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In vitro flowering of *Petunia* × *atkinsiana* D. Don

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ABSTRACT

The aim of this study was an attempt to determine the influence of PGRs – auxins, cytokinins and gibberellic acid – on the flowering and morphogenesis of 'Flash Red' *Petunia* × *atkinsiana* D. Don *in vitro*. The best results at the propagation stage were obtained on an MS medium supplemented with 0.5 mg dm^{-3} GA_3. The petunia plants propagated under these conditions developed high main shoots and a large number of leaves and adventitious shoots. The plants with the best-developed root system were regenerated on an MS medium supplemented with 0.5 and 1.0 mg dm^{-3} IAA. Flowering initiation should be performed using an MS medium supplemented with 0.5 mg dm^{-3} KIN.

Key words: micropropagation, morphogenesis, plant growth regulators

INTRODUCTION

Petunia × *atkinsiana* D. Don is a hybrid derived from two endemic species of South America, *Petunia integrifolia* and *Petunia axillaris*. This species has been grown all over the world in gardens and on balconies (Stehmann et al. 2009). At present, many varieties of this plant are available, varying in resistance to drought and diseases, soil requirements, conformation, flower size and colour. Due to its great popularity among consumers, breeding works are being conducted in order to develop new interesting varieties and propagate them on a large scale (Tsuda et al 2004).

In recent years, there has been a great interest among breeders in biotechnological methods including *in vitro* culture, which can accelerate and intensify the breeding process. They are used, first of all, in order to obtain a large population of progeny genetically identical with the maternal plants in a short amount of time. Several reports indicate sporadic flowering and seed setting under these conditions in: roses (Wang et al. 2002), *Spathiphyllum* (Dewir et. al. 2007), orange jessamine (Jumin and Ahmad 1999), orchids (Tee et al. 2008), bamboo (Lin et al. 2003, 2007, Nadgauda et al. 1997, Ramanayake et al. 2001), gentian (Zhang and Leung 2002), *Dioscorea zingiberensis* (Huang et al. 2009) and date palm (Masmoudi-Allouche et al. 2010). Developing the methods of inducing petunia flowering *in vitro* would facilitate the fast selection of plants with a desired phenotype for plant breeders. This system would be very useful for researchers studying flower development. Flowering plantlets *in vitro* have good commercial potential as ornamentals as well.

Plant flowering is controlled by many factors, both genetic and environmental. The most important factors include: medium pH (Nadgauda et al. 1997), the content of sucrose (Lin et al. 2003, Aneesh et al. 2007) or agar in the medium (Kachonpadungkitti et al. 2001), light intensity and even the size of explants (Taylor et al. 2005). However, the most important of these is the content of plant growth regulators added to media *in vitro* (Levy and Dean 1998, Taylor and van Staden 2006). However, the application of phytohormones is not easy, as their effects depend on their concentration, *in vitro*

conditions, and plant species. Therefore, in each case the kind and concentration of applied growth regulators must be chosen experimentally.

Hence, the aim of this research was to determine the influence of the concentration of auxins, cytokinins and gibberellic acid in the medium on petunia ('Flash Red' *Petunia* × *atkinsiana* D. Don) propagated *in vitro* and also the choice of the optimal media for propagation and rooting as well as the induction of flowering *in vitro.*

MATERIAL AND METHODS

'Flash Red' *Petunia* × *atkinsiana* D. Don grown on an MS medium (Murashige and Skoog 1962) without growth regulators was the plant material. Explants for the experiment consisting of one-node shoot fragments, ca 1-cm long, were placed on the MS medium supplemented with 0.5 and 1.0 mg dm^{-3} KIN (kinetin), BAP (6-benzylaminopurine), IAA (indole-3-acetic acid) and GA_3 (gibberellic acid). Plants on an MS medium without growth regulators were the control group, and 100 explants were placed on each medium.

After adding growth regulators into the media, their pH was adjusted to 5.7 using 0.1 M solution of HCl and NaOH. The media were supplemented with 7 g dm^{-3} agar and 30 g dm^{-3} sucrose and heated to cause agar polymerization; 300-ml jars filled with 30 ml of medium were autoclaved at 121°C for 20 minutes.

