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Establishment of Efficient *in vitro* Method for Drought Tolerance Evaluation in *Pelargonium*

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Abstract: The present work aimed to establish an efficient *in vitro* method for evaluation of drought tolerance in *pelargonium*. Several factors including osmotic stress agent (polyethylene glycol "PEG" or mannitol), media type (liquid or solid), osmotic stress level (115, 260, 505 or 1135 milliosmol kg^{-1} H_2O) and explants (rooted plants or subcultured shoots) were studied for the optimization of method. Experiments were carried out on four *pelargonium* species including *P.* x *domesticum*, *P. graveolens*, *P.* x *hortorum* and *P.* x *peltatum*. Using mannitol for inducing osmotic stress was found to be more selective than PEG. The liquid media was better than solid media in enhancing rooting under osmotic stress. The intermediate and the highest level of osmotic stress were found effective in evaluation of studied species for drought tolerance. Similar evaluation and very good correlation (r= 0.91) was obtained from the two tested explants. So, the subcultured shoots were preferred as explants for the simplicity and rapidity of evaluation method. Survival and rooting percentages decreased with increasing osmotic stress. All growth parameters including stem length, leaves number, plant dry weight and humidity percentage also decreased starting from the low level of osmotic stress. Effect of osmotic stress was highly correlated with survival percentage, rooting percentage and stem length. Correlations among parameters make it possible to use only survival and rooting percentages for evaluation. The studied species showed variability in drought tolerance where *P.* x *domesticum* was the most tolerant followed by *P. graveolens*. However, *P.* x *hortorum* and *P.* x *peltatum* were more sensitive to osmotic stress. The established method is efficient and simple enough to be used for evaluation of drought tolerance in a large number of genotypes in very short time. It can be used for identification and selection of tolerant and sensitive genotypes needed for improvement. It can be also used for the characterization of different morphological, physiological and molecular responses of plants under osmotic stress conditions.

Key words: Biotechnology · *in vitro* · Osmotic · Drought · Stress · Media · Water potential · Osmolarity · mannitol · Polyethylene glycol · Evaluation · Selection · Tolerance · *Pelargonium* · Geranium

INTRODUCTION

Abiotic stress is the most serious threat to agriculture in many parts of the world resulting in increased desertification. Drought or water stress is a major abiotic factor that limits plant growth and productivity. About 40-60% of the agricultural land around the world suffers from drought [1]. It is usually manifested as osmotic stress causing several morphological, physiological and molecular changes in plants.

Pelargonium spp. L'Hér. ex Ait. (Geranium) belonging to the family *Geraniaceae* with about 280 species are among the most popular garden and house plants in the World, with annual global sales reaching US $700 million [2]. The widely cultivated *Pelargonium spp.* fall into four major groups, *P.* x *hortorum*, *P.* x *peltatum*, *P.* x *domesticum* and fragrant leaf species and cultivars as *P. graveolens*.

Breeding for drought tolerance should be given a high research priority in all future plant biotechnology programs [3]. The most important stage needed for improvement of drought tolerance is the evaluation stage for the identification of drought tolerant and sensitive genotypes. Many previous studies have concentrated on the physiological, biochemical and molecular responses of stressed plants [4]. *In situ* evaluation for drought tolerance was studied by several researchers. Peuke *et al.* [5] identified drought-sensitive beech ecotypes basing on physiological parameters of plants exposed to drought period in greenhouse. We have previously reported a simple method to evaluate and select for water stress-tolerance in *P.* x *hortorum* genotypes using few

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morphological and physiological parameters. Cultivars were classified following to their tolerance to water stress induced by withholding water under greenhouse conditions [6].

In vitro culture techniques minimize environmental variations due to defined nutrient media, controlled conditions and homogeneity of stress application. In addition, the simplicity of such manipulations enables studying large plant population and stress treatments in a limited space and short period of time. Simulation of drought stress under *in vitro* conditions during the regeneration process constitutes a convenient way to study the effects of drought on the morphogenic responses. Applying osmotic stress during the regeneration phase was found to be the most efficient for the selection for drought tolerance [7]. It had also a great potential in screening drought-tolerant coconut germplasm [8]. Polyethylene glycol (PEG) of high molecular weights have been used to simulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying. It is used to screen tomato germplasm using seed culture under *in vitro* conditions [9]. It is used to induce water stress in two soybean cultivars at callus and shoot regeneration stages [10]. It is also used for the selection for drought tolerance after UV-C radiation of *in vitro* grown calli in alfaalfa and potato [11, 12]. Mannitol is a sugar alcohol widely used as osmotic agent for the characterization and selection for drought tolerance under *in vitro* conditions. It is used for the selection of tolerant clone of many plant species [13, 14].

