Open access • Journal Article • DOI:10.1007/S11240-011-0045-1

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Muhammad Arshad, Muhammad Arshad, Muhammad Arshad, Jérôme Silvestre ...+9 more authors

Institutions: University of the Sciences, University of Toulouse, Ecolab

Published on: 01 Feb 2012 - Plant Cell Tissue and Organ Culture (Springer Netherlands)

Topics: Murashige and Skoog medium and Pelargonium capitatum

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Muhammad Arshad, Jérôme Silvestre, Georges Merlina, Camille Dumat, Eric Pinelli, Jean Kallerhoff

Thidiazuron-induced shoot organogenesis from mature leaf explants of scented *Pelargonium capitatum* cultivars

M. Arshad · J. Silvestre · G. Merlina · C. Dumat · E. Pinelli · J. Kallerhoff

Abstract Shoot organogenesis from mature leaf tissues of two scented *Pelargonium capitatum* cultivars, 'Attar of Roses' and 'Atomic Snowflake', grown in the greenhouse, were optimized in the presence of thidiazuron (TDZ). The protocol involved preculture of leaf sections on basal Murashige and Skoog (MS) medium supplemented with 10 μM TDZ, 4.4 μM of 6-benzyladenine (BA) and 5.4 μM α-naphtaleneacetic acid (NAA) for a period of 2 weeks and followed by subculture of explants to a fresh medium containing 4.4 μM BA and 5.4 μM NAA. Frequency of regeneration reached approximately 93% for both cultivars, with the induction of more than 100 shoots per explant. Regenerated plantlets were rooted on half-strength MS medium supplemented with 4.4 mM sucrose and 8.6 μM of Indole-3-acetic acid (IAA). All regenerated shoots from both cultivars developed roots when transferred to organic soil mix, acclimatized, and successfully transferred to greenhouse conditions. When regenerated shoots were transferred to hydroponic conditions, frequency of survival was 76.2 and 61.9% for 'Attar of Roses' and 'Atomic Snowflake', respectively.

Keywords Leaf explants · In vitro culture · Organogenesis · Regeneration · TDZ

M. Arshad · J. Silvestre · G. Merlina · C. Dumat · E. Pinelli · J. Kallerhoff (✉)
Université de Toulouse, INP-ENSAT, Ave. de l'Agrobiopôle, BP32607, 31326 Auzeville Tolosane, Castanet-Tolosan, France
e-mail: kallerho@ensat.fr

M. Arshad · J. Silvestre · G. Merlina · C. Dumat · E. Pinelli · J. Kallerhoff
UMR 5245 CNRS-INPT-UPS, EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), Ave. de l'Agrobiopôle, BP32607, 31326 Auzeville Tolosane, Castanet-Tolosan, France

Present Address:
M. Arshad
Institute of Environmental Sciences and Engineering (IESE-SCEE), National University of Sciences and Technology (NUST), H-12, Islamabad 44000, Pakistan

Introduction

Pelargonium species are commercially important crop plants, being used as a source of essential oils in aromatherapy, perfumery and cosmetics, as insect repellents, anti-inflammatory agents and as bedding or potted plants. Improvement of *Pelargonium* species by conventional breeding is hampered by sterility, very low fertility or sexual incompatibility. Therefore, the use of biotechnological approaches is being increasingly considered as alternative strategies for genetic improvement. To this end, in vitro regeneration systems of economically important species have been the subject of focus for more than two decades.

A regeneration system is dependent on the type of explants, the genotype, salt composition of basal medium, growth regulators, light and their altogether interactions (Faizal et al. 2011; Garcia et al. 2011; Song et al. 2011; Vasudevan and Staden 2011). The majority of regeneration systems for *Pelargonium* species reported in the literature have focused on Zonal (*Pelargonium* × *hortorum*), Regal (*Pelargonium* × *domesticum*) and Ivy-leaf pelargoniums (*Pelargonium peltatum*), (Mithila et al. 2001; Haensch 2007), compared to fewer reports for scented *P. capitatum* and *P. graveolens* (Rao 1994; KrishnaRaj et al. 1997; Saxena et al. 2000; Hassanein and Dorion 2005). Regeneration in different species of *Pelargonium* has been studied from diverse starting material, either by indirect

organogenesis (Abo El Nil et al. 1976, Dunbar and Stephens 1989) or by adventitious shoot organogenesis (Boase et al. 1998, Agarwal and Ranu 2000). In the case of scented *Pelargonium captitatum* cv 'Bois joly' and *Pelargonium graveolens* cv. 'Grey Lady Plymouth', Hassanein and Dorion (2005) optimized direct shoot regeneration systems, using leaf disks from in vitro-grown plants. Both direct and indirect organogenesis were established for *Pelargonium graveolens* cv. 'Hemanti' (Saxena et al. 2000) contrarily to *Pelargonium graveolens* (L'Hert) which regenerated shoots only after an intervening callus phase (Rao 1994). None of these studies involved the use of thidiazuron (TDZ) in the culture medium.

