Vol. 16, No. 7 2009

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INFLUENCE OF COPPER IONS ON PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN PLANT MATERIAL REGENERATED FROM EMBRYOS OBTAINED IN ANDROGENIC CARROT CULTURE

WPŁYW JONÓW MIEDZI NA FIZJOLOGICZNE I BIOCHEMICZNE ZMIANY W MATERIALE ROŚLINNYM REGENEROWANYM Z ZARODKÓW ANDROGENNYCH MARCHWI

Abstract: Androgenic embryos of 3 different carrot genotypes were cultivated on the medium containing different Cu^{2+} concentrations: 0.1 μM (control), 1 μM, 10 μM and 100 μM. Carrot sensitivity to Cu^{2+} was evaluated on the basis of growth inhibition, changes in peroxidation of membranes and proline content. The cultivation on the medium supplemented with Cu^{2+} resulted in dose-dependent inhibition of the growth and organogenic ability of Narbonne carrot embryos, while in the genotype 1014 treated with 10 μM Cu^{2+} the significant regeneration ability was observed. The same Cu^{2+} concentration greatly increased the level of free proline in the genotype 1014 which was accompanied by relatively low TBARS content. In the genotype Feria 100 μM Cu^{2+} concentration triggered large increase in proline content associated with high regeneration capacity of carrot embryos.

Keywords: copper ions, anther cultures, lipid peroxidation, proline

Copper (Cu^{2+}), an essential microelement for growth and plant development in higher doses can become very toxic and disturb basic physiological processes [1]. Acting as a cofactor in many enzymatic reactions in cells it causes the formation of harmful reactive oxygen species (ROS) and induces oxidative damage of important macromolecules such as DNA, proteins and lipids [2]. Although plants have evolved a variety of

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mechanisms for metal tolerance, species and cultivars vary widely in this respect, and closely related genotypes can be valuable tools in studying the mechanisms of toxicity or tolerance.

Carrot is one of the most important vegetables and therefore it has become an object of intensive research aimed at obtaining new cultivars. Production of haploids in carrot through another culture allows carrot breeders to release new lines more quickly and screen for metal accumulation more efficiently. Carrot easily accumulates toxins that is why it is important to use *in vitro* cultures in selecting and eliminating the cultivars which accumulate excessive amounts of metals.

Differences in plant responses to Cu^{2+} seem to depend not only on its concentration but also on the ability of plants to increase the antioxidative protection against negative consequences of heavy metal stress. One of the first symptoms of oxidative stress in plant tissues is lipid peroxidation of cell membranes. Proline, which accumulates in response to various kinds of abiotic stresses eg non-optimal temperatures, heavy metals, wounding [3], contributes to osmotic adjustment in cells and it has been shown that this amino acid can be an effective antioxidant [4]. Also phenolic compounds play an important role in protecting plants against biotic and abiotic stresses [5], and the enhancement of their metabolism is one of the responses to heavy metal stress. The purpose of the current work was to characterize the influence of Cu^{2+} on the regeneration of androgenic embryos of 3 different carrot genotypes and on the extent of lipid peroxidation.

Material and methods

The roots of three carrot (*Daucus carota* L.) genotypes, 1014, Feria and Narbonne were the donors of experimental material. The plants developed from the roots were kept in a greenhouse at about 20 °C. The detailed description of another culture procedure was previously given in [6]. The carrot cultures were kept in darkness at 27 °C and after emerging of the embryos, they were transferred to continuous light at the same temperature. When the embryos became green they were transferred onto the regeneration medium B5 [7] supplemented with Cu^{+2} in the form of $CuSO_4 \cdot 5H_2O$ at concentrations: 0.1 μM (control), 1 μM, 10 μM and 100 μM. The embryos were incubated under light (30 μmol m^{-2} sec^{-1}, 20 °C, photoperiod 16/8) for 24 weeks.

TBARS contents in carrot rosettes were measured according to [8], proline was extracted and estimated according to [9], whereas phenolic acids were extracted as described by [10] and analysed according to [11].

Results and discussion

Table 1 shows the effect of Cu^{2+} on the numbers of regenerated carrot rosetes after 24 weeks. Cultivation on the regeneration medium supplemented with Cu^{2+} resulted in dose-dependent inhibition of the growth and organogenic ability of Narbonne carrot embryos, while in the genotype 1014 treated with 10 μM Cu^{2+} the significant regeneration ability was observed. The same Cu^{2+} concentration greatly increased the level of free proline in the genotype 1014 which was accompanied by relatively low TBARS content in comparison with the control (0.1 μM) (Fig. 1A). It is known that TBARS levels can be a good marker of stress and plants which accumulate more TBARS are more sensitive to stressor than those which accumulate less [12]. However, in the genotype Feria Cu^{2+} at 100 μM triggered large increase in proline content (Fig. 1B) associated with high regeneration capacity of carrot embryos in comparison with the genotypes 1014 and Narbonne (Table 1). It seems that accumulation of free proline may be involved in a protective mechanisms against Cu^{2+} stress during regeneration of androgenetic embryos of carrot [13]. On the other hand, increased level of free phenolic acids in the Narbonne genotype presented in Fig. 2 seems to be not sufficient for carrot rosetes to protect them against damaging influence of Cu^{2+}.

