Somatic embryogenesis and bulblet production in *Narcissus papyraceus* cv. Shirazi: effect of plant growth regulators, light intensity, sucrose concentration, methyl jasmonate and anti-gibberellins

Ramin Hosseini[1*], Mousa Moradnejad[1], Esmaeil Nezami-Alanagh[1], Shahrokh Ashrafi[1] and Farzan Ghane-Golmohammadi[1]

[1]Department of Agricultural Biotechnology, Imam Khomeini International University (IKIU), Postal Code: 3414916818, Qazvin, Iran.
**Corresponding Author, E-mail: raminh_2001@yahoo.com. Tel: 02833901160. Fax: 02833780073.*

ABSTRACT

Generally, Narcissus is propagated through vegetative methods, but due to their low yield their are not economical. This study was conducted to develop a suitable method for the production of somatic embryos and bulblets in *Narcissus papyraceus* cv. Shirazi. Bulb scales were placed on the MS medium supplemented with 2,4-D and BAP each at 0.0, 0.25, 0.5 or 1 mg/l concentrations to form somatic embryos (SEs). For proliferation, the bulblets were cut in small segments and placed on either MS medium supplemented with sucrose at 30, 60 or 90 g/l, kept in dark or light intensities of 54 or 108 µmol m^{-2} s^{-1} or the MS medium including Methyl jasmonate (Me JA), growth retardants Paclobutrazol or Cycocel (PBZ or CCC) each at 0.0, 0.5, 1.0 or 2.0 mg/l. The highest SE percentage (75 ± 9.0) was obtained when 1.0 mg/l 2,4-D and 0.5 mg/l BAP were incorporated into the medium. Using sucrose at 60 g/l under a light intensity of 54 µmol m^{-2} s^{-1} or utilizing 1.0 mg/l PBZ produced the greatest bulblet number (4.45 ± 0.51 and 5.0 ± 0.25, respectively). None of the scales produced bulblets in dark at any sucrose concentration.

Keyword: Bulblet production, Growth retardants, Light intensity, Methyl jasmonate, Somatic embryogenesis, Sucrose.

Abbrevations: Benzyl aminopurine: BAP; Cycocel: CCC; Methyl jasmonate: Me JA; Murashige and Skoog: MS; Paclobutrazol: PBZ; Plant growth regulators: PGRs; Somatic embryogenesis: SEs.

INTRODUCTION

Narcissus papyraceus cv. Shirazi, a perennial plant with large bulbs, belonging to Amaryllidaceae family, has become increasingly popular in recent years not only for its attractive scent and diversity in colors, low energy requirement and long vase life but also for its alkaloids, exhibiting various antiviral and antitumor properties (Moraes-Cerdeira *et al*., 2007; Weniger *et al*., 2007). Therefore, its micropropagation based on twin-scales as primary explants has been developed to accelerate the multiplication efficiency (Ozel *et al*., 2008; Rice *et al*., 2011; Santos *et al*., 2002). Recently, bulblet regeneration in Narcissus via SEs has been reported based on the induction of callus from mature bulbs. However, this procedure takes a long time (Anbari *et al*., 2007). Our comprehensive quest revealed that a reliable and rapid protocol for direct SEs of this genotype has not been reported yet. Light and sucrose play key roles in growth and development of *in vitro* grown plantlets (Gautheret, 1955; Kumar *et al*., 2005). While standard conditions of 3% sucrose and 60 µmol m^{-2} s^{-1} light for bulblet production in Narcissus and other bulbous species are well accepted (Anbari *et al*., 2007; Cheesman *et al*., 2010; Gangopadhyay *et al*., 2010), the interaction effect of both variables, light and sucrose, on *in vitro*

plant proliferation still remains a controversial issue (Gago *et al*., 2010). These would indicate the necessity of studying the interaction effects of light intensity and sucrose concentrations on bulblet production.

