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THE INFLUENCE OF PACLOBUTRAZOL IN THE ROOTING MEDIUM ON THE QUALITY OF CHRYSANTHEMUM VITROPLANTS

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A B S T R A C T

The quality of vitroplants depends on the factors influencing the last stage before weaning. Vitroplants should be able to survive the transfer to *ex vitro* conditions and start growing as early as possible. The maturity of the coating and transmission tissues and the functionality of roots and photosystems are among the most important traits. Different measures have been advised to increase the ability for adaptation to *ex vitro* life, including adding paclobutrazol (PBZ) to the rooting medium. In this paper, we report on the reaction of the chrysanthemum cv. 'Ludo' to the presence of this growth regulator at concentrations of 0.5, 1.0 and 3.0 mg 1^{-1} in the rooting medium. The post-weaning analysis revealed that paclobutrazol caused an increase in the whole microshoot and root fresh mass and the chlorophyll content. The length of shoots decreased only on the medium containing 3.0 mg 1^{-1} of PBZ. After one month in the greenhouse, shoots that had been rooted on the media containing paclobutrazol grew taller and developed flowers earlier than the control vitroplants. The paclobutrazol-treated microshoots produced less proline than the controls at deflasking and a week later. The parameters characterizing chlorophyll fluorescence were not strongly affected, although Fv/Fm and Fv/Fo were lower in the control vitroplants at deflasking and to a lesser extent after one month in the greenhouse, indicating better adaptation to heterotrophic life.

Key words: chlorophyll content, chlorophyll fluorescence, growth, morphological characters, proline content

INTRODUCTION

The quality of vitroplants should be at least the same as that of traditionally propagated planting material. Vitroplants are produced in very humid conditions, so their coating and transportation tissues and photosynthetic systems are immature, and their adaptation to heterotrophic life can be challenging (Pospišilowá et al., 1999; Hazarika, 2006). It is therefore important to use in the micropropagation procedure such treatments that increase the ability to survive the acclimatization stress and enable a quick adaptation to heterotrophy. Paclobutrazol (PBZ) is among the compounds that can positively affect the acclimatization of microplants (Smith et al., 1990; Roberts et al., 1992; Gilley and Fletcher, 1997; Sopher et al., 1999). The following effects were found as a result of application of PBZ *in vitro*: increases in the chlorophyll content, the net photosynthetic rate, the internal CO_2 concentration, the thickness of the leaves, epidermis, cuticle, palisade and spongy parenchyma, and the diameter of the phloem elements, a decrease in the diameter of the xylem vessels, reductions in transpiration rate (Jaleel et al., 2007) and shoot length, and an improvement of rooting (Messina and Costa, 1990).

In this paper, we present the results from experiments on the influence of PBZ added to the rooting medium for chrysanthemum on the root and shoot characteristics related to acclimatization, and on the growth and development of plants in the greenhouse.

MATERIAL AND METHODS

2-cm long shoots of the chrysanthemum cultivar 'Ludo', obtained from cultures micropropagated for two years, were subcultured on a rooting medium consisting of MS salts (Murashige and Skoog, 1962), WPM vitamins (Lloyd and McCown, 1981), 0.5 mg 1^{-1} indole-3-acetic acid (IAA), 30 g 1^{-1} sucrose and 7 g 1^{-1} Phyto agar. Batches of the medium were autoclaved, then cooled, and paclobutrazol dissolved in ethanol was added to obtain final concentrations 0.5, 1.0 or 3.0 mg 1^{-1}. The medium for the control contained no paclobutrazol.

Each treatment consisted of 5 jars with 4 shoots, the rooting experiments were repeated twice (series). For the first 5 days, the cultures were incubated in the dark, and then under diffused fluorescent light at an intensity of 60-70 µmol m^{-2} sec^{-1}. After 4 weeks of rooting, the following parameters were defined after deflasking: the fresh weight (FW) of the microplants and roots, the contents of chlorophyll a and b and proline, and the parameters of chlorophyll fluorescence. The vitroplants were planted in a mixture of peat and perlite (4:1), pH 6.7, with 0.5 g 1^{-1} Azofoska fertilizer and treated with Rovral Flo 255 SC 0.2% and Previcur 607 SL 0.2% against pathogenic fungi. The vitroplants were protected from water loss by enclosure in a polyethylene tent, ventilated gradually the second week on. After 1 week in the greenhouse, the proline content, and after 1 month, the shoot length and chlorophyll fluorescence parameters were analyzed.

