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The after-effect of paclobutrazol on morphological characteristics of *in vitro* *Narcissus poeticus ssp. radiiflorus* plants

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Summary: After different pre-culturing period (12, 23 or 34 days) on ½ MS medium with 1 mg l^{-1} paclobutrazol, 1 mg l^{-1} N^6-benzyladenine and 0.1 mg l^{-1} 1- naphthaleneacetic acid , 3 groups of *Narcissus poeticus ssp. radiiflorus* bulb scales were kept on the same medium without hormones. The results were evaluated monthly and the final one happened after 7 month. The best results were achieved due to the shortest pre-culturing period (12 days; Group 1), with 4.9 bulblets and 4.54% hyperhydricity. The result of the second treatment (pre-culturing period of 23 days; Group 2) was not different significantly but the number of bigger bulblet were higher (4.54 bulblets). After the longest pre-culturing period (34 days; Group 3), the number of bulblets was low (3.68) and more hyperhydricity (18.18%) was detected. The highest number of roots (13.91) was observed in this group very likely due to the strong after-effect of paclobutrazol.

Keywords: paclobutrazol, multiplication, hyperhydricity, *Narcissus*

Introduction

Narcissus poeticus ssp. radiiflorus is a nice bulbous perennial (suitable as ornamental plant) with narrower leaves and short, reddish-orange lateral perianthiums (*Chopik* et al., 1977; *Matvejev,* 1980). This plant (which represented as several synonyms in the IUCN Red List of Threatened Species of Ukraine: *N. angustifolius*, *N. poëticus* ssp. *angustifolius*, *N. poëticus* ssp. *radiiflorus, N. poëticus* ssp. *stellaris*) is an actually endangered subspecies with disjunct areas, occur in Albaina, Hungary, Romania. The largest population was found in the Daffodils' Valley (a unique natural wilderness reserve protected by UNESCO), near Khust in Transcarpatia (*Ziman & Bulah*, 2009), Ukraina. In Hungary, it is a rare, protected plant which can be found only in West and South Transdanubia. Harvesting of beautiful flowers also prohibited in Ukraine despite the higher habitat (*Artemchuk* et al., 1966; *Chopik,* 1976; *Kostenko & Sheljag-Sosonka*, 1996).

Paclobutrazol (PB) was effectively used mainly for stimulating multiplication or *in vitro* rooting in the case of different ornamental plants, like *Digitalis obscura* (*Gavidia & Pérez-Bermúdez*, 1997), *Gladiolus* (*Nagaraju* et al., 2002), *Hemerocallis* (*Chen* et al., 2005), *Syringa x hyacinthiflora* (*Hongxia* et al., 2009) and some close relatives belong to the *Amaryllidaceae* family, for example *Leucojum aestivum* (*Jevcsák* et al., 2012) and *Galanthus elwesii* (*Mosonyi* et al., 2013).

Only few data are available in the literature concerning PB application in the micropropagation of *Narcissus*. *Chamani* et al. (2012) used different concentration of PB during micropropagation of unspecified *Narcissus*. Significantly the highest bulb and root number was achieved on medium containing 2 mg l^{-1} PB, and PB at all concentrations decreased the leaf production. For shoot multiplication of *radiiflorus* subspecies of *N. poeticus*, 1 mg l^{-1} N^6-benzyladenine (BA) + 0.25 mg l^{-1} PB + 0,1 mg l^{-1} 1-naphtaleneacetic acid (NAA) combination was optimal, but hyperhydricity and 70% rooting were obtained (*Jevcsák* et al., 2013). In another trial (*Jevcsák* et al., 2014) effects of PB and/or NAA were studied. The highest root number (6.2) and the best weight of bulbs (1.8 g) were detected in the case of using 0.2 mg l^{-1} NAA. As for rooting and bulb formation, the combination of PB and NAA (0.1 + 0.1 mg l^{-1}) proved to be worse than the effect of NAA alone, but better than the media with only PB. However, combined application of PB and PB + NAA resulted in the longest leaves (32.99 mm) but a higher PB concentration caused deformation.