Jasmonates as signal molecules in plant stress responses, play a significant role in plant growth and development (Takahashi *et al*., 1995). This group may also be involved in the process of storage organ formation (Žel *et al*., 1997). Jasmonic acid as one of the derivates of jasmonates promisingly increased bulblet number in some bulbous species (Ondo Ovono *et al*., 2010; Rayirath *et al*., 2011; Santos and Salema 2000). However, studying the similar effect of Me JA) as another component of the jasmonate group on Narcissus would widen our knowledge on the SE and bulblet production.

To assess the possible role of GAs, growth regulators known to inhibit the biosynthesis of GAs have been widely used. Growth retardants with an N-containing heterocycle, e.g. PBZ act as an inhibitor of monooxygenase, catalyzing the oxidative steps from ent-kaurene to ent-kaurenoic acid. CCC, belonging to ammonium group of onium-type, inhibits the conversion of geranyl geranyl pyrophosphate to (-)-kaurene in GA biosynthesis (Rademacher, 2000). In different literatures both anti-gibberellins, PBZ and CCC, have been reported to enhance tuberization and bulbing in potato, shallot, and lily through a decrease in vegetative growth (Kumar *et al*., 2005; Le Guenle Saos *et al*., 2002; Simko, 1994), but there are no reports on the effect of these growth retardants on Narcissus bulbing.

This study was conducted in order to determine the optimum hormonal concentration in embryogenesis, the interacting effect of carbon concentrations with light intensity and also Me JA and anti-gibberellins on bulblet production.

MATERIALS AND METHODS

Plant material

Pre-cooled bulbs (4ºC for 12 weeks) of *Narcissus papyraceusr* cv. Shirazi were purchased from Isfahan Pakan-Bazr Company (www.pakanbazr.com) and used for the experiments. Bulb scales derived from terminal bulbs were used as explants. About 1.5 cm long segments were surface sterilized, using 1% (w/v) commercial bleach solution for 15 min followed by rinsing by sterile distilled water for three times and then placed on the culture media.

Media and culture conditions

MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g/l sucrose was used and pH was adjusted to 5.8 prior to autoclaving at 121°C, 104 kPa for 20 minutes. All cultures were incubated at 25 ± 2°C in a culture room under a 16-h photoperiod provided by cool white fluorescent light giving intensity of 54 µmoles m^{-2} s^{-1} PAR.

Effect of 2,4-D and BAP on SEe and bulblet production

The sterile explants were placed on the MS medium supplemented with 30 g/l sucrose, 2,4-D at the concentrations of 0.0, 0.5, 1.0 or 2.0 mg/l in combination with 0.0, 0.25, 0.5 or 1.0 mg/l of BAP. This study was conducted in factorial with a complete random design using four replications with five explants in each. Regeneration percentage and the mean number of embryos per explant were recorded 10 weeks after culture. The produced bulblets were also recorded after transferring the regenerated embryos to the hormone free MS medium, followed by incubation at a 16-h light condition for onward four weeks.

Effect of sucrose and light intensity on bulblet production

This study was designed to explore the possible interacting effects of sucrose (at 30, 60 or 90 g/l) with light intensity (dark, 54 or 108 µmol m^{-2} s^{-1}) on bulblet production. The basal MS medium was used supplemented with 2 mg/l BAP and 0.5 mg/l NAA (Santos *et al*., 2002). The number of produced bulblets, length, weight and diameter were recorded 90 days after the beginning of the experiment. This study was carried out in a complete random design using four replicates with five explants in each.

Effect of Me JA and anti-gibberllins on bulblet production

Me JA, PBZ and CCC each at 0.0, 0.5, 1.0 and 2.0 mg/l were separately added into the MS medium supplemented with 30 g/l sucrose and 0.5 mg/l NAA. All the three chemicals were added to the media through filtration after autoclaving the media. Experimental conditions and the recorded traits were carried out the same as mentioned above.

