no basal callusing and no malformation of roots (Fig. 1 G) were used for this experiment. The plantlets washed with tap water were transferred on to the small plastic pots containing sterilized soil mix (garden soil and compost in 1:1 ratio). Transferred plantlets were maintained 25-30 days

Table 2. Effects of plant growth regulators on adventitious bulblets proliferation from bulb and leaf explants of *M. armeniacum*.

Types of explants	Plant growth regulators(µM)				Number of bulblets(mean ± SE)	Length of bulblets (cm)(mean ± SE)
	BA	Kn	NAA	IBA		
Bulb	2.0	-	1.0	-	1.6 ± 0.24^{ij}	3.1 ± 0.17^{h}
	4.0	-	1.0	-	8.4 ± 0.24^{a}	5.2 ± 0.12^{a}
	4.0	-	2.0	-	6.7 ± 0.37^{b}	4.8 ± 0.26^{ab}
	6.0	-	1.0	-	4.5 ± 0.22^{cd}	4.6 ± 0.11^{bc}
	6.0	-	2.0	-	3.2 ± 0.20^{fg}	3.1 ± 0.19^{h}
	2.0	-	-	1.0	1.5 ± 0.22^{ij}	2.6 ± 0.12^{ijk}
	4.0	-	-	1.0	6.9 ± 0.33^{b}	4.3 ± 0.15^{cd}
	4.0	-	-	2.0	4.6 ± 0.24^{c}	3.8 ± 0.15^{ef}
	6.0	-	-	1.0	4.2 ± 0.20^{cde}	3.2 ± 0.13^{gh}
	6.0	-	-	2.0	3.7 ± 0.20^{ef}	2.1 ± 0.13^{kl}
	-	2.0	1.0	-	1.4 ± 0.24^{ij}	2.4 ± 0.15^{ijk}
	-	4.0	1.0	-	6.8 ± 0.37^{b}	4.5 ± 0.14^{bcd}
	-	4.0	2.0	-	6.2 ± 0.20^{b}	4.2 ± 0.15^{cde}
	-	6.0	1.0	-	3.2 ± 0.20^{fg}	4.2 ± 0.17^{cde}
	-	6.0	2.0	-	2.6 ± 0.24^{gh}	2.6 ± 0.16^{ij}
	-	2.0	-	1.0	1.2 ± 0.20^{j}	2.3 ± 0.17^{jk}
	-	4.0	-	1.0	6.4 ± 0.24^{b}	4.1 ± 0.13^{de}
	-	4.0	-	2.0	3.9 ± 0.24^{cdef}	3.6 ± 0.14^{fg}
	-	6.0	-	1.0	3.8 ± 0.20^{def}	2.8 ± 0.05^{hi}
	-	6.0	-	2.0	2.1 ± 0.10^{hi}	1.8 ± 0.12^{l}
Leaf	2.0	-	1.0	-	1.2 ± 0.20^{k}	1.7 ± 0.23^{jk}
	4.0	-	1.0	-	10.8 ± 0.58^{a}	5.2 ± 0.11^{a}
	4.0	-	2.0	-	8.7 ± 0.30^{b}	4.1 ± 0.10^{b}
	6.0	-	1.0	-	3.6 ± 0.24^{ef}	3.7 ± 0.17^{bcd}
	6.0	-	2.0	-	1.3 ± 0.30^{k}	2.8 ± 0.16^{fg}
	2.0	-	-	1.0	1.3 ± 0.30^{k}	1.3 ± 0.17^{k}
	4.0	-	-	1.0	8.3 ± 0.43^{b}	3.8 ± 0.19^{bcd}
	4.0	-	-	2.0	6.8 ± 0.20^{c}	3.3 ± 0.10^{def}
	6.0	-	-	1.0	2.2 ± 0.20^{hij}	3.5 ± 0.10^{cde}
	6.0	-	-	2.0	1.8 ± 0.20^{ijk}	2.1 ± 0.15^{ij}
	-	2.0	1.0	-	1.5 ± 0.22^{jk}	2.6 ± 0.16^{gh}
	-	4.0	1.0	-	8.2 ± 0.20^{b}	3.9 ± 17^{bc}
	-	4.0	2.0	-	4.3 ± 0.20^{e}	2.8 ± 0.18^{fg}
	-	6.0	1.0	-	3.4 ± 0.24^{fg}	2.6 ± 0.18^{gh}
	-	6.0	2.0	-	1.6 ± 0.24^{ijk}	2.2 ± 0.13^{hi}
	-	2.0	-	1.0	1.2 ± 0.20^{k}	1.4 ± 0.12^{k}
	-	4.0	-	1.0	5.7 ± 0.30^{d}	3.5 ± 0.15^{cde}
	-	4.0	-	2.0	2.7 ± 0.20^{gh}	3.9 ± 0.12^{bc}
	-	6.0	-	1.0	2.4 ± 0.24^{hi}	3.3 ± 0.23^{def}
	-	6.0	-	2.0	1.6 ± 0.24^{ijk}	3.1 ± 17^{efg}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the Duncan's test.

