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MICROPROPAGATION OF *MANDEVILLA SANDERI* (HEMSL.) WOODSON

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Abstract: *Mandevilla sanderi* (syn. *Dipladenia sanderi*) is a liana, originating in South America, also known as Brazilian Jasmine. There is a wide variety in color: white, rose, red, yellow, and two-color varieties which are mainly propagated vegetatively. Plantlets were successfully regenerated from nodal segments, excised from young stems. MS medium with vitamins plus 1 mg^{-1} BAP was used as starting medium. In multiplication stage, the effect of two cytokines was tested: BAP and kinetin with supplemented 0.1 mg^{-1} IBA. Rooting was achieved by transfer of the isolated segments to fresh MS medium without plant growth regulators. Good response was obtained after acclimatization to ex vitro conditions.

INTRODUCTION

Mandevilla sanderi aerial system consists of indefinite and continuously growing stems with axillary inflorescences with beautiful and abundant flowering. The species is characterized by the presence of laticifers, which are presented in all of the plant's organs, whether they are below or above ground. Biochemical analysis revealed that the tuberous roots have a high starch storage capacity. These reserves commonly confer a large capacity for adaptation to stressful conditions to the plant (Boutebtoub *et al.,* 2009).

In its natural habitats (the region of Rio de Janeiro), Brazilian Jasmine reaches a length of up to 6.5 m and blooms from May to October. In a moderate continental climate it cannot survive winter conditions and no seed formation is observed. It is mainly propagated vegetatively by cuttings. In Bulgaria it blooms outdoors from July to the middle of September. When plants are kept in temperatures under 8°C, they enter dormancy, characterized by defoliation.

The aim of the study was to develop an efficient protocol for *in vitro* propagation of *Madevilla sanderi* by using nodal segments as staring material.

MATERIALS AND METHODS

Vegetative material from *Mandevilla sanderi* was collected from the greenhouse of the Institute of Ornamental and Medicinal Plants – Sofia (IOMP). Fifty explants with one nodal segment were first wash with soapy water by stirring and after that with distilled water. They were pre-sterilized by immersion in 70% ethanol for 40 s and then surface-sterilized with 1.5 % solution of NaClO for 20 min and rinsed three times in sterile distilled water. Each explant was then transferred to culture tube with 10 ml of MS medium (Murashige and Skoog, 1962), including vitamins with 1 mg.l-1 N^6-Benzil adenine (BAP), 6 $g.l^{-1}$ agar and 30 $g.l^{-1}$ sucrose. The pH of the medium was adjusted to 5.73 before sterilization (20 min, 121°C, 1 215.9 hPa in autoclave). Cultures were maintained at 22-23 °C with 16 h/8 h light/dark photoperiod with 30 μmol/m^2.s photon flux density in all cases.

After 60 days, nodal segments (approx. 1-2 cm long) were excised from explants and inoculated onto multiplication medium: MS with vitamins, supplemented with BAP or kinetin in different concentrations (Table 1) and 0.1 $mg.l^{-1}$ Indole-3-butyric acid (IBA).

Table 1 Different concentrations of plant regulators in multiplication mediums

Variant	MS	BAP	Kinetin	IBA	Agar	Sucrose
	/$g.1^{-1}$/	/$mg.l^{-1}$/	/$mg.l^{-1}$/	/$mg.l^{-1}$/	/$g.1^{-1}$/	/$g.1^{-1}$/
I – control	4.anp	-	-	-	6	30
II	4.anp	0.1	-	0.1	6	30
III	4.anp	0.3	-	0.1	6	30
IV	4.anp	0.5	-	0.1	6	30
V	4.anp	1	-	0.1	6	30
VI	4.anp	2	-	0.1	6	30
VII	4.anp	-	0.1	0.1	6	30
VIII	4.anp	-	0.3	0.1	6	30
IX	4.anp	-	0.5	0.1	6	30
X	4.anp	-	1	0.1	6	30
XI	4.anp	-	2	0.1	6	30

Percentage of multiplied plants and number of cuttings by explant was recorded on the 30th day of cultivation. Multiplication was estimated as a "proliferation rate", which is calculated using the formula:

$$\mathbf{PR = N \times A/100}$$

where **PR** – proliferation rate, **N** - number of new nodal segment observed per explant, **A** – percentage of multiplied plants.