Statistical analysis

Since the data did not follow a normal distribution, the

number of embryos and bulblets were transformed to square root and embryogenesis percentage was transformed to arcsine. Following transformation, the SEs data, sucrose and light intensity were analyzed with the analysis of variance using the GLM procedure. In all experiments, SAS (Software Version 9.1) in a complete randomized design (CRD) was implemented. Then Duncan's multiple range test was employed to evaluate the treatment differences.

RESULTS

Effect of 2,4-D and BAP on SEe and bulblet production

After 15 days of incubation on the SEe induction medium, early SEe appeared and globular embryos formed within next four weeks. After transferring the embryos to the hormone free MS medium, whole plantlets formed (Figure 1A-D). The results presented in Table 1 indicate that the highest SEs percentage (75 ± 5.0) was obtained when 1.0 mg/l 2,4-D with 0.5 mg/l BAP were integrated into the medium. These concentrations were also enough to obtain the greatest mean number of embryos and bulblets per explant (3.25 ± 0.47 and 2.5 ± 0.28, respectively, Table 1). Results also revealed that although the presence of both BAP and 2,4-D was necessary for embryogenesis, their usage at higher concentrations significantly decreased SEs efficiency.

Effect of sucrose concentrations and light intensity on bulblet production

Results showed that there were highly significant differences for the effect of sucrose, light and their interaction on bulblet number, length and diameter (Table 2). The greatest number of bulblets (4.45 ± 0.51) were achieved on the media supplemented with 60 g/l sucrose under a light intensity of 54 μmol m^{-2} s^{-1}, whilst the mean number of bulblets significantly decreased under darkness and/or the highest light intensity regardless of the carbon source of the medium (30-90 g/l sucrose). It would be a noticeable impact of light intensity compared to carbon source on the bulblet production. In regards to the bulblet length, results also revealed that the longest bulblets (19.68 ± 0.52 mm) were optained on the media supplemented with 90 g/l sucrose under 108 μmol m^{-2} s^{-1} light intensity. Bulblet diameter was influenced by both sucrose concentration and light intensity, giving rise to the formation of bulblets with 11.43 ± 0.92 mm in diameter on the medium containing 90 g/l sucrose under the light intensity of 108 μmol m^{-2} s^{1} (Table 3 and Figure 1E-H).

Table 1. Effect of growth regulators on direct SEe from twin-scales of *Narcissus papyraceus* cv. Shirazi.

PGRs		Embryogenesis percentage	Mean No. of embryos per explant	Mean No. of bulblets produced per explant
2,4-D (mg/l)	BAP (mg/l)			
0.0	0.0	-	-	-
0.0	0.25	-	-	-
0.0	0.5	-	-	-
0.0	1.0	-	-	-
0.5	0.0	-	-	-
0.5	0.25	15 ± 9.50	0.5 ± 0.28	-
0.5	0.5	30 ± 5.77	1.25 ± 0.25	0.5 ± 0.28
0.5	1.0	15 ± 5.00	1.0 ± 0.40	0.75 ± 0.25
1.0	0.0	-	-	-
1.0	0.25	50 ± 5.77	2.25 ± 0.47	2.0 ± 0.40
1.0	0.5	75 ± 5.00	3.25 ± 0.47	2.5 ± 0.28
1.0	1.0	35 ± 5.00	1.5 ± 0.29	1.25 ± 0.25
2.0	0.0	-	-	-
2.0	0.25	15 ± 5.00	0.75 ± 0.25	0.5 ± 0.28
2.0	0.5	30 ± 5.77	1.5 ± 0.28	0.75 ± 0.25
2.0	1	-	-	-

Values are the means of four replicates, each including five explants (n = 20).

Table 2. ANOVA for the effect of sucrose, light and their interaction on bulblet number, length and diameter.

Source of variation	DF	MS		
		Bulblet number	Bulblet length (cm)	Bulblet diameter (cm)
Sucrose	2	6.66**	29.14**	4.51**
Light	2	55.30**	64.98**	31.03**
Sucrose × Light	4	22.90**	8.13**	0.93**
Error	147	0.33	0.25	0.18
Total	155			
CV (%)		26.35	14.09	18.27

** significant at the 1 percent probability level.

Table 3. Effect of light intensity and sucrose concentration on bulblet production in *Narcissus papyraceus* cv. Shirazi.