N-phenyl-N′-1,2,3-thidiazol-5-ylurea or thidiazuron (TDZ), a substituted phenyl-urea, has proven to be a highly effective regulator of plant morphogenesis. Originally TDZ was considered as a cytokinin and induced many responses typical of natural cytokinins (Murthy et al. 1998). However, later research demonstrated that TDZ, unlike traditional cytokinins, was capable of fulfilling both cytokinin (bud formation) and auxin (somatic embryogenesis) functions involved in various morphogenetic responses of different plant species (Jones et al. 2007). In recent studies, BA has been replaced by TDZ to induce organogenesis in explants from various plant species (Dai et al. 2010; Li et al. 2010; Huang and Dai 2011). It was shown that TDZ could initiate organogenesis in leaves and petioles of *Pelargonium zonale* and *Pelargonium peltatum* hybrids (Winkelmann et al. 2005). The response was genotype dependent with cultivars of *P. peltatum* expressing higher efficiencies than those of the *P. zonale* hybrids. Adventitious shoot regeneration from petiole explants of *Pelargonium* × *hederaefolium* 'Bonete' was also described both by organogenesis and somatic embryogenesis using high TDZ concentrations i.e. 9–18 µM (Wojtania et al. 2004). TDZ has however never been proven to be an initiator of organogenesis in the case of scented *Pelargonium* species.

The objective of the present study was to develop a highly efficient regeneration protocol for scented *Pelargonium* cultivars, prior to optimising genetic transformation for genetic improvement. Two cultivars ('Attar of Roses' and 'Atomic Snowflake'), both belonging to *Pelargonium capitatum* species were selected, essentially on the basis of their high performances for lead (Pb) phytoextraction (Arshad et al. 2008). Indeed, optimizing genetic transformation for these cultivars would be a useful tool in functional genomics of *Pelargonium* cultivars, especially relevant to metal hyperaccumulation. The regeneration protocol for scented *Pelargonium capitatum* cv. 'Bois Joly', reported by Hassanein and Dorion (2005) was assessed towards both cultivars, but could not be reproduced as reported by the authors. In this report, we describe the optimization of an efficient plant regeneration method by organogenesis, in the presence of TDZ, for the scented *P. capitatum* cv. 'Attar of Roses' and 'Atomic Snowflake'.

Materials and methods

Plant material and disinfection

Scented *P. capitatum* cultivars ('Attar of Roses' and 'Atomic Snowflake'), propagated from cuttings of commercial plantlets from the Heurtebise nursery, Clansayes, France, were grown in pots containing special substrate for *Pelargonium* made up of a mixture of white peat, humic peat and clay granulate at pH_{CaCl_2} 5.5–6.1 (Hawita Flor). Plants were maintained in a greenhouse and were regularly irrigated with tap water. Scented *Pelargonium* cultivars 'Attar of Roses' and 'Atomic Snowflake' will be referred to hereafter as 'Attar' and 'Atomic' respectively.

The two latest fully developed leaves from greenhouse-grown 4 months plants were harvested and washed with tap water for 30 min. The leaves were then dipped in 95% ethanol for 30 s followed by immersion in filtered 2.5% Calcium hypochlorite during 20 min and rinsed thrice in double de-ionized sterile water.

Culture media and conditions

The basic medium used for regeneration experiments was MS with 8.8 mM sucrose (Murashige and Skoog 1962). Three growth regulators, BA, NAA and TDZ were chosen and the general scheme followed for the optimization of the protocol is presented in Fig. 1. Basically, sterilized explants were subjected to pre-culture in the dark during 2 weeks on different combinations of growth regulators (Table 1) and then moved to light conditions. All media were adjusted to pH 5.8 ± 0.05 and autoclaved (autoclave; SMI 134) during 20 min at 121°C, solidified by 0.8% bacto-agar (Fischer). Sterilized leaves were cut into 0.25 cm^2 pieces, and 10 pieces were placed adaxial side down on a 94 × 16 mm Petri dish containing 25 mL of different regeneration media. For each experiment, thirty explants were cultured on each medium and all experiments were repeated thrice. Table 1 represents various combinations of growth regulators. Treatment 1 was devoid of hormones. Treatments 2–5 only contained different levels of BA and NAA in the media. In treatments 6–12, the media had been supplemented with 10 µM TDZ in combination with various levels of BA and NAA during 2 weeks pre-incubation period in the dark. After pre-incubation, explants were transferred to the media without TDZ and containing only BA and NAA.