From the above mentioned works, the tissue culture approach seemed to be the best system for testing and selecting for drought tolerance. However, many methods were used including different osmotic agents as PEG or mannitol and different explants as calli, seeds or shoots. The present work aimed to evaluate efficiency of several methods in the selection for drought tolerance under *in vitro* conditions. *Pelargonium* species will be used for establishing the method basing on their variability in drought tolerance previously shown under greenhouse conditions [6, 15, 16]. The simple, rapid and efficient evaluation of a large number of genotypes using the establishing method is the final goal of work.

MATERIALS AND METHODS

Plant Materials and Growth Conditions: Four *pelargonium* species including *P.* x *domesticum*, *P. graveolens*, *P.* x *hortorum* and *P.* x *peltatum* were studied throughout experiments. *In vitro*-grown plants of all species were used as donor plants or explants source. Plants were established *in vitro* from buds as reported by Hassanein and Dorion [17] and Dorion *et al.* [18], then propagated by apex and node microcuttings. *In vitro* mother plants were sub-cultured every 4-5 weeks in jars (three apices per jar) on 100 ml "basal medium" containing half-strength MS [19] macronutrients, normal MS micronutrients, vitamins [20], Fe EDTA (7.4 g l^{-1} Na_2EDTA + 5.57 g l^{-1} $FeSO_4$.7H2O), 20 g l^{-1} sucrose, 1.0 mg l^{-1} indole-3-acetic acid (IAA) and 6 g l^{-1} Agar (Sigma).The pH was adjusted to 5.8 and the medium was autoclaved at 113°C for 20 min, except for the growth regulators that were 0.22 μm-filter sterilized and added to the cooled medium. Donor plants were grown in a growth chamber under fluorescent lighting with 16 h photoperiod.

Explants and Tested Factors: *Pelargonium* specie, explant, media type, osmotic agent and osmotic stress level were studied for the establishment of evaluation method. The four above mentioned *pelargonium* species were used in different experiments. Elongated apical shoots of *in vitro* grown plants were cut into 1 cm lengths and used directly as explants (subcultured shoots) or after rooting on basal medium one month later (rooted plants). The basal medium was used as above described (solid medium) or without the gelling agent (liquid medium). Two osmotic agents were used at four different levels. Polyethylene glycol (PEG 6000, Sigma) was used at 0, 10, 15 or 25% and mannitol (Sigma) was used at 0, 200 mM (3.6%), 400 mM (7.3%) or 800 mM (14.6%). Osmotic agents were added to media before pH adjustment then osmotic stress level was measured in all media using electric osmometer. The four tested levels of both osmotic agents gave similar osmolarity in culture media of 115, 260, 505 and 1135 milliosmol kg^{-1} H_2O respectively. Subcultured shoots or rooted plants were inserted upright into 25 x 120 mm glass test tubes containing 10 ml of solid or liquid basal medium with one of PEG or mannitol levels. For culture in liquid medium, pieces of papers containing small holes (paper bridges) were used to support explants. Three replicates of six explants (18) were used per treatment and all experiments were repeated twice. Cultures were maintained in growth chamber under the same conditions of donor plants.

Data Collection and Statistic Analysis: Different measurements were collected from plants after four weeks of culture on selection media. After washing up the agar or media residues with sterile water, the following

parameters were taken for all experiments. The survival percentage (%) was calculated as the percent of survived plants after four weeks of culture on selection medium. Growth parameters including shoot length (cm), number of green and dry leaves per plant and plant fresh weight (g) were recorded at the end of experiments. Plant dry weight (g) was recorded after drying at 80°C for 48 h. Humidity percentage (%) was calculated by dividing the difference between fresh and dry weight by the fresh weight. The rooting percentage (%) was also recorded for subcultured shoots experiments as the percent of rooted shoots after four weeks of culture on selection media. All data were subjected to analysis of variance followed by Tukey's mean comparison method (S-Plus 6.0 Professional release 1; 1988-2001). Coefficient of correlation (r) between data sets and parameters was measured using the same statistic program.