Treatments		Mean number of bulblet / Explant ± SE	Bulblet length / Explant ± SE	Bulblet diameter / Explant ± SE
Light intensity	Sucrose concentration (g/l)			
Darkness	30	1.0 ± 0.0^{e}	7.125 ± 0.64^{f}	4.5 ± 0.49^{e}
	60	1.0 ± 0.0^{e}	8.25 ± 0.8^{ef}	5.37 ± 0.49^{de}
	90	1.0 ± 0.0^{e}	8.43 ± 0.57^{e}	6.06 ± 0.52^{d}
54 µmol m^{-2} s^{-1}	30	1.5 ± 0.24^{d}	7.55 ± 0.52^{ef}	5.2 ± 0.66^{de}
	60	4.45 ± 0.51^{a}	13.15 ± 1.07^{c}	7.45 ± 0.5^{c}
	90	3.1 ± 0.25^{b}	11.65 ± 0.71^{d}	7.75 ± 0.62^{c}
108 µmol m^{-2} s^{-1}	30	3.31 ± 0.25^{b}	11.31 ± 0.57^{d}	9.93 ± 0.5^{b}
	60	2.18 ± 0.23^{c}	14.68 ± 0.62^{b}	9.37 ± 0.54^{b}
	90	1.62 ± 0.26^{d}	19.68 ± 0.52^{a}	11.43 ± 0.92^{a}

The data presented are the means of four replications, each including five explants (n = 20), compared by Duncan's New Multiple range Test, *P = 0.05*.

Effect of Me JA and anti-gibberellins on bulblet production

The results presented in Table 4 indicate that there were significant differences among the treatments implemented on bulblet production. Results obtained from Duncan's multiple range test assigned treatments into different groups specified for bulblet number, weight, length, and diameter (Table 5). In terms of bulblet number, PBZ at 1 mg/l (5.00 ± 0.26) and in the second and third ranks, 1 mg/l Me JA (4.06 ± 0.21) and 0.5 mg/l CCC (3.87 ± 0.27) produced the highest numbers of bulblets. In bulblet diameter, the control showed the highest value (12.54 ± 0.33) followed by 0.5 mg/l PBZ and Me JA (10.43 ± 0.39 and 10.37 ± 0.28, respectively). A similar trend was observed for bulblet length. Whereas, in fresh weight, 0.5 mg/l CCC and 1.0 mg/l PBZ caused the highest increases in bulblet weight compared to the control (Table 5; Figure 1I-K).

DISCUSSION

The results presented here could be used as a basis for the rapid and economical production of *N. papyraceus* cv. Shirazi through SEs and also bulblet production. Based on our previous report (Anbari *et al.*, 2007) 2,4-D and BAP were known as dominant PGRs to induce

Table 4. ANOVA for the effect of Me JA, PBZ and CCC on bulblet number, diameter, length and fresh weight.

Source of variation	DF	MS			
		Bulblet number	Bulblet diametre (cm)	Bulblet length (cm)	Fresh weight (g)
Treatment	9	30.88**	20.52**	23.63**	1.26**
Error	150	0.54	1.72	1.91	0.001
Total error	159	30.10	20.47	16.26	10.52

** significant at 1 percent probability level.

Table 5. The effect of Me JA, PBZ and CCC on bulblet number, diameter, length and fresh weight.