The aim of our present study was to decrease the rate of hyperhydricity (with lower concentration of paclobutrazol) and to find an efficient method for *in vitro* multiplication of *Narcissus poeticus ssp. radiiflorus*.

Materials and methods

The experiments were carried out from sterile bulbs (sized 8–10 mm diameter) originated from the previous *in vitro* works in the laboratory of the Department of Floriculture and Dendrology, Corvinus University Budapest.

On January 18th 2012, bulbs were cut in four equal segments (10-12 mm) and placed into 100 ml Erlenmeyer flasks containing half-strength (½) MS medium (*Murashige & Skoog*, 1962) supplemented with 1 mg l^{-1} N^6-benzyladenine (BA), 1 mg l^{-1} paclobutrazol (PB), 0,1 mg l^{-1} 1-naphtaleneacetic acid (NAA) and 30 g l^{-1} sucrose, 11 g l^{-1} agar (Reanal). The pH adjusted to 5.6 with KOH. The media were autoclaved on 10^5 Pa pressure for 30 minutes.

Three groups (3 x 22 bulb clusters) were separated. These plants were transferred to hormone-free ½ MS medium (containing as much sucrose and agar) after 12 (Group 1), 23 (Group 2) and 34 (Group 3) days. Culture conditions were: 23 °C, 16/8 light/dark photoperiod (37.2 µmol m^{-2} s^{-1} photosynthetic photon flux density).

Morphological characteristics (the number and length of shoots and roots, vitrification rate) were recorded six times (from 27th March to 21st September, 2012). Data were evaluated by ROPStat statistical software (*Vargha*, 2002, 2008). Means comparison were done by using Games-Howell, Tukey-Kramer tests at $p<0.01$. Each experiment was replicated twice.

Results and discussion

Plantlets of the of the Group 1 *(Table 1)* produced the highest number of bulblets from 27th April to 29th May. But in September, increasing of proliferation was detected in the other two groups of plantlets and there was significant difference between values of the Group 1 (4.9) and Group 3 (3.68). As the number of bulblets was the highest in the Group 1 and 2, the hyperhydricity of bulblets proved to be lower (4.5 and 9 % respectively), similarly as in the previous experiments (15–30%, *Jevcsák* et al., 2013).

The root number was the highest on plants of Group 3 almost in every sampling time between 27th April and 21st September). The most roots (13.91) were achieved by September after 34-day-long pre-culturing period (Group 3). Root length was measured only at the last sampling time (21st September) and averagely the longest roots (41.19 mm) were observed in the Group 1 (date were not shown). There was no positive correlation between the length of pre-culturing period and root length whereas in our earlier trial (*Jevcsák* et al, 2013) PB in every concentration shortened the roots (2.9–4.75 mm) and lack of PB resulted in significantly longer roots (8.68–33.88 mm).

All (100%) plantlets of Groups 1 and 2 developed roots *(Figure 1)* by the end of the observation period. The maximal rooting percentage was 77.27% when plants were cultured previously for the longest period on medium with PB (Group 3). As it was described previously, PB was not efficient for rooting especially in the case of higher concentration (2.5 mg l^{-1}), and the best results (100% rooting and averagely 9.85 roots) were obtained on hormone-free medium (*Jevcsák* et al., 2013).

The hyperhydrated bulblets were found first at the second sampling time (27th April). By the next sampling time (8th May) the percentage of hyperhydricity was increased (up to 31.81%), but no more changes were observed until 19th June. At the time of final sampling (21st September) values of hyperhydricity in every group decreased (4.54–18.18%, *Figure 2)*. Consequently, after-culturing on hormone-free ½ MS medium resulted in lower hyperhydricity in spite of applying more BA and PB in the pre-treatment. In another trial (*Jevcsák* et al, 2013) lower concentration of BA and PB (0.5 + 0.25 mg l^{-1} instead of 1 + 1 mg l^{-1}) resulted in more hyperhydrated bulblets (15-30%) when plantlets were cultured on BA + PB-containing medium continuously (without a rest period on hormone-free medium). In our study, lower hyperhydricity was achieved if every groups were transferred to hormone-free medium earlier (i.e. hyperhydratation % was the lowest in the Group 1 and the highest in the Group 3).