RESULTS

Selection of Osmotic Stress Agent and Media Type: Data presented in Table (1a) show the effect of osmotic agent, media type and osmotic stress level on growth of subcultured shoots of *P.* x *domesticum*. Survival percentage decreased significantly with the highest level of osmotic stress only. Mannitol was found to be more effective in selection than PEG especially in solid media. Rooting was highly affected by osmotic stress where it was fully inhibited with the intermediate level in solid medium and the highest level in liquid medium. Shoot length decreased significantly compared to control regardless osmotic stress level. Moreover, PEG in solid medium gave significantly lower shoot length compared to mannitol in liquid medium. Little number of leaves was produced and high number of leaves was lost with increasing osmotic stress in media. Plant dry weight decreased dramatically with osmotic stress but reduction was gradually in liquid media where significantly higher dry matter was obtained with the liquid media compared to the solid one for both osmotic agents. Humidity percentage also decreased with osmotic stress and the highest reduction was recorded with the highest level and with PEG in solid media. It must be noted that PEG avoided solidification of solid media and semi liquid medium was obtained. It is clear that effect of osmotic stress was highly affected by the studied factors. All studied parameters significantly decreased with increasing osmotic stress level (Table 1b). Despite a lower survival percentage was obtained with mannitol, it allowed higher rooting percentage compared to PEG. The liquid media also showed similar effect on rooting. Moreover, the above mentioned results showed an intermediate effect for mannitol and liquid medium on *P.* x *domesticum* compared to other tested conditions. It could be beneficial for the evaluation for osmotic stress tolerance in many species. So, this condition was maintained for the next experiments.

Selection of Explant and Osmotic Stress Level: Evaluation of four *pelargonium* species for their tolerance to osmotic stress induced by mannitol in liquid media was studied using rooted plants or subcultured shoots as explants. Results obtained with rooted plants are shown in Table (2). *Pelargonium* species showed different responses to osmotic stress. The intermediate level of osmotic stress was selective where survival percentage significantly decreased in *P.* x *peltaum* and *P.* x *hortorum*. The highest level was more selective where it significantly affected survival percentage of all species. However, the lowest level didn't affect this characteristic. All growth parameters significantly reduced starting from the lowest level of osmotic stress compared to control. However, stem length was the least affected parameter. The intermediate and the highest level of osmotic stress gave similar effect on growth parameters in most cases.

Results obtained in the same species using subcultured shoots as explants showed similar indicators (Table 3). The highest level of osmotic stress significantly affected survival percentage of all species and no shoots survived for *P.* x *peltatum* and *P.* x *hortorum*. The intermediate level showed similar indicator where survival percentage varied from 75-100% for *P.* x *graveolens* and *P.* x *domesticum* to 25% for *P.* x *peltatum* and *P.* x *hortorum*. Rooting was fully inhibited for all species with the highest level of osmotic stress and the rooting percentage ranged from 50% for *P.* x *graveolens* and *P.* x *domesticum* to 12.5% for *P.* x *peltatum* and *P.* x *hortorum*. Survival and rooting percentages were not significantly affected, in most cases, by the lowest level of osmotic stress. All growth parameters significantly decreased starting from the lowest level of osmotic stress except humidity percentage that decreased with the intermediate level. No significant difference was found between the effect of the intermediate and the highest level of osmotic stress on growth parameters in most cases.