Treatment	Concentration (mg/l)	Bulblet number	Bulblet diametre (mm)	Bulblet length (mm)	Fresh weight (g)
Control		1.00 ± 0.00^{f}	12.54 ± 0.33^{a}	15.20 ± 0.33^{a}	0.15 ± 0.00^{fg}
Me JA	0.5	2.00 ± 0.20^{d}	10.37 ± 0.28^{b}	12.68 ± 0.36^{b}	0.23 ±0.00^{e}
	1	4.06 ± 0.21^{b}	4.81 ± 0.32^{de}	6.93 ± 0.33^{e}	0.52 ± 0.01^{c}
	2	1.50 ± 0.13^{def}	2.87 ± 0.24^{f}	4.62 ± 0.27^{g}	0.13 ± 0.00^{g}
PBZ	0.5	1.37 ± 0.12^{ef}	10.43 ± 0.39^{b}	12.75 ± 0.37^{b}	0.17 ± 0.00^{f}
	1	5.00 ± 0.26^{a}	7.50 ± 0.39^{c}	9.31± 0.36^{c}	0.79 ± 0.02^{b}
	2	2.62 ±0.20^{c}	4.00 ± 0.37^{e}	5.87 ±0.40^{f}	0.26 ± 0.00^{d}
CCC	0.5	3.87 ± 0.27^{b}	5.62 ±0.34^{d}	8.12 ±0.34^{d}	0.89 ± 0.01^{a}
	1	1.81 ± 0.16^{de}	2.93 ± 0.23^{f}	4.87 ± 0.25^{g}	0.25 ± 0.00^{de}
	2	1.25 ± 0.11^{f}	3.06 ± 0.30^{f}	4.75 ± 0.39^{g}	0.15 ± 0.00^{fg}

The data presented are the means of four replications, each including five explants (n = 20), compared by Duncan's New Multiple range Test, *P = 0.05*.

SEs in comparison to IBA, NAA and Kinetin. Here, the various concentrations of 2,4-D and BAP were incorporated into the medium. The results indicated that 1 mg/l 2,4-D and 0.5 mg/l played a key role on SEs, giving rise to the production of the highest no of SEs. The SE production efficiency however, decreased significantly at higher concentrations. This was in agreement with the reports documented by (Marija *et al*., 2011; Sage *et al*., 2000; Zdravkovic-Korac *et al*., 2010) in bulbous species where 2,4-D induced SEs in these species.

Results also revealed that light greatly influenced the effect of sucrose and as a result, no bulblets were produced in dark. The greatest numbers of bulblets (4.45 ± 0.51 and 3.31 ± 0.25) were obtained on the media supplemented with 60 g/l sucrose at 54 µmol m^{-2} s^{-1} and at the second rank, 30 g/l sucrose at 108 µmol m^{-2} s^{-1} light intensities, respectively (Table 3). This result is consistent with those reports on bulbous species, e.g. tulips and onions, that increasing sucrose concentration had a direct effect on bulblet formation (Kahane *et al*., 1992; Keller, 1993). Based on the results obtained here, it seems likely that light intensity was clearly more effective than sucrose concentration on bulblet production. This may be in coincidence with the hypothesis presented by Hazarika (2003) reporting that *in vitro* plant growth may be possible on a sucrose-free or sucrose-reduced medium (photoautotrophy) by increasing light intensity.

In the case of bulblet length and diameter, the highest bulblet length (19.68 ± 0.52 mm) and bulblet diameter (11.43 ± 0.92 mm) were produced when 90 g/l sucrose was incorporated into the medium at a 108 µmol m^{-2} s^{-1} light intensity (Table 3). One can conclude that by increasing light intensity, the bulblets initiate to store