The statistical processing of the data was analyzed by t-test. The significant differences between the control and variants were presented as *($P<0.05$), ** ($P<0.01$), *** ($P<0.001$) and the non-significant – ns. Rooting of micro cutting, obtained in the multiplication phase was achieved by transferring to basal MS medium (without vitamins). Plantlets, obtained from the *in vitro* rooting phase were acclimatized to e*x vitro* conditions, establishing them in a potting mix (peat : perlite, 1 : 0.1) and growing in greenhouse.

RESULTS AND DISCUSSION

A high percentage of non-contaminated explants was obtained after sterilization. After 30 days of cultivation on MS medium with 1 $mg.l^{-1}$ BAP, explants regenerated and grew. Between the 45^{th} and the 60^{th} day they reached optimal size and shoots were transferred to multiplication medium.

The study of the specific response of each species to the cytokinin concentration has practical meaning, thus producing accurate *in vitro* proliferation protocols and calculating the expected final number of plants, obtained in the multiplication phase. Many authors claim that BAP is among the most effective and available cytokines used in the commercial micropropagation of plants (Bell and Reed, 2002; Nowell, 2003; Bairu *et al.,* 2007; Vasil, 2008; Hassan *et al.* 2013; Manolova *et al.,* 2014).

In multiplication stage *Mandevilla sanderi* explants showed different response to the two synthetic cytokines BAP (0.1^{-2} $mg.l^{-1}$) and kinetin (0.1^{-2} $mg.l^{-1}$). High percentage (83 % and above) of multiplied plants was observed when cytokines BAP (0.1^{-1} $mg.l^{-1}$) and kinetin (0.3-0.5 $mg.l^{-1}$) were added (**Figure 1**).

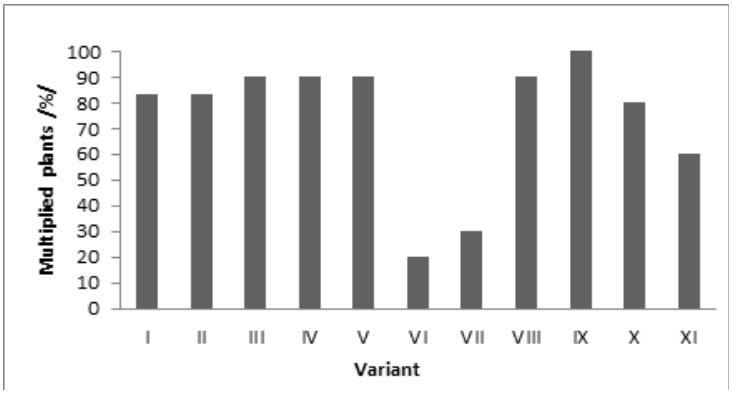

Fig. 1 Effect of different proliferation mediums on the percentage of multiplied plants in M*andevilla sanderi*

In variants with high concentration (2 mg.l^{-1}) BAP and kinetin, unsatisfactory results for regenerated plants were reported. The optimum result of 100 % multiplied plants was obtained only in variant IX – MS + 0.5 mg.l^{-1} kinetin + 0.1 mg.l^{-1} IBA.

Plants, cultivated on medium containing BAP had slow apical growth, compared to those, cultivated on medium with kinetin (**Figure 2**). The addition of BAP caused thickening at the basal part of the nodal segment and callus formation, which probably was the reason for abnormal development. Due to the smaller number of new micro cuttings, the proliferation rate was lower.