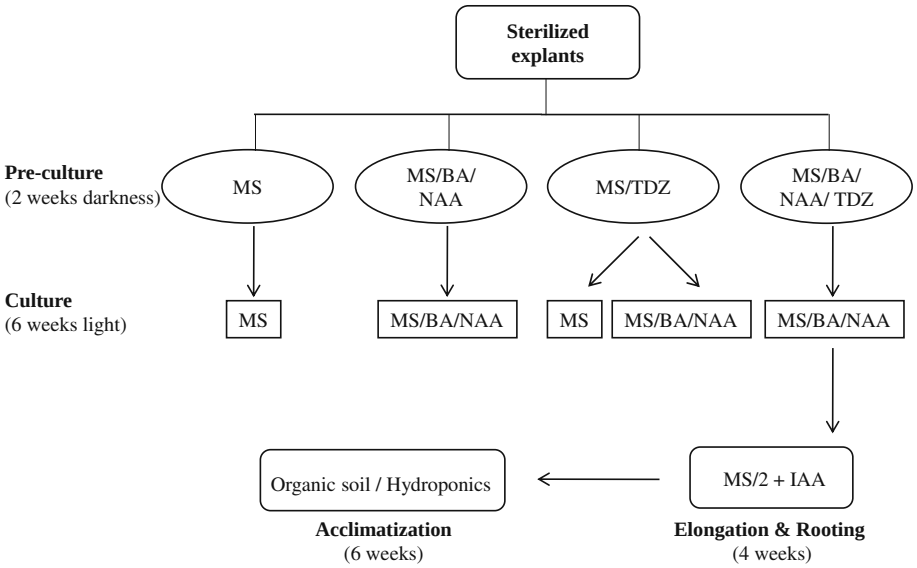

Fig. 1 Schematic representation of the regeneration protocol for *Pelargonium capitatum* cv. 'Attar' and 'Atomic'. Explants were pre-cultured on different media, in the dark during 2 weeks and then transferred to light with a 14-h photoperiod (150 μmol m^{-2} s^{-1}). On each medium, 30 sterilized explants were cultured and replicated thrice. The culture room was maintained at 25°C with 70% relative humidity. Duration of culture for each step is figured in *brackets*

Table 1 Effect of plant growth regulators on organogenesis potential of *Pelargonium capitatum* cv. 'Attar' and 'Atomic'

Treatment	Plant growth regulators (μM)					Frequency of shoot regeneration (%)		Number of shoots per explant	
	Pre-culture			Culture					
	BA	NAA	TDZ	BA	NAA	Attar	Atomic	Attar	Atomic
1	–	–	–	–	–	0.0	0.0	–	–
2	4.4	2.7	–	4.4	2.7	24.4 ± 10.3^{f}	10.9 ± 1.8^{g}	13.4 ± 3.6^{f}	12.8 ± 1.6^{e}
3	4.4	5.4	–	4.4	5.4	75.7 ± 8.6^{c}	86.2 ± 3.3^{b}	28.4 ± 7.4^{e}	20.2 ± 6.5^{de}
4	8.8	10.7	–	8.8	10.7	89.3 ± 3.8^{ab}	96.3 ± 3.8^{a}	35.4 ± 7.1^{e}	28.2 ± 11.3^{d}
5	13.2	5.4	–	13.2	5.4	80.9 ± 5.4^{bc}	54.7 ± 5.9^{d}	31.2 ± 9.2^{e}	27.2 ± 7.9^{d}
6	–	–	10	–	–	51.1 ± 9.6^{d}	34.6 ± 7.3^{f}	61.4 ± 5.2^{c}	45.4 ± 12.5^{cd}
7	–	–	10	4.4	5.4	69.1 ± 12.8^{cd}	54.4 ± 13.9^{d}	72.8 ± 7.4^{c}	56.8 ± 11.8^{c}
8	–	–	10	8.8	10.7	44.8 ± 0.9^{e}	11.1 ± 1.9^{g}	64.4 ± 10.3^{c}	47.0 ± 6.0^{c}
9	–	–	10	13.2	5.4	32.0 ± 2.3^{f}	87.4 ± 4.9^{b}	51.2 ± 2.6^{d}	57.2 ± 14.0^{c}
10	4.4	5.4	10	4.4	5.4	93.5 ± 0.2^{a}	93.0 ± 6.5^{ab}	102.8 ± 2.9^{a}	103.2 ± 5.4^{a}
11	8.8	10.7	10	8.8	10.7	18 ± 14.6^{fg}	65.6 ± 9.4^{cd}	85.8 ± 4.2^{b}	81.8 ± 8.5^{b}
12	13.2	5.4	10	13.2	5.4	58.1 ± 16.1^{cd}	34.4 ± 9.4^{ef}	89.8 ± 8.0^{b}	59.8 ± 7.0^{c}