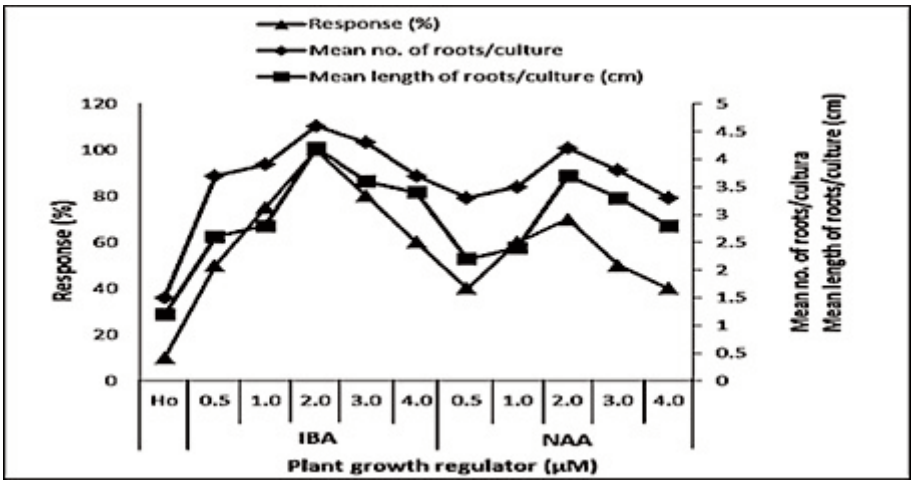

Fig. 2. Effects of auxins (IBA and NAA) on adventitious rooting of *in vitro* derived bulblets of *M. armeniacum*.

in culture room conditions and then transferred to the outdoor condition. After transferring them to outdoor condition approximately 60 % plantlets were survived well (Fig. 1 H).

DISCUSSION

The results presented in the foregoing section reveal that bulb and leaves segments of *M. armeniacum* are successfully established for producing axillary and adventitious bulblets through *in vitro* technique. However, *in vitro* responses were found to be different, and the possible causes of these differences have been discussed in below.

In this study, contamination-free cultures with expected survivability of the explants were primarily achieved by treating the explants with 0.1% $HgCl_2$ solution for 15 minutes where 80% bulb segments produced contamination free explants. There are also many other reports using $HgCl_2$ for surface sterilization of explants from field grown plants (Apurva and Thakur, 2009; Biswas *et al*., 2009; Robinson *et al*., 2009; Sudersan and Aboel-Nil, 2002).

Axillary bulblets were produced directly from bulb-scale explants after 7-8 weeks of culture. BA-NAA combination was found to be better growth regulator combination than BA-IBA, Kn-NAA and Kn-IBA combinations, and 4.0 µM BA with 2.0 µM NAA enhanced maximum axillary bulblets formation from the bulb explants. The variable increase in the bulb size could be attributed to the variable effects of plant growth regulators in the culture medium. Ozel *et al.* (2007) reported that the BAP-NAA combination had significant effects on axillary shoot multiplication of *M. macrocarpum*. They obtained highest number (12.64) of shoots per explants from bulb-scale explants on medium containing 2.0 mg/l BA in combination with 2.0 mg/l NAA. In *Muscari mirum*, Nasircilar *et al.* (2011) reported MS medium supplemented with 4.0 mg/l BA and 0.25 mg/l NAA as the best growth regulator combination for the highest percentage of bulb formation (23.5 per explant) from bulb-scale explants. Recently, Uzun *et al.* (2014) reported that MS medium with 4.0 mg/L BA and 0.50 mg/L NAA showed the best bulblets regeneration after 1 year of culture condition. Ozel *et al.* (2015) also observed, the best results of *in vitro* bulblet regeneration of *M. muscaimi* using twin scale explant on MS medium containing 17.76 µM BAP with 10.74 µM NAA.

The type of explants is extremely important in the establishing of as efficient micropropagation and regeneration system (Koroch *et al*., 2002; Uranbey *et al*., 2003; Basalma *et al*., 2008; Uranbey, 2011). Two types of explants viz. bulb-scale and leaf were used during this investigation to test the effect of explant type on direct adventitious bulblets proliferation. The results indicated

that an auxin-cytokinin ratio 1:4 is the best combination for producing maximum number of adventitious bulblets per culture. For bulb explant, among the various concentration of BA with NAA, the maximum number (8.4 ± 0.58) of adventitious bulblets per explants were found on MS medium containing of 4.0 µM BA + 1.0 µM NAA. Whereas, leaf explants produced the maximum number (10.8 ± 0.24) of adventitious bulblets per explants containing MS medium with 4.0 µM BA + 1.0 µM NAA. Our study indicates that leaf explants of *M. armeniacum* have a high regenerative capacity than bulb explants for producing adventitious bulblets directly. This finding is in agreement with several previous reports (Suzuki and Nakano, 2001; Wang *et al*., 2013). On the basis of the present results between the two cytokinins BA in combination with NAA was found to be better than Kn with NAA for proliferating bulblets from the leaf and bulb-scale explants. Uranbey (2010) also found similar observation of *Muscari azurium*. In conclusion, this technique could be utilized for preparing largescale plant materials with a view to field trial and to establish this plant as a new cut flower variety. It also could be an optimum method for developing clonal variation in laboratory conditions.

CONCLUSION

In conclusion, the present study described a successful regeneration and acclimatization protocol of *M. armeniacum* using bulb scale and leaf explants through direct organogenesis pathways. Among different plant growth regulators, BA-NAA combination was found to the best for both axillary and adventitious bulblet proliferation. Results also demonstrated the leaf-derived explants as the best explant for adventitious bulblet proliferation than that of bulb-derived explant. However, for *in vitro* rooting, IBA in half strength MS medium was recorded to be the best. Taken together, the developed *in vitro* protocol could be helpful for commercial production of *M. armeniacum* and subsequently for its acclimatization in the new environment.

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