

Establishment of an *in vitro* culture of *Pelargonium* × *domesticum* cultivars characterized by different growth requirements

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Abstract

Experiments were carried out to develop micropropagation protocols for cultivars of hybrid origin which have been not cultivated *via* tissue culture. Proliferating cultures of *Pelargonium* × *domesticum* “Tip Top Duet” and “Black Knight” were obtained even if initially the number of excised aseptic nodal pieces was considerably reduced due to contamination with endogenous bacteria. The supplementation of a maintenance medium with either 100 or 200 mg/l peptone proved beneficial. The best propagation coefficient, exceeding 82 adventitious shoots from one initial microcutting, was obtained on a modified MS medium with an addition of 200 mg/l peptone and 0.5 mg/l adenine. The regenerated shoots readily developed new, anatomically properly formed leaves. In the plant material studied, the leaf epidermis produced glandular trichomata which were similar in structure to those observed in a standard cultivar.

Keywords: micropropagation, *Pelargonium*, plant tissue culture, Geraniaceae, peptone

Supplementary Table 1. The composition of propagation media P_K, P₁, and P₂ used for multiplication of *Pelargonium* cultivar shoot cultures

		P _K	P ₁	P ₂
MS	Macroelements	250 ml/l		
	Microelements	125 ml/l		
Fe-EDTA		20 ml/l		
Vitamins and amino acids	Glycine	4 mg/l		
	Thiamine	0.5 mg/l		
	Pyridoxine	0.5 mg/l		
	Nicotinic acid	0.5 mg/l		
	Ascorbic acid	2 mg/l		
Mezoinozytol		100 g/l		
Sucrose		30 g/l		
Peptone		100 mg/l	200 mg/l	
Adenine		–	0.5 mg/l	
PGR	BAP	0.2 mg/l	0.3 mg/l	0.4 mg/l
	IAA	0.05 mg/l	0.05 mg/l	0.1 mg/l
pH		5.8		
Agar Difco		8 mg/l		

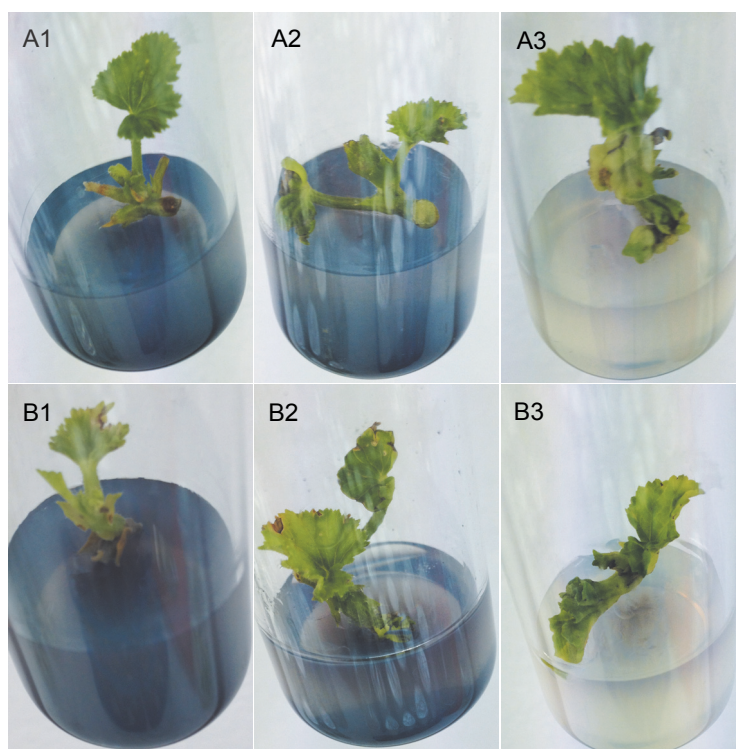
Supplementary Table 2. Percentage of regenerated and contaminated primary explants of *Pelargonium* × domesticum cultivars during culture initiation stage on media E₅, and E_{5c} (supplemented with charcoal)

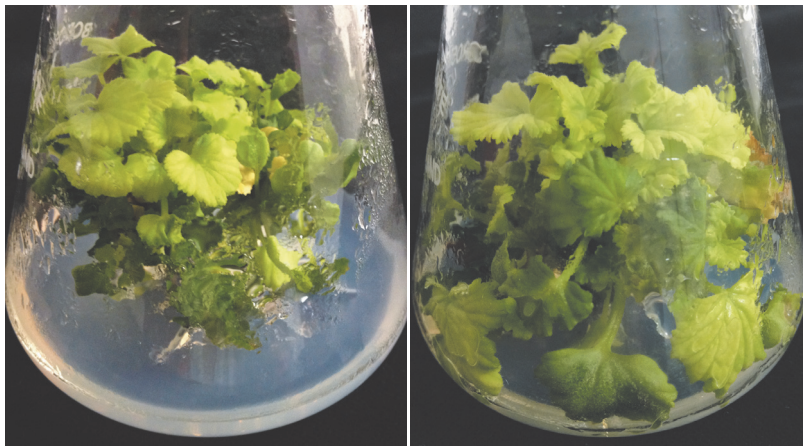
Cultivar	I verification after 28 days						II verification after 56 days					
	Regeneration			Remove index ^x			Regeneration			Remove index ^x		
	E ₅	E _{5c}	Mean	E ₅	E _{5c}	Mean	E ₅	E _{5c}	Mean	E ₅	E _{5c}	Mean
“Black Knight”	67	100	83.5	33	1	17	100	100	100	2	1	1.5
“Tip Top Duet”	100	100	100	2	3	2.5	85	100	92.5	0	0	0

Supplementary Table 3. Mean proliferation rate in shoot cultures obtained from secondary explants of *Pelargonium* × domesticum cultivars on different propagation (P) media

Cultivar	Medium	I passage		II passage
		Propagation coefficient after 14 days	Propagation coefficient after 28 days	Propagation coefficient after 56 days
<i>Pelargonium</i> “Black Knight”	P _K	16.5	50.5 cd*	64.0 bc
	P ₁	21.5	49.7 cd	65.0 bc
	P ₂	21.0	42.0 e	52.8 c
<i>Pelargonium</i> “Tip Top Duet”	P _K	17.6	49.8 cd	71.3 b
	P ₁	22.0	54.5 cd	82.5 a
	P ₂	22.2	52.5 c	74.5 b

* mean values are given, means followed by the same letter do not differ significantly $\alpha = 0.05$

**Supplementary Figure 1.** Representative shoots of *Pelargonium* × domesticum obtained in 21 days from primary explants on medium E_C (1-2) and on medium E (3): A) “Black Knight”, B) “Tip Top Duet”



Supplementary Figure 2. Proliferative shoot culture on P₁ propagation medium
- *Pelargonium* × *domesticum* “Black Knight” (left), “Tip Top Duet” (right)