BA 6-Benzyladenine, *NAA* α-naphthalene acetic acid, *TDZ* Thidiazuron. All media consisted of Murashige and Skoog (MS) basal medium. Explants were pre-cultured in darkness on the different pre-culture media during 2 weeks and then cultured in light conditions on culture media. All data was recorded after 8 weeks as from the start of experimentations. Frequency of shoot regeneration refers to the percentage of explants forming at least 10 shoots per explant. Mean values from three replications (30 explants per replication) with different letters are significantly different ($P < 0.05$) as measured by LSD Fisher test

All Petri dishes and culture vessels for all experiments were maintained in a culture room during regeneration and rooting phase at 25°C with 70% relative humidity and a 14-h photoperiod (150 μmol m^{-2} s^{-1}) provided by Sodium Vapor Lamps (Philips 400 W, Eindhoven, Netherlands).

Elongation and rooting

After 8 weeks of culture, 16 shoots per Petri dish, from the best culture medium, were picked on a random basis and further transferred to 900 cm^3 *Vitrovent* vessels (Duchefa, The Netherlands) for rooting and development. For each cultivar, three Petri dishes were selected in order to have three replications. The *Vitrovent* vessels contained 100 ml of half-strength MS medium with 4.4 mM of sucrose supplemented with 5.7, 8.6 or 11.4 μM of IAA and solidified by 0.8% bacto-agar (Fischer).

Acclimatization of plants

Seven well developed and rooted plants were removed from *Vitrovent* vessels after 4 weeks (12th week from the start of

experiment), washed from the gelling agent, and acclimatized either in pots containing organic soil or in hydroponics (aerated non-circulating nutrient solution) in the greenhouse. These were replicated thrice for both conditions. Plants were placed in a closed mini growth-chamber, in the greenhouse during 2 weeks, after which they were opened every day to allow gradual exposure to greenhouse conditions. Plants transferred to organic soil were regularly irrigated with tap water and every 2 weeks with the following nutrient solution (Uzu et al. 2009) comprising of macronutrients: 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4, 1.5 mM $MgSO_4$, and micronutrients: 46 μM H_3BO_3, 9 μM $MnSO_4{\cdot}H_2O$, 0.1 μM $MoNaO_4{\cdot}2H_2O$, 0.9 μM $CuSO_4{\cdot}5H_2O$, 15 μM $ZnSO_4{\cdot}7H_2O$ and 180 μM Fe–EDTA**.** In the case of hydroponic cultures, the nutrient solution was renewed every 2 weeks and the level was maintained with de-ionized water. Acclimatization efficiency was recorded after 18 weeks of culture initiation. It is defined as the percentage of plants that survived the transition from in vitro to greenhouse conditions.

Data collection and analysis

Explants were regularly examined to monitor morphogenic responses. Explants forming at least 10 shoots were considered as responding explants and scored for frequency of shoot regeneration, calculated as the percentage of explants forming more than 10 shoots per explant. Data obtained was subjected to analysis of variance (ANOVA) with two factors, i.e. cultivar and growth regulators using the software Statistica, Edition'98 (Stat Soft Inc., Tulsa, OK, USA). For each bioassay, mean values with different letters represent significant difference ($P < 0.05$) as measured by LSD Fisher test.

Results

Influence of pre-incubation period in the dark

Frequency of shoot regeneration of 'Attar' and 'Atomic' cultivars was assessed under different light/dark regimes together with two hormonal combinations (Fig. 2). Frequency of shoot regeneration for 'Attar' cultivar on direct exposure to light was 7.2 ± 4.4% on medium containing 4.4 μM BA + 5.4 μM NAA and 3.3 ± 2.7% on medium containing 8.8 μM BA + 10.7 μM NAA. However, using the same hormonal combinations, frequency of regeneration was dramatically increased when explants were pre-incubated in the dark during 15 or 22 days. Best results were achieved for a period of 15 days darkness leading to a frequency of 81.3 ± 5.4% for 'Attar' cultivar on medium supplemented with 8.8 μM BA and 10.7 μM NAA. Organogenesis was not very severely inhibited by direct exposure to light for 'Atomic' cultivar. The minimum frequency of shoot regeneration was 49.3 ± 3.3% and the maximum reached 92.3 ± 4.2% on medium having 8.8 μM BA and 10.7 μM NAA. All further experiments were thus carried out with a 15 day darkness pre-incubation period for both cultivars.

