IMPROUVED METHOD FOR THE "IN VITRO" REGENERATION OF TRUE TO TYPE PLANTS OF *PELARGONIUM PELTATUM* L.

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Abstract. *The economical importance of „in vitro" tissue culture at ornamental plants is a actual problem, the existing results underlying the fact that over 500 million of plants are micro propagated annually, the biggest part of them being ornamental plants.*

The objective of this study was to develop a rapid system for regeneration of the important ornamental plant, Pelargonium peltatum L, from nodal explant. Single node explants were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) and GA_3 for direct plant regeneration. Maximum numbers of shoot (14.3±1.5) were observed on the medium containing 2.0 mg/l BAP and 1.0 mg/l NAA after two - three weeks of culture.

Regenerated shoots were separated and rooted on same MS medium supplemented with NAA alone (in different concentrations) for 8-10 days. Well-developed complete plantlets were transferred on to plastic cup containing soil substrate. Acclimatized plantlets were successfully grown in greenhouses.

Rezumat. *Importanța culturilor de țesuturi"in vitro" la plantele ornamentale este o problemă de actualitate, rezultatele obținute până acum subliniind o dată în plus acest aspect. Din cele peste 500 de milioane de plante multiplicate anual, proporția cea mai mare o dețin plantele ornamentale*

Principalul obiectiv al acestui studiu este dezvoltarea unui sistem rapid de regenerare la Pelargonium peltatum L,utilizând ca inocul inițialexplantele nodale. Acestea au fost inoculate pe un mediu bazal MS (Murashige and Skoog, 1962) conținând 3% zaharoză, suplimentat cu diferite concentrații şi combinații de 6-benzilaminopurină (BAP), acid indolil-3 acetic (IAA) sau acid indolil 3 butiric (IBA) şi GA_3. Numărul maxim de lăstari (14.3±1.5) a fost obținut, după 2-3 săptămâni de cultură, pe mediu conținând 2.0 mg/l BAP şi 1.0 mg/l NAA.

Lăstarii regenerați au fost înrădăcinați pe acelaşi mediu de bază MS suplimentat cu NAA, în diferite concentrații, pe o perioadă de 8-10 zile. Plantele complet dezvoltate au fost transferate pe substrat de sol, după aclimatizare fiind crescute în sere.

INTRODUCTION

The genus *Pelargonium* L. is a rather big one and is comprised of well over 200 species. The majority are native to the Southern part of the African continent.

Because of their ability to survive in arid conditions, because of their adaptability, and, last but certainly not least, because of their beautifully colored flowers, there are probably only a few gardeners who have never grown pelargoniums. Most of the balcony and windowsill plants are, however, cultivars. Controlled hybridization started two centuries after their arrival to Europe in 1600 and reached its peak in Victorian England.

Micropropagation (in vitro propagation of axillary and/or adventitious buds as well as somatic embryos) is presently used as an advanced biotechnological system for the production of identical pathogen-free plants. Conventional techniques of vegetative propagation of *P. peltatum* based on cuttings are difficult because of the low rates of rooting. The cells and tissues cultures "in vitro" assure a unique opportunity to manipulate the morphogenesis in a perfectly controlled medium, thus offering a powerful complementary instrument that can help in overcoming such problems.

Plant regeneration in vitro is dependent on the manipulation of the inorganic and organic constituents in the medium, as well as the type of explant and the species. In most plants, successful regeneration from the callus or directly from the explants takes place after a series of subcultures in various media, in a sequence which is often specific to the species, variety, or the newly introduced genotype. The determining factors are the combination of the concentration in relation to medium volume and the composition of growth promoting and retarding regulators in the medium, the physiological status and competence of the cells and their capability for morphogenetic expression.

MATERIALS AND METHODS

Explants were collected from healthy, mature mother plants maintained at Vegetable Research and development Station Bacau. The young shoots excised from this actively growing plant,.were cut into 1.0 to 2.0 cm nodal segments and used for induction of multiple shoots.