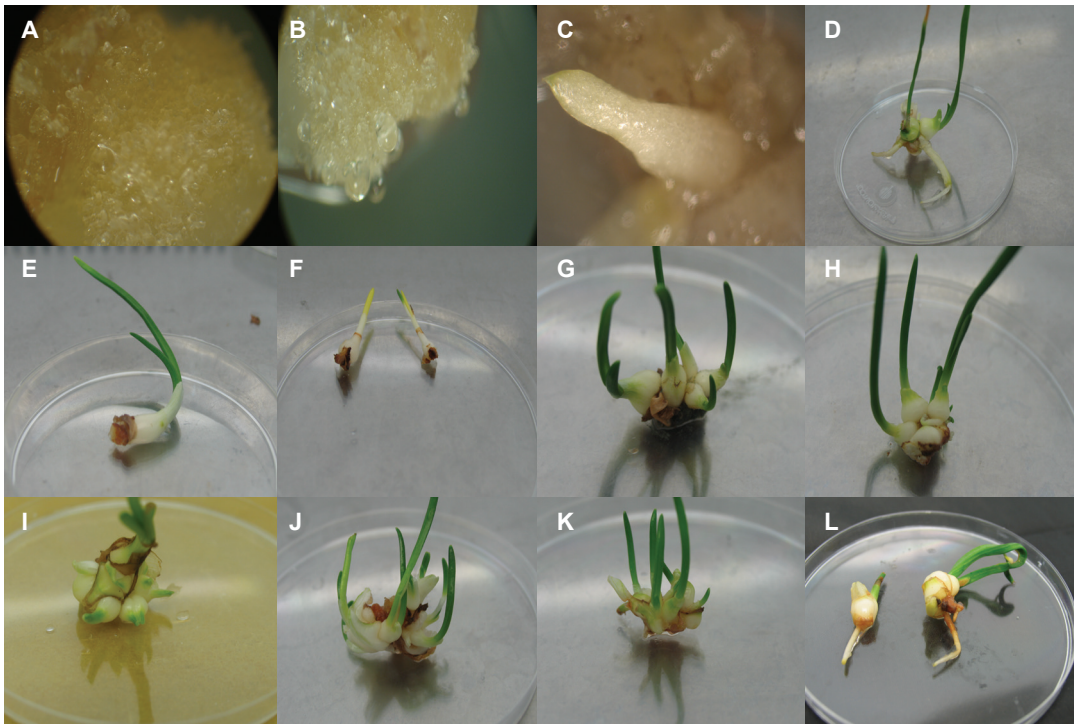

Figure 1. Somatic embryogenesis and bulblet production in *Narcissus papyraceus* cv. Shirazi. **A:** Development of early somatic embryos after culture of scale explants for 15 days on a standard induction medium; **B:** Development of early and the advanced somatic embryos after four weeks of culture; **C:** A somatic embryo after four weeks; **D:** Production of plantlets after transferring the produced embryos to the hormone free MS medium; **E:** Shoot cultures on MS medium supplemented with 30 g/l sucrose, 2 mg/l BAP and 0.5 mg/l NAA grown under 54 µmol m^{-2} s^{-1} (control); **F:** Plantlets grown on the MS medium supplemented with 30 g/l sucrose, 2 mg/l BAP and 0.5 mg/l NAA grown in dark; **G:** Bulblet production on the MS medium supplemented with 60 g/l sucrose, 2 mg/l BAP and 0.5 mg/l NAA grown at 54 µmol m^{-2} s^{-1}; **H:** Bulblet production on the MS medium supplemented with 30 g/l sucrose, 2 mg/l BAP and 0.5 mg/l NAA grown under 108 µmol m^{-2} s^{-1}; **I-K:** Bulblet production on the MS medium supplemented with 30 g/l sucrose, 0.5 mg/l NAA, grown at 54 µmol m^{-2} s^{-1} containing **I:** 1.0 mg/l Me JA **J:** 1.0 mg/l PBZ and **K:** 0.5 mg/l CCC; **L:** Rooting on the MS medium supplemented with 90 g/l sucrose, 2 mg/l BAP and 0.5 mg/l NAA grown at 54 µmol m^{-2} s^{-1}.