Effect of plant growth regulators on shoot organogenesis

Results showing frequency of shoot regeneration in various treatments involving BA, NAA and TDZ are given in Table 1. As expected, no morphogenic response was observed when plant growth regulators were omitted from the culture medium. Among the different combinations of BA and NAA, the one containing 4.4 μM BA and 2.7 μM NAA (Treatment 2) resulted in low frequency of shoot regeneration i.e. 24.4 ± 10.3 and 10.9 ± 1.8% for 'Attar' and 'Atomic' cultivars, respectively. Contrarily to this, medium containing 8.8 μM BA and 10.7 μM NAA (Treatment 4) displayed maximum frequency of shoot regeneration i.e. 89.4 ± 3.8 and 96.3 ± 3.8% for 'Attar' and 'Atomic' cultivars, respectively. The maximum number of shoots per

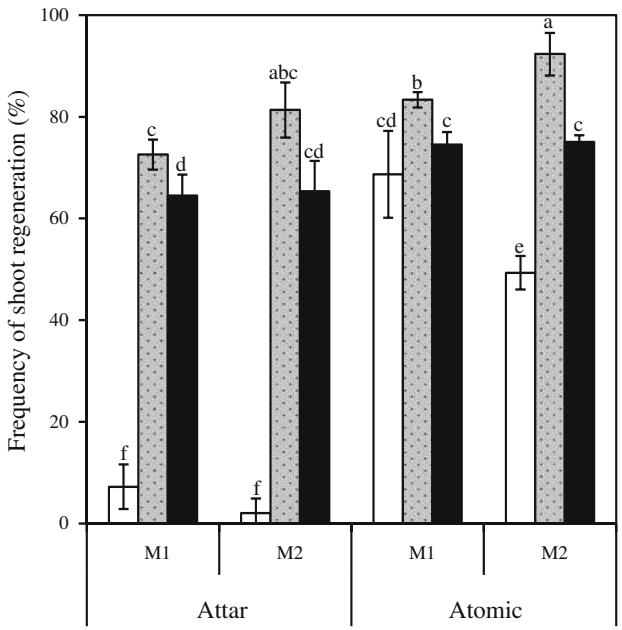

Fig. 2 Effect of light/dark pre-incubation period on frequency of shoot regeneration of *Pelargonium capitatum* cv. 'Attar' and 'Atomic'. Explants were cultured on MS medium containing 4.4 μM BA + 5.4 μM NAA (*M1*) and 8.8 μM BA + 10.7 μM NAA (*M2*). Frequency of shoot regeneration for 'Attar' and 'Atomic' cultivars directly exposed to light (*white bar*) or pre-incubated during 15 days (*gray bar with dot*) and 22 days (*black bar*) in the dark. Data was recorded after 8 weeks of culture initiation. *Different letters* denote a statistically significant difference at $P < 0.05$, as determined by LSD Fisher test

explant was obtained in treatment 4 for both cultivars i.e. 35.4 ± 7.1 and 28.2 ± 11.3 for 'Attar' and 'Atomic' cultivars, respectively. Despite the high frequency of shoot regeneration (percentage of explants with more than 10 shoots) for both cultivars, this protocol needed to be further improved in order to increase the number of newly formed shoots randomly distributed on each explant. Therefore, a second strategy, involving a 15 day TDZ pulse in the absence or presence of the same hormonal combinations in darkness followed by removal of TDZ from the media was attempted.

Preliminary experiments showed that both the cultivars were sensitive to high concentrations of TDZ i.e. 15 and 20 μM, resulting in rapid tissue browning and inhibition of morphogenesis. Lower concentrations (1 and 5 μM TDZ) did not give rise to more than 20% frequency of shoot regeneration. Therefore, results are presented for only one concentration i.e. 10 μM (Table 1, treatments 6–12). During the first 2 weeks in culture on regeneration media containing TDZ in darkness, explants generally expanded, swelled and thickened. Before sub-culturing on TDZ free medium, explants were cut into two or three (depending on the final size) in order to allow direct contact with the medium. They became dark green after 2 weeks in the light (Fig. 3a). Morphogenesis started in 6th week resulting into small outgrowths (Fig. 3b) which developed into shoots after 8 weeks in culture (Fig. 3c).