The content of free proline was measured according to Bates et al. (1973). Well expanded leaves (500 mg 1^{-1} FW) without their leaf stalk were extracted overnight with 10 ml 3% sulphosalicylic acid and 2 ml of filtrate were used for the acid ninhydrin reaction. The reaction mixture was extracted with 4 ml toluene and the absorbance read at 520 nm using toluene for a blank. The chlorophyll content was measured according to Bruinsma (1963). The proline and chlorophyll contents were means per 5 of samples in each treatment. The chlorophyll fluorescence was measured on the fourth leaf from the top. The time of flowering was also observed. The photosynthetic activity was evaluated with the PEA Hansatech model, measuring the maximal fluorescence (Fm), ground state value fluorescence (Fo), variable fluorescence (Fv), time of increase in signal from Fo to Fm (Tfm), maximum quantum yield of photochemistry (Fv/Fm) and efficiency of oxygen complex release (Fv/Fm).

The morphological parameters were evaluated on 20 and chlorophyll fluorescence on 10 microshoots, and the results were subjected to variance analysis. The data were transformed according to the Freeman-Tukey's function. The significance of differences between the means was evaluated with the Duncan test at p = 5%.

RESULTS

All the vitroplants survived transplantation to the greenhouse. The presence of PBZ in the rooting medium significantly influenced some morphological parameters at the weaning stage, increasing the FW of the microplants and roots, but decreasing the length of the shoots at a concentration of 3.0 mg 1^{-1}. The leaf number and area were not affected (Tab. 1). The microshoots rooted on the media containing PBZ were stronger with visibly thicker stems, which led to a higher growth potential, as the shoots were taller than the controls after two months in the greenhouse. PBZ at each concentration brought flowering on faster, in 6-8 days (Fig. 1). The chlorophyll content at weaning was higher when the shoots had been rooted in the media with 0.5 and 1.0 mg1^{-1} PBZ (Tab. 2). The control shoots rooted without PBZ produced significantly more proline at weaning and after 1 week of acclimatization. PBZ significantly improved the parameters characterizing leaf photosystem functioning (Fo/Fm and Fv/Fo) at weaning. Fm and Fv were significantly higher than the controls when measured after 1 month of acclimatization (Tab. 3).

DISCUSSION

Paclobutrazol (PBZ) is a member of the triazole family. Triazoles are considered plant multiprotectants against various stresses (Gilley and Fletcher, 1997). PBZ affects the content of plant growth regulators by inhibiting gibberellin synthesis, reducing ethylene evolution, and increasing cytokinin level (Kamountsis and Chronopoulou-Sereli, 1999). The morphological changes observed in

Table 1. The influence of paclobutrazol added to the rooting medium on the physiological parameters of vitroplants of the chrysanthemum 'Ludo' at weaning and after one month in the greenhouse

Concentration of paclobutrazol [mg 1^{-1}]	At weaning					Length of shoots after 1 month in the greenhouse [cm]
	FW of shoots [g]	FW of roots [g]	length of shoots [cm]	No. of leaves	leaf area [cm^2]	
0	1.13a	0.14a	5.24ab	14.72a	17.06a	5.5a
0.5	1.23ab	0.18ab	4.16ab	13.98a	16.25a	6.45ab
1.0	1.47c	0.22b	5.9b	15.32a	17.96a	7.85c
3.0	1.37bc	0.20ab	3.5a	14.3a	15.85a	7.2bc

Table 2. The influence of paclobutrazol added to the rooting medium on the contents of chlorophyll a and b and proline in vitroplants of the chrysanthemum 'Ludo' at weaning and after 1 week in the greenhouse