Comparison between evaluation of *pelargonium* species using rooted plants (Table 2) and subcultured shoots (Table 3) showed very good coefficient of correlation ($r = 0.91$). It means that similar indictors can be

Table 1a: Effect of osmotic agent and media type on efficiency of *in vitro* evaluation for drought tolerance in *P.* x *domesticum* after four weeks of shoots subculture

Osmotic agent	media type	levels	O.S.*	survival (%)	Rooting (%)	shoot length (cm)	green leaves no/plant	dry leaves no/plant	dry weight (mg)	humidity (%)
Mannitol	solid	initial	-	-	-	1.0	1.0	-	-	-
		0	115	100.0a	100.0a	2.2a	5.3a	0.0e	47.9a	90.0a
		200 mM (3.6%)	270	100.0a	40.0d	1.1 bc	1.4bcd	0.8cd	10.6 cd	80.1 cdef
		400 mM (7.3%)	513	100.0a	0.0d	1.0c	1.2bcd	0.8cd	7.7cd	79.0 def
		800 mM (14.6%)	1145	0.0d	-	-	-	-	-	-
		Mean	*75.0 c*	*46.7 b*	*1.4*	*2.6 a*	*0.5 b*	*22.1*	*83.0*	
	liquid	0	115	100.0a	100.0a	2.3a	4.7a	0.0e	53.5a	89.0ab
		200 mM (3.6%)	270	100.0a	100.0a	1.3b	1.0cde	1.4bc	24.1b	84.8bc
		400 mM (7.3%)	513	100.0a	40.0c	1.0 c	0.2ef	2.4a	9.2cd	82.0 cde
		800 mM (14.6%)	1145	40.0c	0.0d	1.0 c	0.0f	2.0ab	3.9d	77.1ef
		Mean	*85.0 b*	*60.0 a*	*1.4*	*1.5 b*	*1.5 a*	*22.7*	*83.2*	
Poly-ethylene glycol (PEG)	solid	0	115	100.0a	100.0a	2.1a	5.4a	0.0e	50.0a	90.0a
		10%	246	100.0a	60.0b	1.0 c	1.6bc	0.0e	13.1c	82.9cd
		15%	494	100.0a	0.0d	1.0c	1.2bcd	0.4de	12.6 cd	79.6 def
		25%	1126	100.0a	0.0d	1.0c	0.8cdef	0.6de	13.1c	75.6f
		Mean	*100.0 a*	*40.0 b*	*1.3*	*2.3 a*	*0.3 b*	*22.2*	*82.0*	
	liquid	0	115	100.0a	100.0a	2.3a	4.9a	0.0e	50.5a	90.0a
		10%	246	100.0a	60.0b	1.2bc	2.0b	0.8cd	21.9b	83.9cd
		15%	494	100.0a	60.0b	1.0 c	0.6def	1.4bc	12.0 cd	82.0 cde
		25%	1126	80.0b	0.0d	1.0 c	0.0f	2.0ab	5.6cd	77.2ef
		Mean	*95.0 a*	*55.0 a*	*1.4*	*1.9 b*	*1.1 a*	*22.5*	*83.3*	

* Osmolarity (osmotic stress) of culture media in milliosmol kg^{-1} H_2O. Mean of two repetitions of three replicates. Means or values with the same letter in the same column are not significantly different at (p=0.05)

Table 1b: Effect of principal factors on the evaluation for drought tolerance

Osmotic agent	media type	levels	survival (%)	rooting (%)	shoot length (cm)	green leaves no/plant	dry leaves no/plant	dry weight (mg)	humidity (%)
Mannitol	-	-	80.0 b	54.3 a	1.4	2.0	1.1 a	22.4	83.1
PEG	-	-	97.5 a	47.5 b	1.3	2.1	0.7 b	22.4	82.7
-	Solid	-	87.5	42.9 b	1.3	2.4 a	0.4 b	22.1	82.5
-	Liquid	-	90.0	57.5 a	1.4	1.7 b	1.3 a	22.6	83.3
-	-	Control	100.0 a	100.0 a	2.2 a	5.1 a	0.0 d	50.5 a	89.8 a
-	-	Level 1	100.0 a	65.0 b	1.2 b	1.5 b	0.8 b	17.4 b	82.9 b
-	-	Level 2	100.0 a	25.0 c	1.0 b	0.8 c	1.3 a	10.4 c	80.7 b
-	-	Level 3	55.0 b	0.0 d	1.0 b	0.3 d	1.5 a	7.5 c	76.6 c

Means with the same letter in the same column in each part are not significantly different at (p=0.05)

Table 2: Evaluation of different *Pelargonium* species for drought tolerance (osmotic stress) induced *in vitro* by mannitol after four weeks of rooted plants transfer on liquid medium