Pre-incubation of both cultivars on media containing 10 μM TDZ followed by culture on media with or without BA and NAA had significant effects on the numbers of shoots per explant. Frequency of shoot regeneration was lower in treatments 6–9 (no BA and NAA during the pre-incubation period in the dark) than in treatments 2–5 (No TDZ during the pre-incubation). However the number of shoots per explant had almost been doubled. Treatments 10–12 resulted into the highest number of shoots per explant for both cultivars. The combination of BA, NAA and TDZ in treatment 10 i.e. 4.4 μM BA + 5.4 μM NAA + 10 μM TDZ produced the best results both in terms of frequency of shoot regeneration and number of shoots per explant. The frequency was 93.5 ± 0.2 and 93.0 ± 6.5% for 'Attar' and 'Atomic' cultivars, respectively. The numbers of shoots per explant were 102.8 ± 2.9 and 103.2 ± 5.4 for 'Attar' and 'Atomic' cultivars, respectively.

Shoot elongation, rooting and acclimatization of in vitro plants

After 8 weeks of culture initiation, shoots developed on regeneration media were sub-cultured on Elongation and Rooting Medium (ERM). The shoots from best regeneration medium (treatment 10) were elongated and rooted within 4 weeks (Fig. 3d). Results concerning the influence of IAA on rooting efficiency are shown in Fig. 4. The rooting efficiencies were 47.9 ± 7.2 and 50 ± 6.3% for 'Attar' and 'Atomic' cultivars, respectively on ERM containing 5.7 μM IAA. The respective rooting efficiencies for 'Attar' and 'Atomic' cultivars were 91.7 ± 3.6 and 89.6 ± 9.5% on media supplemented by 8.6 μM IAA. There was no significant difference between 8.6 and 11.4 μM IAA for both cultivars. However, extremely dense rooting, enhanced root diameter and restricted growth were observed on the ERM containing 11.4 μM IAA. So the best level of IAA was 8.6 μM IAA for root development of plantlets.

Rooted plants of both cultivars were transferred to organic soil and hydroponics for acclimatization to greenhouse conditions. Acclimatization efficiency was 100% on organic soil for both cultivars. In hydroponics, the acclimatization frequencies were 76.2 ± 8.3 and 61.9 ± 8.2% for 'Attar' and 'Atomic' cultivars, respectively. All the plants were maintained in the greenhouse till flowering (Fig. 3e), after 18 weeks from culture initiation. In spite of lower efficiencies of acclimatization in hydroponics, growth of plants was more vigorous and quicker in hydroponics and, flowering occurred earlier as compared to plants grown on organic soil.

Discussion

This study was conducted to develop an efficient regeneration system for *P. capitatum* cv. 'Attar' and 'Atomic'. Initially, explants were cultured on various auxins and cytokinins such as BA and NAA. For both cultivars, media containing only BA and NAA performed considerably well in terms of frequency of shoot regeneration (approximately 90%). However, the number of shoots per explant for 'Attar' and 'Atomic' (35.4 and 31.2, respectively) was considered to be a fairly poor response in the light of using the process to genetic transformation. Saxena et al. (2000), obtained 44.2 ± 2.5 shoots per leaf explant with *Pelargonium graveolens* cv. 'Hemanti' on medium containing another hormonal combination; 23 μM kinetin and 5.4 μM NAA. Hassanein and Dorion (2005) found that the combination of Zeatin and BA associated with 4 weeks pre-incubation in the dark was necessary to achieve high regeneration frequency. In our experiments, the effects of darkness were significant for both cultivars. Direct exposure to light and incubation period in the dark more than 2 weeks decreased frequency of shoot regeneration (Fig. 2). 'Attar' cultivar proved to be very light sensitive as explants rapidly showed signs of necrosis resulting in poor frequency of shoot regeneration ($\leq$ 7.2%) whereas 'Atomic' cultivar had the lowest frequency of 49.3% upon