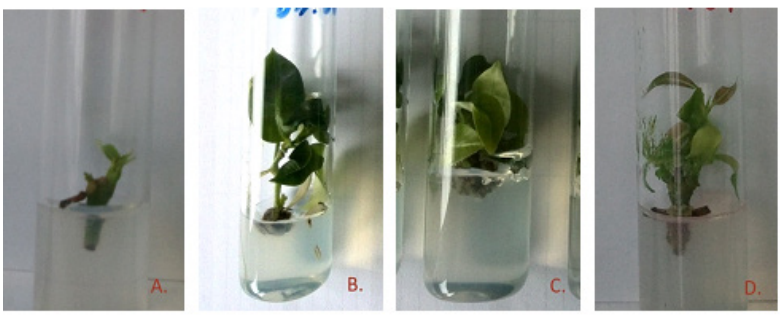

Fig. 2 . Different response in *Mandevilla explants* to cytokines – BAP and kinetin:
A. Control – MS; **B.** Variant IV - MS + 0.5 mg.l^{-1} BAP;
C. Variant V – MS + 1 mg.l^{-1} BAP; **D.** Variant IX – MS + 0.5 mg.l^{-1} kinetin.

The highest proliferation rate of 2.25 in variants with added BAP was obtained at 1 mg.l^{-1} (**Figure 3**). The concentration of 2 mg.l^{-1} slowed the growth of plants and shortened the internodes, making it difficult to take new cuttings. This and the low percentage of multiplied plants were the reasons for the low proliferation rate.

In our experiment we observed the growing of plants after replacing the initially applied cytokine BAP with kinetin. The results showed that plants growing in the presence of kinetin had better formed internodes and were easier to work with. Concentration of kinetin from 0.3 to 1 mg.l^{-1} resulted in high proliferation rate and had very good significant differences with the control variant ($p \leq 0.05$). The highest proliferation rate – 3.2 was obtained in IX variant: MS + 0.5 mg.l^{-1} kinetin + 0.1 mg.l^{-1} IBA.

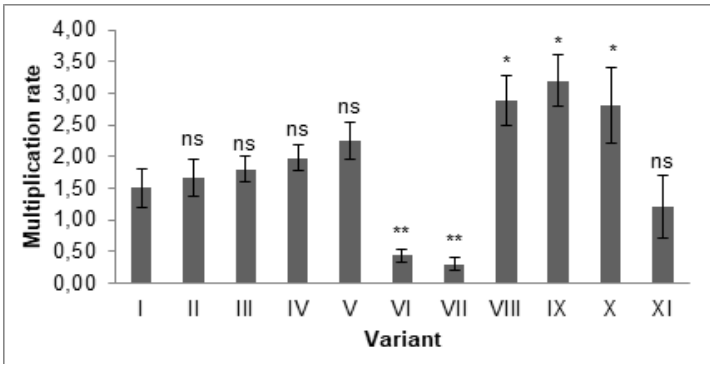

Fig. 3 Effect of different multiplication mediums on the proliferation rate in *Mandevilla sanderi.* * (P≤0.05), ** (P≤0.01), *** (P≤0.001), non-significant – ns

Replacing the initially applied cytokine BAP with kinetin in multiplication phase increased *in vitro* multiplication possibilities of *Mandevilla sanderi*. Our results supports the suggestion of Pratibha *et al.* (2010) that exposure of explants (nodal segments) to higher BAP concentration during induction phase may lead to accumulation of cytokines, which inhibit further shoot growth. After transferring the plants to MS medium without vitamins and growing regulators, high percentage of rooted plant (> 90) was obtained. All plants (100%) were acclimatized to ex vitro conditions which support Boutebtoub et al. (2009) assumption that the species has a large capacity for adaptation to difficult conditions.

CONCLUSIONS

Initial regeneration and shoot formation was observed in the presence of BAP, but further cultivation on medium containing this cytokine inhibits shoot elongation. Replacing BAP with kinetin in multiplication medium increased the proliferation rate. The maximum percentage of multiplied plants and the highest proliferation rate was obtained in variant IX - MS + 0.5 mg. l^{-1} kinetin + 0.1 mg. l^{-1} IBA + 6 g. l^{-1} agar + 30 g. l^{-1} sucrose.

DECLARATION OF INTEREST

The author declare no existing conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

DM designed and performed the experiments, analyzed the data and wrote the manuscript.

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