Pelargonium specie	mannitol (mM)	O.S.*	survival (%)	shoot length (cm)	green leaves no/plant	dry leaves no/plant	dry weight (mg)	humidity (%)
P. x *domesticum*	initial	-	-	1.5	4.9	-	-	-
	0	112	100.0 a	3.0 a	8.4 a	0.5 d	76.7 a	90.8 a
	200	271	100.0 a	2.3 b	4.4 b	1.4 c	66.9 a	82.7 b
	400	512	100.0 a	1.8 bc	1.4 c	3.3 b	31.6 b	80.4 bc
	800	1190	62.5 b	1.5 c	0.4 d	4.7 a	32.4 b	75.0 c
P. graveolens	initial	-	-	1.4	3.9	-	-	-
	0	112	100.0 a	1.8 a	6.4 a	0.0 c	76.3 a	91.7 a
	200	271	100.0 a	1.6 ab	2.6 b	1.9 b	48.0 b	85.7 b
	400	512	100.0 a	1.4 b	1.5 c	2.4 b	38.4 bc	83.2 b
	800	1190	50.0 b	1.4 b	1.0 c	3.2 a	31.8 c	77.4 c
P. x *hortorum*	initial	-	-	1.0	3.0	-	-	-
	0	112	100.0 a	1.4 a	4.5 a	0.3 c	80.1 a	92.5 a
	200	271	100.0 a	1.2 ab	1.5 b	0.9 bc	39.3 b	86.3 b
	400	512	75.0 b	1.2 ab	1.3 b	2.3 a	36.5 b	83.4 bc
	800	1190	16.5 c	1.0 b	1.0 b	2.0 a	36.8 b	78.9 c
P. x *peltatum*	initial	-	-	1.2	1.8	-	0.0	0.0
	0	112	100.0 a	1.7 a	4.0 a	0.0	47.6 a	92.5 a
	200	271	100.0 a	1.2 b	2.0 b	0.0	18.2 b	79.6 b
	400	512	0.0 b	-	-	-	-	-
	800	1190	0.0 b	-	-	-	-	-

*Osmolarity (osmotic stress) of culture media in milliosmol kg^{-1} H_2O. Species were analyzed separately. Means with the same letter in the same column in each part (specie) are not significantly different at (p=0.05)

Table 3: Evaluation of different *Pelargonium* species for drought tolerance (osmotic stress) induced *in vitro* by mannitol after four weeks of shoots subculture on liquid medium

Pelargonium specie	mannitol (mM)	O.S.*	survival (%)	rooting (%)	shoot length (cm)	green leaves no/plant	dry leaves no/plant	dry weight (mg)	humidity (%)
P. x *domesticum*	initial	-	-	-	1.0	1.0	-	-	-
	0	118	100.0 a	100.0 a	2.7 a	7.2 a	0.2 b	71.2 a	91.9 a
	200	268	100.0 a	100.0 a	1.5 b	2.3 b	1.5 a	23.2 b	85.5 ab
	400	513	100.0 a	50.0 b	1.1 bc	0.4 c	1.7 a	14.6 c	80.2 bc
	800	1100	40.0 b	0.0 c	1.0 c	0.0 c	2.0 a	9.8 c	75.5 c
P. graveolens	initial	-	-	-	1.0	1.0	-	-	-
	0	118	100.0 a	100.0 a	1.8 a	6.7 a	0.2 b	50.6 a	93.1 a
	200	268	100.0 a	100.0 a	1.4 ab	3.0 b	2.7 a	25.0 b	87.5 ab
	400	513	75.0 b	50.0 b	1.1 b	0.2 c	2.1 a	11.9 c	83.4 b
	800	1100	20.0 c	0.0 c	1.0 b	0.5 c	2.5 a	9.5 c	70.9 c
P. x *hortorum*	initial	-	-	-	1.0	1.0	-	-	-
	0	118	100.0 a	100.0 a	2.1 a	5.2 a	0.1 b	60.2 a	91.9 a
	200	268	100.0 a	100.0 a	1.5 b	2.2 b	1.9 a	31.4 b	85.5 ab
	400	513	25.0 b	12.5 b	1.0 b	0.3 c	2.0 a	15.0 c	81.7 b
	800	1100	0.0 c	-	-	-	-	-	-
P. x *peltatum*	initial	-	-	-	1.0	1.0	-	-	-
	0	118	100.0 a	100.0 a	1.9 a	2.5 a	0.3 b	36.1 a	91.0 a
	200	268	75.0 b	100.0 a	1.2 b	1.0 b	1.7 a	12.9 b	86.1 ab
	400	513	25.0 c	12.5 b	1.1 b	0.7 b	1.7 a	14.7 b	81.6 b
	800	1100	0.0 d	-	-	-	-	-	