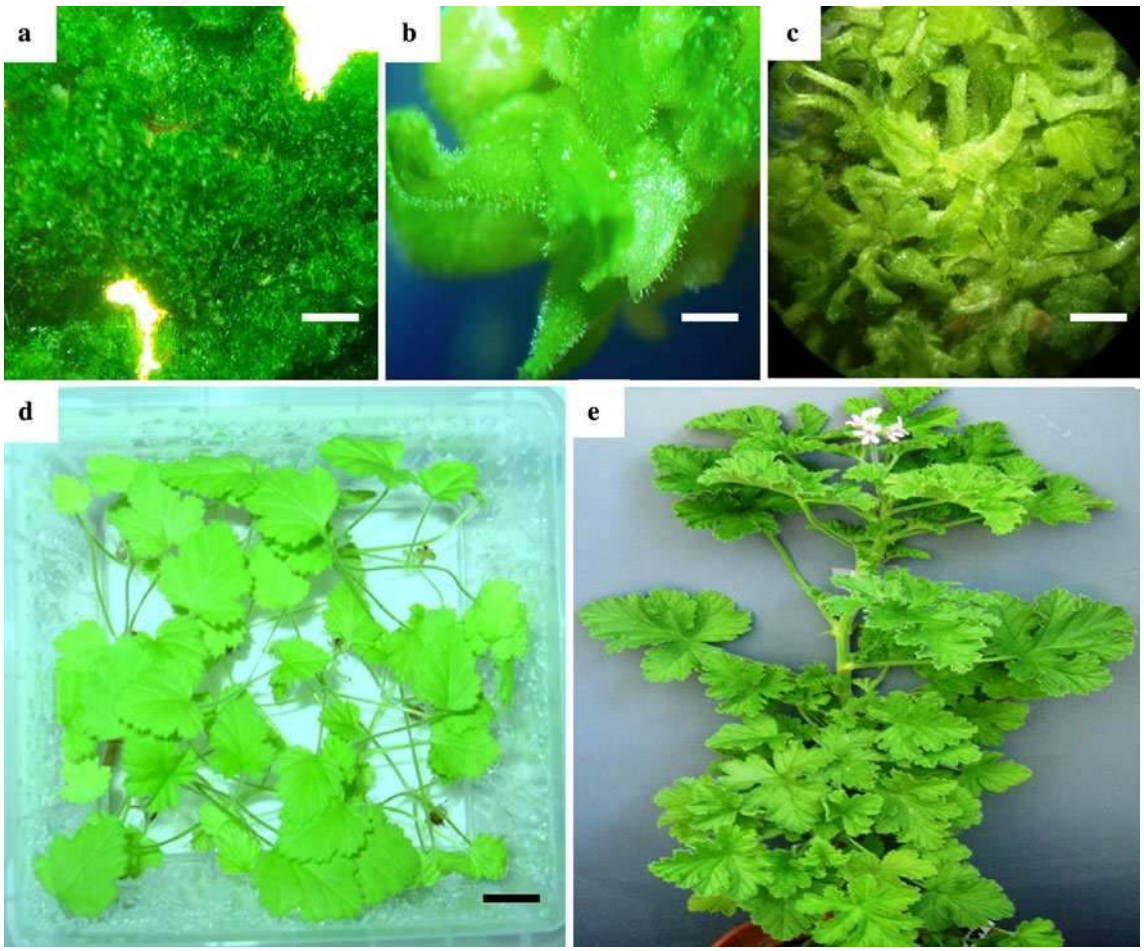

Fig. 3 TDZ-induced shoot regeneration on leaf explants from *Pelargonium capitatum* cv. 'Attar'. **a** Expansion and greening of explants after 2 week pre-incubation in the dark on 10 μM TDZ plus 4.4 μM BA and 5.4 μM NAA followed by culture on same medium without TDZ. *Bar* = 2 mm. **b** Onset of organogenesis, 6 weeks after culture initiation. *Bar* = 2 mm. **c** An explant with multiple shoots after 8 weeks from culture initiation. *Bar* = 2 mm. **d** Rooting of in vitro shoots on half strength MS medium supplemented with 8.6 μM IAA after 12 weeks of culture initiation. *Bar* = 2 cm. **e** Acclimatized plant in the greenhouse after 18 weeks from culture initiation

direct exposure to light. Sukhumpinij et al. (2010) have reported 30 days incubation in the dark as being an optimal period for regeneration from mature leaf explants of *Pelargonium rapaceum* (L.) L'Hérit. This effect was also described in several other genera (Perez-Tornero et al. 2000; Zobayed and Saxena 2003) and could be explained by an inhibitory effect of light on hormone efficiency (Suzuki et al. 2004). The combination of BA and NAA coupled with a 2 week pre-incubation in the dark led to high frequency of shoot regeneration but low number of shoots. This could hamper the recovery of transgenic plants during genetic improvement procedures as the number of cells responding in the morphogenic process would be low.