Table 1. Average number of bulblets and roots of *in vitro N. poeticus subsp. radiiflorus*

Data of examination (2012)	Group	Average bulblet number ± SD	Average root number ± SD
27th March	1.	0.95 ± 1.21 a	1.54 ± 2.11 a
	2.	0.5 ± 0.8 a	1.09 ± 1.5 a
	3.	0.4 ± 0.66 a	0.54 ± 1.22 a
27th April	1.	1.31 ± 1.17 a	2.72 ± 2.27 a
	2.	1.18 ± 1.29 a	2.54 ± 2.08 a
	3.	1.68 ± 1.64 a	3.4 ± 3.2 a
8th May	1.	1.9 ± 1.3 a	4.59 ± 2.93 a
	2.	1.86 ± 1.39 a	4.4 ± 3.2 a
	3.	2.54 ± 2.13 a	6.54 ± 6.37 a
29th May	1.	2.4 ± 1.36 a	6.13 ± 3.72 a
	2.	2.27 ± 1.63 a	5.09 ± 3.63 a
	3.	2.95 ± 2.25 a	8.36 ± 8.28 a
19th June	1.	3.22 ± 1.41 a	7.72 ± 2.41 a
	2.	2.63 ± 1.56 a	5.63 ± 3.59 b
	3.	3.13 ± 1.78 a	9.04 ± 8.34 ab
21st Sept	1.	4.9 ± 1.37 a	8.71 ± 2.83 a
	2.	4.54 ± 1.29 ab	8.04 ± 2.4 a
	3.	3.68 ± 1.46 b	13.91 ± 8.55 b

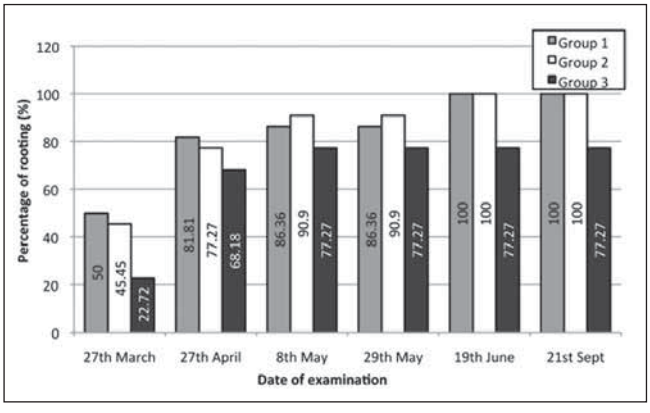

Figure 1. Rooting rate of *in vitro Narcissus poeticus subsp. radiiflorus*

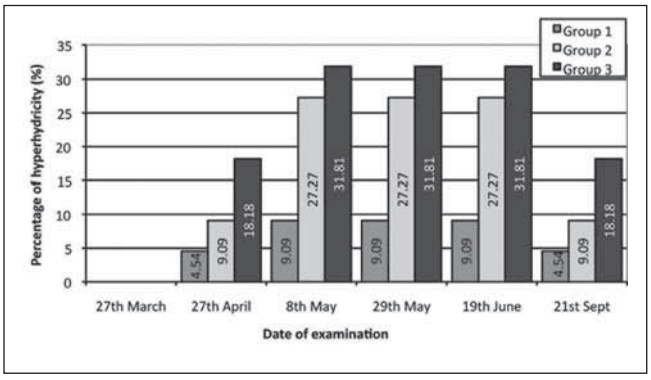

Figure 2. Rate of hyperhydrated bulblets of *in vitro Narcissus poeticus subsp. radiiflorus*