* Osmolarity (osmotic stress) of culture media in milliosmol kg^{-1} H_2O. Species were analyzed separately. Means with the same letter in the same column in each part (specie) are not significantly different at (p=0.05)

Table 4: Correlation coefficient (r) between studied parameters used for drought tolerance evaluation of *Pelargonium* species

	O.S.*	survival (%)	rooting (%)	shoot length (cm)	green leaves no/plant	dry leaves no/plant	dry weight (mg)	humidity (%)
O.S.*	1.000	-0.860	-0.913	-0.886	-0.711	0.122	-0.763	-0.748
survival (%)	-	1.000	0.921	0.829	0.650	0.005	0.701	0.748
rooting (%)	-	-	1.000	0.819	0.718	-0.141	0.723	0.633
shoot length (cm)	-	-	-	1.000	0.844	-0.164	0.930	0.796
green leaves no/plant	-	-	-	-	1.000	-0.453	0.953	0.474
dry leaves no/plant	-	-	-	-	-	1.000	-0.407	0.376
dry weight (mg)	-	-	-	-	-	-	1.000	0.586
humidity (%)	-	-	-	-	-	-	-	1.000

•Osmolarity (osmotic stress) of culture media in milliosmol kg^{-1} H_2O. The general correlation between the two methods of evaluation (rooted plants and subculture shoots) is 0.912187

obtained using one of the two tested methods. So, subcultured shoots was maintained for time economy compared to rooted plants needing one month for rooting before evaluation. The highest and/or the intermediate level of osmotic stress can be used for evaluation basing on the above mentioned results.

Selection of Pertinent Parameters: Correlations between studied parameters used for evaluation of *pelargonium* species using the subcultured shoots as explants are presented in Table (4). Osmotic stress level was found to be very well negatively correlated with rooting percentage (r= 0.91), survival percentage (r= 0.89) and shoot length (r= 0.86). It gave determination coefficient (r^2) of 0.83, 0.78 and 0.74 respectively. It means that any of these parameters can explain from 74% to 83% of variability found among species. These three parameters correlated with other parameters which make it possible to use them as good indicators (Table 4). They were also positively correlated together with correlation coefficient ranged from 0.82 to 0.92 which make it possible to evaluate species using one of them only. Because of simplicity and importance of survival and rooting percentages, these parameters can be used for stress tolerance evaluation.

Evaluation of *Pelargonium* Species: Basing on the obtained results, induction of osmotic stress using mannitol in liquid media, using subcultured shoots as