To improve the number of shoots regenerated per explant, TDZ was tested in combination with or without BA and NAA. TDZ has been considered important for in vitro morphogenesis of plants (Huetteman and Preece 1993; Singh et al. 2003), embryogenesis (Mithila et al. 2001) and adventitious shoot formation (Winkelmann et al. 2005) in *Pelargonium* species. Explants were pre-incubated during 2 weeks as prolonged exposure to TDZ (3–4 weeks) caused necrosis and death of primary shoot and callusing of primary root in most of the seedlings of pigeon pea (Singh et al. 2003). During pre-incubation on media containing TDZ, expansion, swelling and thickening of explants were observed. This could probably due to intense cell division as in the work reported on lentils (Chhabra et al. 2008). The application of TDZ during the pre-incubation period, followed by culture on MS medium without BA and NAA, resulted in an increase in the number of shoots per explant for both cultivars (Table 1, treatments 6–9 vs. 2–5). Pre-incubation in the presence of all the three regulators (BA, NAA and TDZ) followed by culture on media without TDZ produced the maximum number of shoots per explant. When 4.4 μM BA and 5.4 μM NAA (Treatment 10) were present in both

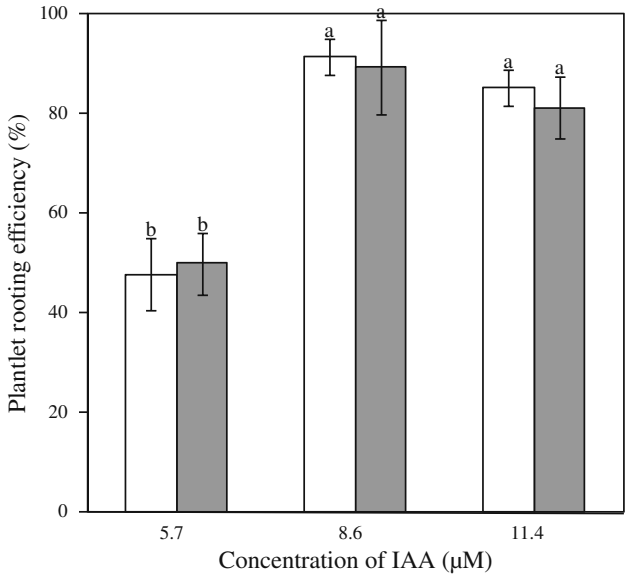

Fig. 4 Effect of IAA concentration on rooting of regenerated plantlets of *Pelargonium capitatum* cv. 'Attar'and 'Atomic'. Plantlets regenerated from explants of *Pelargonium capitatum* cv. 'Attar' (*white bar*) and 'Atomic' (*gray bar*), subjected to treatment 10 (Table 1), were cultured on half-strength MS medium supplemented by different concentrations of IAA. *Small letters* show significant difference ($P < 0.05$) as measured by LSD Fisher test for various levels of IAA and cultivars

pre-culture and culture stages, the frequency of shoot regeneration was 93% for both cultivars coupled with more than 100 shoots per explants (Table 1). The doubling of BA and NAA or tripling of BA concentrations resulted in a decrease in frequency of shoot regeneration for both cultivars (Treatment 10 vs. 11–12). These results show that the response of TDZ is determined by different levels of BA and NAA in the media. TDZ might have a cytokinin action, probably by increasing the level of endogenous cytokinins (de Melo Ferreira et al. 2006).

For rooting purposes, 8.6 μM IAA appeared the best in terms of rooting frequency and quality of roots. This IAA concentration is higher than reported by Hassanein and Dorion (2005) i.e. 5.7 μM IAA for rooting of plantlets of *Pelargonium capitatum*. This difference for scented *Pelargonium* cultivars may be due to different kinds of growth regulators used to regenerate the buds or the type of cultivar. Rooted plants of both cultivars had 100% acclimatization efficiency on organic soil and 76.2 and 61.9% in hydroponics for 'Attar' and 'Atomic' cultivars, respectively (Fig. 4). Hassanein and Dorion (2005) have reported 100% acclimatization frequency for scented *Pelargonium* cultivars on vermiculite and peat mixture (2:1 v/v). This is to our knowledge, the first report of organogenesis in the two *Pelargonium* cultivars studied here, using a TDZ pulse in the presence of BA and NAA, with subsequent removal of TDZ from the medium after 2 weeks. This strategy will, in future be applied, in order to optimize an efficient genetic transformation methodology for these cultivars.

Acknowledgments The authors would like to thank the Higher Education Commission of Pakistan (www.hec.gov.pk) for sponsoring M. Arshad. We are also thankful to Prof. (Emeritus) Gilbert Alibert for helpful discussions.

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