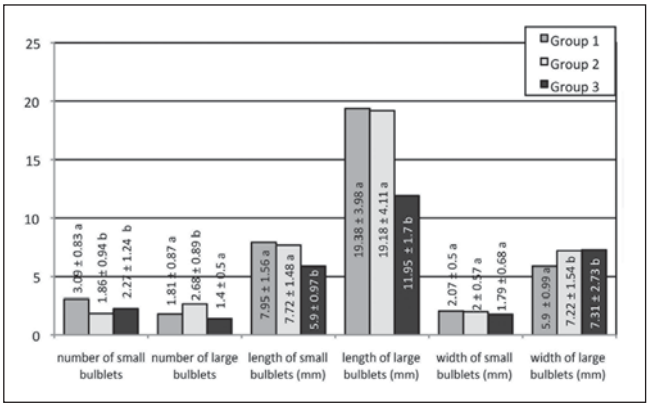

Figure 3. Small and large* bulblet characteristics of *in vitro Narcissus poeticus subsp. radiiflorus* on 21st Sept (*small: shorter than 9 mm, large: longer than 10 mm)

At the last sampling time (21st Sept), bulblets were separated according to their sizes *(Figure 3).* The highest number of small bulblets was found in the Group 1 (3.09), and same result of large ones was detected in the Group 2 (2.68). *Jevcsák* et al. (2013) found more (2.35–7.75) small bulblets on every medium (except for the hormone free one), although PB was not suitable for producing more ones, because higher concentration (2.5 mg l^{-1}) of PB significantly decreased multiplication.

Length of small and large bulblets was the highest in the Group 1 and the lowest in the Group 3. Additionally, large bulblets became wider (7.31 mm in Group 3), and the maximum small bulblets width was only 2.07 mm in the Group 1. Thus, longer time of pre-treatment (on medium + PB, BA and NAA) resulted in shorter and narrower bulblets *(Figure 4).*

Summarizing, if the pre-culturing period was too long, paclobutrazol decreased the number of bulblets (4.9-3.68) and increased hyperhydricity (4.54–18.18). According to the after-effect, this growth retardant increased the bulblet number in the case of the shortest pre-treatment and the hyperhydricity was very low. The highest number of roots (13.91) were developed after the longest pre-culturing period (34 days, Group 3). Therefore the paclobutrazol is recommended as a pre-treatment before *in vitro* rooting of *Narcissus poeticus subsp. radiiflorus* on hormone-free medium.

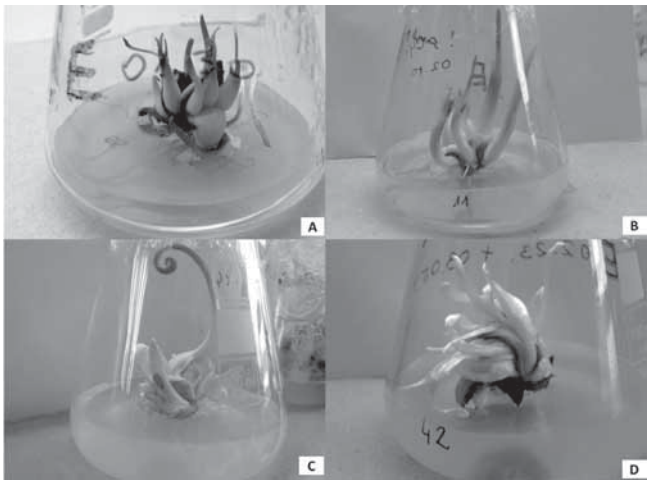

Figure 4. *In vitro* healthy (**A**: Group 1, **B**: Group 2) and hyperhydrated (**C, D**: Group 3) *Narcissus poeticus subsp. radiiflorus* bulblets before the last evaluation (photos by: Jámbor-Benczúr)

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