Next, the cultures were kept in a growth chamber at a temperature of 25°C, with daylight at a 16-h photoperiod. Four weeks later, biometric measurements were taken and the length of the main shoot (mm), number of axillary shoots, plant weight (g), number of leaves, root length (mm), number of roots and number of formed flower buds were determined. The significance of differences was verified using analysis of variance and the Tukey test at $\alpha = 0.05$. Homogenous groups between the examined combinations were labelled in the table with successive letters of the alphabet.

RESULTS AND DISCUSSION

The studies conducted show that the addition of plant growth regulators to the media has a highly significant influence on petunia morphogenesis and flowering. The development of the aboveground part was particularly strongly stimulated by 0.5 mg dm^{-3} of gibberellic acid (Tab. 1). Plants from the media with 0.5 mg dm^{-3} GA_3 produced the longest main shoots (70.97 mm). Petunias regenerated on that medium were characterised by the largest number of leaves (30.26), which is in agreement with the findings of Farhatullah et al. (2007) in the studies on the effects of growth regulator on explants of potato ('Desiree' *Solanum tuberosum*), which like petunia belongs to the family *Solanaceae*. In this case, 1.0 mg dm^{-3} of gibberellic acid significantly affected the development of foliage in comparison with the control group.

IAA was found to be the strongest growth regulator inducing rhizogenesis, irrespective of the applied concentration. Petunias propagated on the media with IAA formed about 40% more roots than the control plants (on average 5.54 and 5.17 roots). The root length of petunias rooted on the media supplemented with IAA was close to those regenerated on the MS medium. A similar relationship was observed in the studies by Witomska and Ładożyńska (2001) on petunia of the group Ursynia, in which the addition of 1.0 mg

Table 1. The influences of growth regulators on shoot and root formation of *Petunia* × *atkinsiana* D. Don.

Plant growth regulators	Concentration (mg dm^{-3})	Length of main shoot (mm)	Number of axillary shoots	Number of leaves	Fresh weight (g)	Number of roots	Length of root (mm)
BAP	0.5	58.07 abc*	1.95 bcd	19.90 b	1.27 b	1.23 e	24.39 abc
	1.0	63.29 abc	2.73 bc	21.01 b	1.42 b	3.40 bcd	47.88 a
GA_3	0.5	70.97 a	4.09 a	30.26 a	1.59 ab	1.56 cde	8.89 bc
	1.0	52.72 bc	4.71 a	32.03 a	1.26 b	0.36 e	2.51 c
IAA	0.5	66.12 ab	2.88 b	21.67 b	2.06 a	5.54 a	40.51 a
	1.0	59.03 abc	1.54 cd	17.12 b	1.66 ab	5.17 ab	44.21 a
KIN	0.5	71.35 a	1.99 bcd	20.06 b	1.44 b	3.64 abc	30.74 ab
	1.0	65.90 ab	2.46 bcd	28.20 a	1.28 b	1.52 de	25.20 abc
MS - Control		46.65 c	1.74 cd	17.87 b	1.27 b	3.77 ab	43.35 a
$LSD_{0.05}$		17.67	1.03	4.88	0.52	2.08	23.52

*Values designated with the same letters within columns do not significantly differ at $\alpha = 0.05$

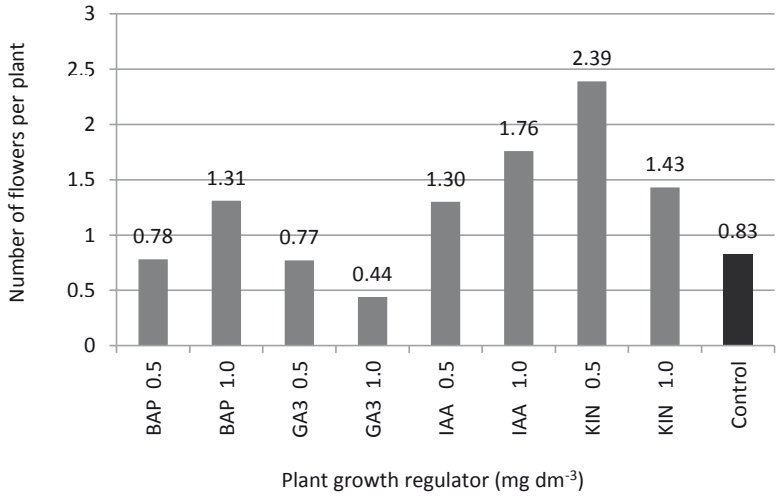

Figure 1. Number of flowers per petunia plant propagated on media with different plant growth regulators