Explants were washed thoroughly under running tap water for 30 min and treated with a surfactant, Tween 20 (10 drops per 100ml of sterilized distilled water). Then these explants were surface sterilized with 0.1% mercuric chloride (w/v) for 15 min and washed repeatedly using sterilized distilled water. Under aseptic conditions, explants were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine, kinetin, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or gibberellins (GA_3) for direct plant regeneration and root induction (Table 1). The pH was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C (1.06 kg/cm2) for 15 min.

Cultures were incubated at 25±1°C under 16 hr photoperiod of 3000-lux light intensity.

The cultures were transferred at a 2 weeks interval on fresh media, for a period of 90 days. Observation of shoot multiplication and growth were recorded at weekly intervals. After two weeks, shoots of above 2.0 cm length were harvested and subcultured on the same medium for the continuation of the regeneration processes.

Table 1

Variants of nutritive medium with different hormonal factors utilized for "in vitro" regeneration

Components	P_1	P_2	P_3	P_4	P_5	P_6	P_7
Macroelements	MS	MS	MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS	MS	MS
BAP	2.0	2.0	3.0	2.0	-	-	-
KIN	-	-	-	-	2.0	2.0	2.0
NAA	1.0	-	-	-	1.0	-	-
IAA	-	-	1.5	-	-	-	1.5
IBA	-	1.0	-	-	-	1.0	-
GA_3	-	-	-	1.0	-	-	-
Sucrose	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l	30g/l
Agar	8 g/l	8 g/l	8 g/l	8 g/l	8 g/l	8 g/l	8g/l
pH	5,8	5,8	5,8	5,8	5,8	5,8	5,8

A part of the newly formed shoots that demonstrated a good development of leafs were transferred to rooting medium containing different concentration of NAA (table 2).

Table 2

Variants of rooting medium with different concentration of NAA auxine

Components	R_1	R_2	R_3
Macroelements	MS	MS	MS
Microelements	MS	MS	MS
Vitamins	MS	MS	MS
NAA	0,6 mg/l	0,8 mg/l	1 mg/l
Sucrose	30 g/l	30 g/l	30g/l
Agar	8 g/l	8 g/l	8g/l
pH	5,8	5,8	5,8

After 2 weeks, the rooted plants were acclimatized and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for acclimatization during 2 weeks before transfer to green house.

RESULTS AND DISCUSSIONS

Shoot buds got initiated on nodal segments after 6 days of culture. Only 0.04% of the shoots didn`t have any positive development, the noted reaction being toward partial or total necrosis of the tissues. The higher frequency (97.5%) formation of maximum number of shoots was observed in 2 mg/l BAP in combination with 1.0 mg/l NAA (variant P1). Initially 1 or 2 buds developed, later upto 12 shoots of above 4 cm length were formed in node in two weeks. KN in combination with NAA was less effective than BAP as it induced only up to 72.0 % formation of 6 - 7 shoots.

Table 3

Effects of different concentrations of BAP, KN alone and in combination with IBA, IAA or GA_3 in MS medium for multiple shoot induction from node explants of *Pelargonium peltatum* L.

Variant	% of explant showing response	Average no. of shoots	Average length of shoots
P_1	97.5	14.3±1.5	3.7±0.38
P_2	85.0	13.9±2.9	3.5±0.28
P_3	90.0	12.0±0.5	4.1±0.52
P_4	83.9	7.9±0.5	4.4±0.46
P_5	72.0	8.0±0.5	3.1±0.60
P_6	75.5	11.0±0.4	3.4±0.29
P_7	74.7	10.8±1.6	3.3±0.34

Preliminary studies proved that nodal explants culture in MS medium individually supplemented with both BAP and KN showed remarkable response. Among cytokinins, 2.0 mg/l BAP responds well compare to KN in medium for shoot proliferation (see Table 3). In order to evaluate the synergistic effect of BAP with IAA, IBA and NAA for direct plant regeneration, NAA combinations responded well compare to IAA or IBA. The maximum induction of multiple shoots (14.3±1.5) was achieved from medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA, 2 to 3 weeks after incubation, with an average shoot length of 3.7 cm (Figures 1 a-c).