nutrients e. g. sucrose and as a consequence, the bulblet diameter and length increase. This is in agreement with the results presented by Takayama and Masanaru (1979). We also observed that at the 108 µmol m^{-2} s^{-1} light intensity, by increasing sucrose concentration, the number of produced bulblets decreased, indicating that probably light at this intensity had an antagonistic effect with sucrose concentration, resulting in the excessive storage of sucrose and a significant reduction in bulblet production. The present study also showed that the incorporation of different growth retardants and Me JA at various concentrations enhanced bulblet number and weight. Me JA had a negative effect on bulblet length and bulblet diameter and they showed a decrease by the increase in Me JA concentration. Both traits (bulblet length and diameter) decreased significantly by the increase in the concentration of growth retardants. The increase in bulblet number and fresh weight using these growth retardants may be related to the nature of this group, i.e. arresting the vegetative growth, while, enhancing the regenerative growth. This coincides with the findings of some reports (Albany *et al*., 2005; Hassan *et al*., 2009a; Hassan *et al*., 2009b; Kumar *et al*., 2005; Zheng *et al*., 2012) where the incorporation of growth retardants such as Alar, CCC and PBZ in the culture media during the multiplication stage of bulbous and other species decreased the excessive growth of stems and leaves, but increased the productivity ra-

tio. The propagation rate of *Narcissus papyraceus* cv. Shirazi is relatively low by the conventional methods. Here, an efficient *in vitro* approach was developed for bulblet production. In addition, a SEs method was optimized for the future studies to be used for the *in vitro* plantlet production. This protocol has the potential to be used as a basis for the mass production of this ornamental and medicinal plant.

ACKNOWLEDGMENTS

This work was supported by Imam Khomeini International University (IKIU).

REFRENCES

Albany N. R., Vilchez J. A., Garcia L., and Jimenez E. (2005). Comparative study of morphological parameters of Grand Nain banana (Musa AAA) after *in vitro* multiplication with growth retardants. *Plant Cell, Tissue and Organ Culture*, 83: 357-361.

Anbari S., Tohidfar M., Hosseini R., and Haddad R. (2007). SEs induction in *Narcissus papyraceus* cv. Shirazi. *Plant Tissue Culture and Biotechnology*, 17: 37-46.

Cheesman L., Finnie J., and Van Staden J. (2010). Eucomis zambesiaca baker: Factors affecting *in vitro* bulblet induction. *South African Journal of Botany*, 76: 543-549.

Gago J., Martínez-Núñez L., Landín M., and Gallego P. (2010). Artificial neural networks as an alternative to the traditional statistical methodology in plant research. *Journal of Plant Physiology*, 167: 23-27.

Gangopadhyay M., Chakraborty D., Dewanjee S., and Bhattacharya S. (2010). Clonal propagation of *Zephyranthes grandiflora* using bulbs as explants. *Biologia Plantarum*, 54: 793-797.

Gautheret R. (1955). The nutrition of plant tissue cultures. *Annual Review of Plant Physiology*, 6: 433-484.

Hassan M., Abd-El-Kareim A., and El-Banna A. (2009b). Using growth retardants for preservation of date palm somatic embryos. *Mansoura University Journal of Agricultural Sciences*, 34: 835-844.

Hazarika B. (2003). Acclimatization of tissue-cultured plants. *Current Science*, 85: 1704-1712.

Kahane R., Serve B., and Rancillac M. (1992). Bulbing in long-day onion (*Allium cepa* L.) cultured *in vitro*: comparison between sugar feeding and light induction. *Annals of Botany*, 69: 551-555.

Keller E. (1993). Sucrose, cytokinin, and ethylene influence formation of *in vitro* bulblets in onion and leek. *Genetic Resources and Crop Evolution*, 40: 113-120.

Kumar S., Kashyap M., and Sharma D. (2005). *In vitro* regeneration and bulblet growth from lily bulbscale explants as affected by retardants, sucrose and irradiance. *Biologia Plantarum*, 49: 629-632.

Le Guenle Saos F., Hourmant A., Esnault F., and Chauvin J. (2002). *In vitro* bulb development in Shallot (*Allium cepa* L. Aggregatum Group): effects of anti-gibberellins, sucrose and light. *Annals of Botany*, 89: 419-425.