Concentration of paclobutrazol [mg 1^{-1}]	At weaning				Proline content after 1 week in the greenhouse [µg g^{-1} F.W.]
	chlorophyll a content [mg g^{-1} F.W.]	chlorophyll b content [mg g^{-1} F.W.]	chlorophyll a+b content [mg g^{-1} F.W.]	proline content [µg g^{-1} F.W.]	
0	126.2a	31.8a	146.1a	209.1b	804.0b
0.5	141.8b	38.6b	170.9b	179.7a	181.4a
1.0	142.6b	38.3b	167.6b	178.6a	181.7a
3.0	131.1a	34.0ab	153.2a	154.3a	428.8a

Table 3. The influence of paclobutrazol (PBZ) added to the rooting medium on the chlorophyll fluorescence of vitroplants of the chrysanthemum 'Ludo' at weaning and after one month in the greenhouse

Stage	PBZ [mg 1^{-1}]	Parameters of chlorophyll fluorescence					
		Fo	Fm	Fv	Tfm	Fv/Fm	Fv/Fo
At weaning	0	341.8a	1749.4a	1407.5a	418.8a	0.804a	4.1a
	0.5	341.9a	1811.3a	1469.5a	419.9a	0.810ab	4.3ab
	1.0	328.1a	1792.1a	1464.0a	447.1a	0.817bc	4.5bc
	3.0	313.0a	1778.3a	1465.3a	460.8a	0.823c	4.7c
After one month in the greenhouse	0	393.4a	2167.4a	1774.0a	417.8a	0.817a	4.5a
	0.5	385.6a	2262.0b	1876.4b	416.1a	0.829a	4.9a
	1.0	399.9a	2260.8b	1860.9b	422.6ab	0.822a	4.7a
	3.0	396.9a	2233.5ab	1835.5ab	462.3b	0.821a	4.7a

Figure 1. The influence of paclobutrazol added to the rooting medium on the development of chrysanthemum 'Ludo' plants. Development assessed 4 months after weaning

PBZ-treated plants include the inhibition of plant growth and internodal elongation, chlorophyll content increase, chloroplast enlarge-ment, leaf tissue thickening, root to shoot ratio increase, and epicuticular wax formation (Watson and Himelick, 2004; Jaleel et al., 2007). Sopher et al. (1999) reported that in maize seedlings, PBZ changed the proportions of stem components, decreasing the xylem and increasing the phloem and cortex thickness and xylem density.

This study shows that the addition of paclobutrazol to the rooting medium for chrysanthemum increased the fresh weight of the roots and of the whole microplants, and increased the chlorophyll content, but did not affect the number or surface area of the leaves, which was a matter of controversy to Smith et al. (1990), where these two parameters were found to decrease. Shoot length was lower only at PBZ concentration of 3 mg 1^{-1}. Smith et al. (1990) also reported for chrysanthemum an increase in the chlorophyll and epicuticular wax contents, shortened stems and thickened roots, and a decrease in shoot and root length in 1-month old plants that had been rooted on media containing from 0.5 to 4 mg 1^{-1} PBZ. In our study, the shoots rooted on PBZ-containing medium were longer after 1 month of growth in the greenhouse. They were less stressed by changing

of conditions than the control plants, which was reflected in the proline content, and they had a higher growth potential thanks to their thicker stems, although the number and surface area of the leaves were not affected. The higher growth potential resulted in faster flower development.

Chlorophyll content is one of the indirect markers of the efficiency of the photosynthetic apparatus, informing as it does about all chlorophyll and not only that which takes part in photosynthesis. Photosynthesis is a physiological marker related to vitroplant quality (Borkowska, 2003). PS II photosystem functioning is most sensitive in reaction to different stresses (Long et al., 1994). Fv/Fm is a measure of the photochemical competence of PS II. It was accepted that 0.800 is the minimal value of this parameter typical for healthy plants (Björkman and Demmig, 1987). In our experiments, the value of this parameter always exceeded 0.800 and although the differences were not large, they were statistically significant between the control and experimental shoots rooted with 1.0 and 3.0 mg 1^{-1} PBZ when measured at weaning. This shows that PBZ-treated vitroplants are better prepared for photosynthesis, and that this preparation usually occurs during their *in vitro* development. This trend was also observed after one month in the greenhouse. The same relationship was found for the Fv/Fo coefficient, which characterizes the effectivity of the complex releasing oxygen, and is also very susceptible to stress factors (Havaux and Lannoye, 1984). An increase in the proline content is approved as a marker of the reaction of plants to the stress resulting from changes in water content in the tissues (Delauney and Verma, 1993) The existence of such a stress, connected with a weaning, which involves removing the microplants from vessels where the air humidity is close to 100%, was confirmed for chrysanthemum by Ritchie et al. (1991) and Roberts and Mathews (1995). They reported on the higher resistance to drying of vitroplants which were treated with PBZ during *in vitro* rooting.