Among the concentrations tested, the best response was noticed with 2.0 mg/l BAP and 1.0 mg/l NAA. Normally, other species like *P. peltatum* shows good response towards plant regeneration in MS medium in the presence of BAP combined with auxins as reported by various authors.

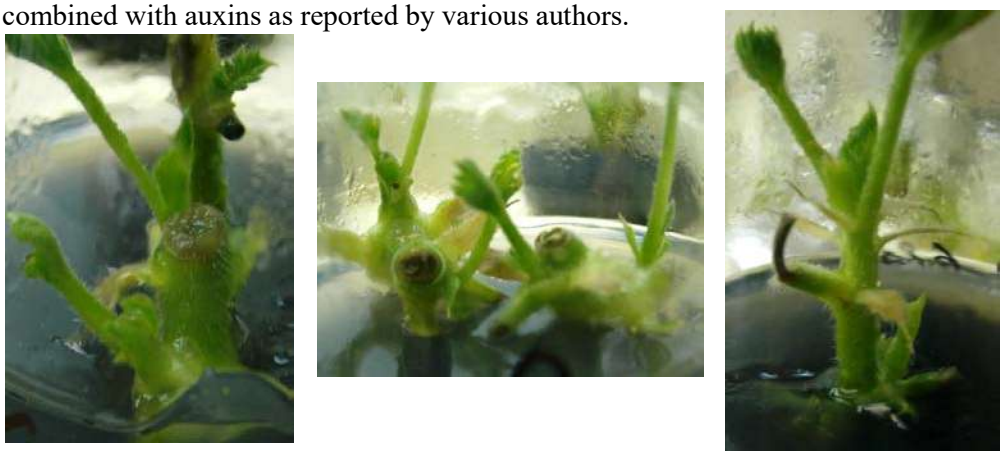

Fig. 1 a-c: Different aspects of shoots proliferation "in vitro"

After 3 to 4 weeks, when regenerated shoots reached a length of more than 4.0 cm, they were separated and transferred on MS basal medium with different concentration of NAA.

Table 4

Effects of different concentrations of NAA in MS medium for root induction from shoots of *Pelargonium. peltatum* L.

Variant	% of rooting response	Average length roots (cm)
R_1	**98.9**	2.6±0.29
R_2	**97.3**	2.9±0.26
R_3	**97.1**	3.1±0.50

The results obtained on the rooting medium shows the fact that NAA is ended the best auxin for root induction, the rooting reaction being positive on all the variants. Whereas root primordial emerged from the shoot base on first week of culture on auxin- supplemented medium. Maximum percent of rooting response (98.9%) were produced on R_1 variant characterised by the addition of 0.6 mg/l NAA in the basal MS medium. (Figure 2).

Fig. 2 – General aspects of shoots on rooting medium

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Fig. 3 – Plants on acclimatization stage

Fig. 4 – Acclimatized plants

For acclimatization – figure 3, plantlets were removed from rooting medium after twenty days of incubation and transferred to plastic pots containing soil substrate – fig. 4 and covered with perforated polythene bags to maintain humidity and were kept under culture room conditions for one week

After two weeks, polythene bags were removed and transferred to green house and placed under shade until growth was observed.

CONCLUSIONS

Direct shoot multiplication is possible for generating true-to-type plants of *Pelargonium peltatum*. This study supports the rapid multiplication of this useful medicinal plant by *in vitro* conditions. This report provides a simple protocol for the micropropagation of *Pelargonium peltatum*. Shoots can be easily derived from node cultures on BAP containing medium and subsequently rooted on NAA containing medium. The efficiency of the system could be improved to give rise to more shoot proliferation. This approach offers a means for producing identical plantlets from node explant of *Pelargonium peltatum.*

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