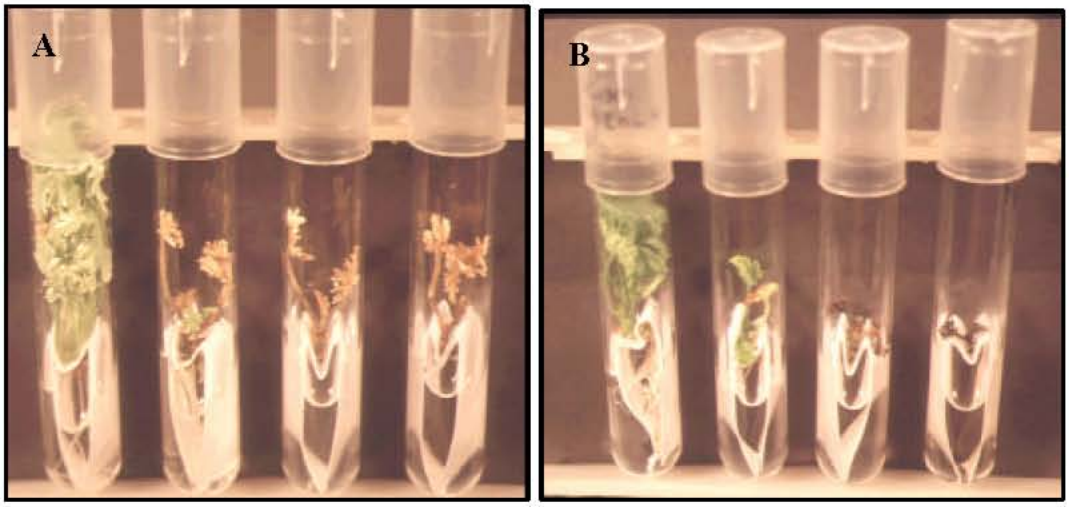

Fig. 1: Growth of subcultured shoots of *P. graveolens* (A) and *P.* x *hortorum* (B) after four weeks on liquid media containing four levels of osmotic stress induced by Mannitol

explants, applying 400 or 800 mM of mannitol as osmotic stress levels and recording survival and rooting as parameters were selected for simple and efficient evaluation of studied species. Survival percentage ranged from 25% for *P.* x *peltatum* and *P.* x *hortorum* to 75% for *P. graveolens* and 100% for *P.* x *domesticum* at 400 mM of mannitol (Table 3 and Figure 1). Rooting percentage was 12.5% for the first two species and 50% for the second two species under the same level. At 800 mM of mannitol, survival percentage ranged from 0% for *P.* x *peltatum* and *P.* x *hortorum* to 20% for *P. graveolens* and 40% for *P.* x *domesticum* but no rooting was obtained for all species. Therefore, *P.* x *domesticum* was the most tolerant followed by *P. graveolens* whereas *P.* x *peltatum* and *P.* x *hortorum* were the most sensitive.

DISCUSSION

Drought stress is a major abiotic stress factor affecting significantly success of plant production. Evaluation for drought tolerance is an essential step for plant improvement. We have previously reported on evaluation of drought tolerance of *Pelargonium* x *hortorum* under greenhouse conditions [6]. The *in vitro* screening method could be an effective method to screen accurately large set of germplasm within short time. The present work aimed to establish an efficient *in vitro* method that can be simply used for evaluation of drought tolerance in *pelargonium*. Mannitol was found to be more efficient than polyethylene glycol (PEG) as osmotic agent. Mannitol is an organic compound and sugar alcohol largely used as osmoticum in protoplast culture and in selection for drought tolerance [13, 17]. It was previously reported that the polyether compound (PEG) had a toxic effect on plant cells [21, 22]. The liquid medium h ad a favorable effect on rooting compared to the solid medium which can be explained by the more nutrients and water availability in such media. The similar indicators obtained using subcultured shoots or rooted plants and the good correlation found between their results allowed the selection of subcultured shoots as explants for the simplicity of achievement and the economy of time. These explants were used successfully in solid medium for evaluation of *Populus euphratica* [14]. The high level of osmotic stress was found to be more selective than the low level which can facilitate distinguishing between tolerant and sensitive genotypes. All growth parameters decreased with increasing osmotic stress. Growth reduction under drought stress may have an adaptive role by restricting the evaporative surface area [23]. Correlation among parameters showed the importance of survival and rooting for selection. Indeed, rooting is needed for absorption of water and nutrients indispensable for growth continuity. Variability was found in osmotic stress tolerance of tested *pelargonium* species. Similar results were previously found under greenhouse conditions [6, 16]. The studied species also showed different responses in regeneration under *in vitro* conditions [24]. The overall results ensure the establishment of simple *in vitro* method capable to evaluate drought tolerance of different species.

CONCLUSION

An efficient *in vitro* method was established for drought tolerance evaluation in *pelargonium*. Two osmotic agents, two media types, four osmotic stress levels and two explants were studied for the optimization of method using four *pelargonium* species. The established method can be achieved by subculture of shoots (1 cm in length) in paper bridges on liquid media containing 400 or 800 mM of mannitol for four weeks. Survival and rooting percentage was found to be efficient as indicators for drought tolerance. Application of this method on the tested *pelargonium* species showed variability in their drought tolerance. *P.* x *domesticum* was the most tolerant followed by *P. graveolens*. However, *P.* x *hortorum* and *P.* x *peltatum* were the most sensitive species. The efficiency and simplicity of established method could make it possible to evaluate a large number of genotypes in very short time.

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