Marija P., Angelina S., Sladana J., and Milana T. (2011). SEs and bulblet regeneration in snakehead fritillary (*Fritillaria meleagris* L.). *African Journal of Biotechnology*, 10: 16181-16188.

Moraes-Cerdeira R. M., Burandt Jr C. L., Bastos J. K., Nanayakkara N. P. D., Mikell J., Thurn J., and McChesney J. D. (2007). Evaluation of four Narcissus cultivars as potential sources for galanthamine production. *Planta Medica*, 63: 472-474.

Murashige T., and Skoog F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.

Ondo Ovono P., Kevers C., and Dommes J. (2010). Tuber formation and growth of *Dioscorea cayenensis*-D. rotundata complex: interactions between exogenous and endogenous jasmonic acid and polyamines. *Plant Growth Regulation*, 60: 247-253.

Ozel C., Khawar K., Karaman S., Ates M., and Arslan O. (2008). Efficient *in vitro* multiplication in *Ornithogalum ulophyllum* Hand.-Mazz. from twin scale explants. *Scientia Horticulturae*, 116: 109-112.

Rademacher W. (2000). Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Biology*, 51: 501-531.

Rayirath U. P., Lada R. R., Caldwell C. D., Asiedu S. K., and Sibley K. J. (2011). Role of ethylene and jasmonic acid on rhizome induction and growth in rhubarb (*Rheum rhabarbarum* L.). *Plant Cell, Tissue and Organ Culture*, 105: 253-263.

Rice L., Finnie J., and Van Staden J. (2011). *In vitro* bulblet production of *Brunsvigia undulata* from twin-scales. *South African Journal of Botany*, 77: 305-312.

Sage D., Lynn J., and Hammatt N. (2000). SEs in *Narcissus pseudonarcissus* cvs. Golden Harvest and St. Keverne. *Plant Science*, 150: 209-216.

Santos A., Fidalgo F., Santos I., and Salema R. (2002). *In vitro* bulb formation of *Narcissus asturiensis*, a threatened species of the Amaryllidaceae. *Journal of Horticultural Science and Biotechnology*, 77: 149-152.

Santos I., and Salema R. (2000). Promotion by jasmonic acid of bulb formation in shoot cultures of *Narcissus triandrus* L. *Plant Growth Regulation*, 30: 133-138.

Simko I. (1994). Effect of PBZ on *in vitro* formation of potato microtubers and their sprouting after storage. *Biologia Plantarum*, 36: 15-20.

Takahashi K., Fujino K., Kikuta Y., and Koda Y. (1995). Involvement of the accumulation of sucrose and the synthesis of cell wall polysaccharides in the expansion of potato cells in response to jasmonic acid. *Plant Science*, 111: 11-18.

Takayama S., and Masanaru M. (1979). Differentiation in Lilium bulb scales grown *in vitro*. Effect of various cultural conditions. *Physiologia Plantarum*, 46: 184-190.

Weniger B., Italiano L., Beck J. P., Bastida J., Bergonon S., Codina C., Lobstein A., and Anton R. (2007). Cytotoxic activity of Amaryllidaceae alkaloids. *Planta Medica*, 61: 77-79.

Zdravkovic-Korac S., Meilojevic J., Tubic L., Calic-Dragosavac D., Mitic N., and Vinterhalter B. (2010). SEs and plant regeneration from root sections of *Allium schoeno prasum* L. *Plant Cell, Tissue and Organ Culture*, 101: 237-244.

Žel J., Debeljak N., Ucman R., and Ravnikar M. (1997). The effect of jasmonic acid, sucrose and darkness on garlic (*Allium sativum* L. cv. Ptujski Jesenski) bulb formation *in vitro*. *In Vitro Cellular and Developmental Biology-Plant*, 33: 231-235.

Zheng R., Wu Y., and Xia Y. (2012). Chlorocholine chloride and PBZ treatments promote carbohydrate accumulation in bulbs of Lilium Oriental hybrids 'Sorbonne'. *Journal of Zhejiang University-Science B*, 13: 136-144.