The results presented here show the significant differences in responses of 3 carrot genotypes regenerated from another cultures to Cu^{2+}. Narbonne seems to be the most sensitive to harmful impact of Cu^{2+}, while Feria appears tolerant even to extremely high Cu^{2+} concentration. We suggest the protective role especially of free proline against Cu^{2+} stress during regeneration of androgenetic embryos of carrot.

Table 1

Effect of Cu^{2+} on the numbers of normally rooted and non-rooted carrot rosettes of genotypes 1014, Feria and Narbonne after 24 weeks of cultivation on B5 medium [6]. Control (C) – 0.1 μM of Cu^{2+}.

Cu^{2+} concentration [μM]	Total No. of regenerated rosettes (with and without roots)		
	1014	Feria	Narbonne
C	5.9 a	12.6 a *	8.7 a *
Cu-1	8.8 a	9.6 a	2.1 a
Cu-10	24.1 b	10.6 a	1.0 a
Cu-100	2.7 b	8.4 a	0.9 a

Asterisks (*) indicate the results obtained from Cu^{2+} – treated carrot cultures that significantly differ from the corresponding control values at $p \leq 0.05$

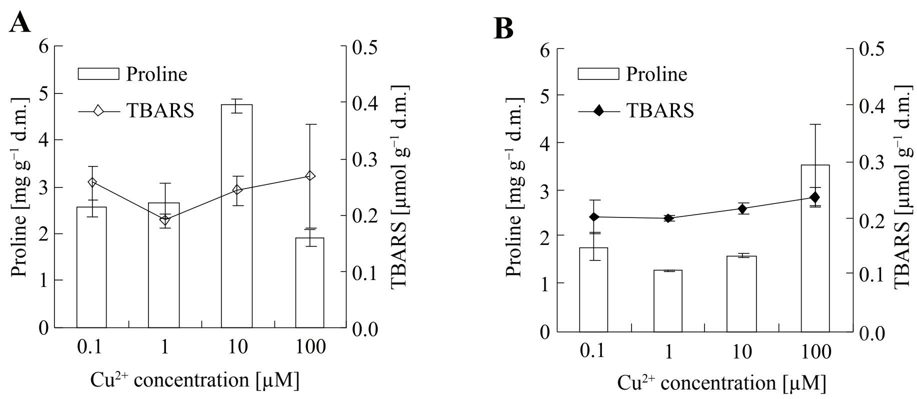

Fig. 1. Proline and TBARS contents in carrot rosetes of genotype 1014 (A) and Feria (B) after 24 weeks of cultivation on medium B5.

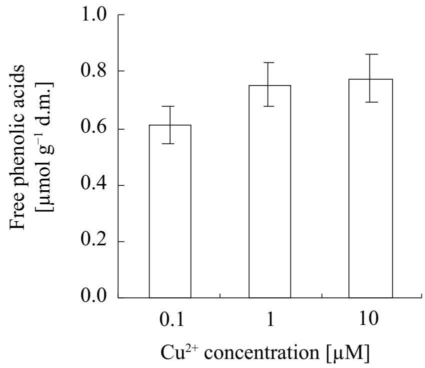

Fig. 2. Contents of free phenolic acids in the rosetes regenerated from carrot tissues of genotype Nar‑bonne

Acknowledgements

Study financed by the Polish Ministry of Sciences and Higher Education in 2006–2009 as a research project No. N305 040 31/1576.

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Abstrakt: Zarodki androgenne 3 różnych odmian marchwi hodowano na pożywce z dodatkiem Cu^{2+} w 4 stężeniach: 0,1 µM (kontr.), 1 µM, 10 µM, i 100 µM. Wrażliwość marchwi na Cu^{2+} oceniano na podstawie zahamowania wzrostu, zmian w utlenianiu lipidów błonowych oraz zawartości wolnej proliny. Hodowla na pożywce wzbogaconej w Cu^{2+} spowodowała stopniowe zahamowanie wzrostu oraz zdolności regeneracyjnych zarodków marchwi odm. Narbonne, podczas gdy ilość prawidłowych rozet genotypu 1014 traktowanych 10 µM Cu^{2+} była największa. Badany metal w tym samym stężeniu znacząco zwiększył zawartość wolnej proliny w rozetach genotypu 1014, co było skorelowane ze stosunkowo niskim poziomem TBARS. Natomiast, w przypadku genotypu Feria, wzrost zawartości proliny skojarzonej z dużymi zdolnościami regeneracyjnymi zarodków zaobserwowano w rozetach traktowanych Cu^{2+} o stężeniu 100 µM.

Słowa kluczowe: jony miedzi, kultury pylnikowe, peroksydacja lipidów, prolina