The obtained result cannot fully reflect the reaction of the chrysanthemum photosystem to acclimatization-related stress, because the stress level in our experiment was too low, as the vitroplants were carefully protected against water loss at weaning and during acclimateization, and no wilting was observed. The increase in the time needed to reach the maximal value of fluorescence in the shoots rooted on medium supplemented with 3.0 mg 1^{-1} PBZ is difficult to explain with the data obtained here.

CONCLUSIONS

The obtained experimental data on the morphological and physiological parameters of vitroplants yielded the conclusion that rooting chrysanthemum on a medium containing 1 mg 1^{-1} PBZ increases the values of the parameters responsible for acclimatization to *ex vitro* conditions.

This helps in the adaptation to greenhouse conditions, with an effect visible already at the *in vitro* stage. Such vitroplants could grow faster and flower earlier.

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WPŁYW PAKLOBUTRAZOLU W UKORZENIANIU *IN VITRO* NA JAKOŚĆ MIKROSADZONEK CHRYZANTEMY

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S T R E S Z C Z E N I E

Sadzonki z *in vitro* wykazują niedostateczne funkcjonowanie tkanek przewodzących i okrywających oraz niedojrzałość aparatu fotosyntetycznego. Istotne jest przygotowanie mikrosadzonek do warunków heterotroficznych na etapie ukorzeniania *in vitro* poprzez wyzwalanie procesów adaptacyjnych i zwiększanie odporności na stres przenoszenia. Jednym z czynników pobudzających aklimatyzację jeszcze na etapie kultur jest paklobutrazol (PBZ).

Celem podjętych badań było zbadanie wpływu PBZ w pożywce do ukorzeniania na morfologiczne parametry jakości, zawartość proliny, fluorescencję i zawartość chlorofilu oraz szybkość zakwitania mikrosadzonek chryzantemy odmiany ‘Ludo’.

Dodatek 1 mg 1^{-1} PBZ do pożywki wpływał istotnie na parametry morfologiczne mikrosadzonek, zwiększał masę korzeni i roślin, nie powodując zmniejszenia liczby i powierzchni liści. Mikrosadzonki po pożywkach z PBZ miały grubsze i silniejsze pędy, co poskutkowało istotną różnicą w wysokości roślin po dwóch miesiącach przebywania w szklarni. Zaznaczył się również pozytywny wpływ tego regulatora wzrostu na przyspieszenie o 6-8 dni zakwitania roślin. Parametry fluorescencji chlorofilu określające aktywność fotochemiczną liści w kulturach *in vitro* wskazują na znaczący wpływ dodatku PBZ na poprawę funkcjonowania fotosystemów roślin na pożywce z tym regulatorem. Zaznaczył się istotny wpływ dodatku 0,5 i 1 mg 1^{-1} paklobutrazolu na zawartość chlorofilu a i b. Zawartość proliny w pędach rosnących na pożywce kontrolnej była istotnie wyższa niż w pozostałych kombinacjach zarówno na koniec etapu ukorzeniania *in vitro*, jak również po tygodniu przebywania w szklarni. Otrzymane wyniki wskazują, że mikrosadzonki ukorzeniane na pożywce z dodatkiem paklobutrazolu były lepiej przystosowane do warunków *ex vitro*.

Słowa kluczowe: fluorescencja chlorofilu, parametry morfologiczne, ukorzenianie *in vitro,* zawartość chlorofilu, zawartość proliny