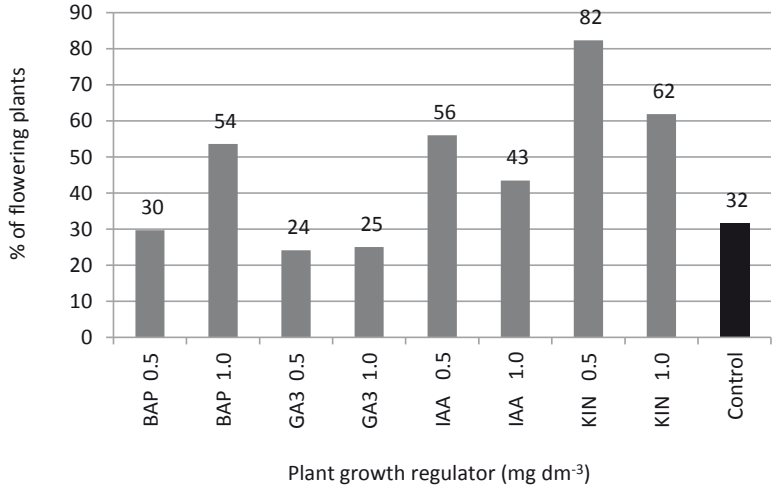

Figure 2. Percentage of flowering petunias propagated on media with different plant growth regulators

dm^{-3} IAA resulted in over three times as many roots as in the control trial and their length was over 20% higher than the roots of petunias regenerated on the medium without growth regulators.

Gibberellic acid and cytokinins (especially BAP and TDZ) are considered growth regulators responsible for inducing flowering *in vitro* (Rout and Das 1994, Nadgauda et al. 1997, Levy and Dean 1998). The application of kinetin for *in vitro* cultures of tomato (*Lycopersicon esculentum*), also belonging to the family *Solanaceae*, resulted in flowering in 66.7% of plants, whereas the lack of plant growth regulators in the media or the addition of gibberellic acid completely inhibited flower morphogenesis (Dielen et al. 2001). Lin et al. (2007) found an advantageous effect in supplementing the media with cytokinis (BAP, TDZ, 2iP, zeatine and kinetin) on flower induction in bamboo. However, only the addition of TDZ and zeatin positively affected the development of inflorescence. Studying the effect of cytokinin on the development of *Dioscorea zingiberensis* in the *in vitro* culture, Huang at al. (2009) observed a beneficial effect of BAP at the concentration 1.0 to 4.0 mg dm^{-3} on inducing flowering. No effect of other growth regulators (IAA, NAA and KIN) was noted. In our research, we found a positive influence of kinetin incorporated in the media on the flowering of regenerated petunias . The addition of 0.5 mg dm^{-3} KIN to the media had the strongest stimulating effect on flower formation – 82% of plants formed flower buds on that medium. These plants also produced the most numerous flowers; on average, their number per plant (2.39) was almost three times greater than in the control group (Fig. 1, 2, Photo 1).

Simultaneously, we observed an inhibiting effect of gibberellic acid on flowering. Petunias growing

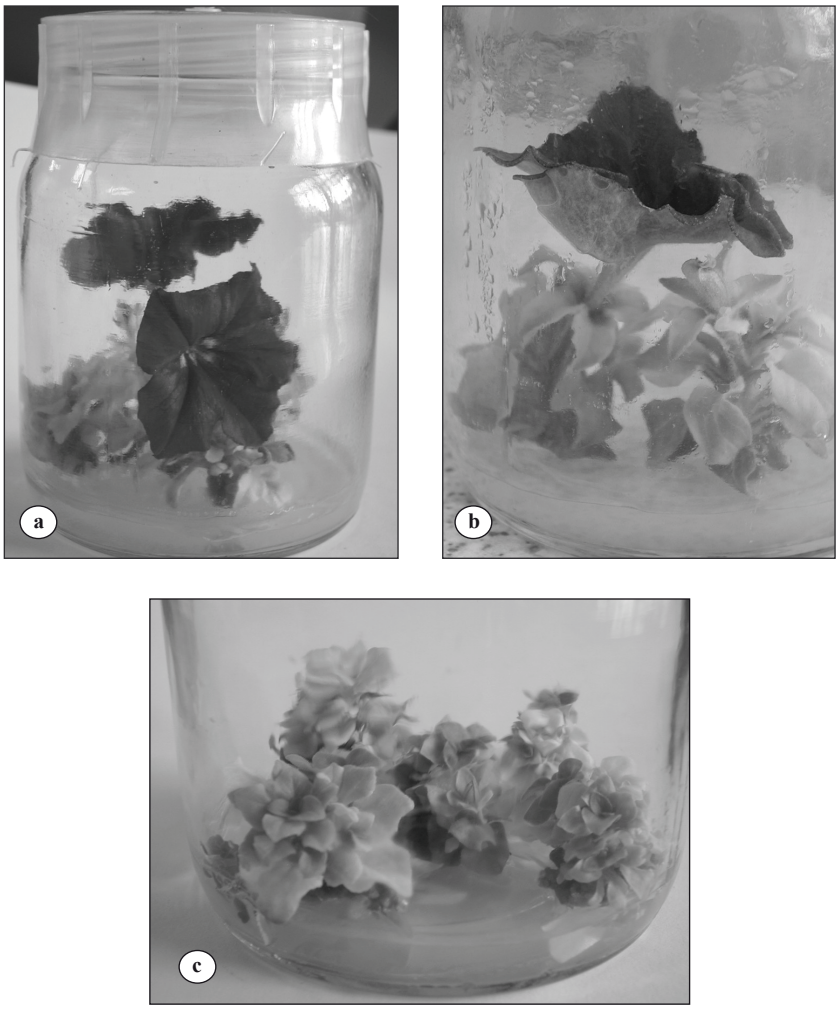

Photo 1. Petunia regenerated on media supplemented with 0.5 mg dm^{-3} KIN (a and b) and 1.0 mg dm^{-3} BAP (c)

on the media with 1.0 mg dm^{-3} GA_3 produced half as many flowers in comparison with the control plants. Huang et al. (2009) obtained similar results in the studies on *Dioscorea zingeberensis*. Gibberellic acid, even at the concentration 0.1 mg dm^{-3}, inhibited bud formation and inactivated the beneficial effect of other growth regulators. However, Dewir et al. (2007), studying the effects of GA_3 on *Spathiphyllum* flowering, found its stimulating influence but at considerably higher concentrations – 10 mg dm^{-3} resulted in flower formation in 83% of explants.

CONCLUSIONS

On the basis of the obtained results, the following conclusions have been drawn:

1. Petunia should be propagated in vitro on a Murashige and Skoog (1962) medium supplemented with 0.5 mg dm^{-3} gibberellic acid (GA_3). Plants propagated under these conditions grow high and form a large number of leaves and auxiliary shoots.
2. Indole-3-acetic acid (IAA) added to an MS medium at a dose of 0.5 and 1.0 mg dm^{-3} appeared to be the most useful for rooting 'Flash Red' *Petunia* × *atkinsiana* D. Don. Petunias rooted in this way had long numerous roots.
3. The optimal medium for initiating flowering in petunia is MS supplemented with 0.5 mg dm^{-3} kinetin. Its application resulted in the largest number of flowers with a normal morphological structure.

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KWITNIENIE PETUNII (*PETUNIA* × *ATKINSIANA* D. DON ‘FLASH RED’) W KULTURACH *IN VITRO*

Streszczenie: Celem badań było określenie wpływu auksyny, cytokinin i kwasu giberelinowego na kwitnienie i morfogenezę roślin *Petunia* × *atkinsiana* D. Don odmiany ‘Flash Red’ w kulturach *in vitro*. Najlepsze wyniki na etapie namnażania uzyskano na pożywce MS uzupełnionej 0,5 mg dm^{-3} GA_3. Petunie namnażane w tych warunkach były wysokie oraz wykształciły dużą liczbę liści i pędów bocznych. Najlepiej rozwinięty system korzeniowy obserwowano u roślin zregenerowanych na pożywce MS z dodatkiem IAA w ilości 0,5 lub 1,0 mg dm^{-3}. Inicjację kwitnienia u petunii należy natomiast prowadzić na pożywce MS uzupełnionej 0,5 mg dm^{-3} KIN.

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