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RESPONSE OF *LYCOPERSICUM PERUVIANUM* L. LINE TO SALINITY IN VITRO CULTURE

REAKCJA LINII *LYCOPERSICUM PERUVIANUM* L. NA ZASOLENIE W KULTURACH IN VITRO

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Streszczenie. Trzy linie *Lycopersicum peruvianum* L. (LA0462, LA1692, LA4316), rozmnożone z nasion zebranych w Peru i Chile, poddane były działaniu stresu solnego w kulturach merystemów i kalusa *in vitro*. W pierwszym etapie badań, na pożywkę MS z dodatkiem 25 do 150 mM NaCl wykładano fragmenty wierzchołkowe pędów. W drugim doświadczeniu na pożywkę z dodatkiem NaCl w stężeniu od 75 do 150 mM wykładano fragmenty tkanki kalusowej. Kontrolę stanowiły fragmenty roślin i tkanka kalusowa, wyłożona na pożywkę bez dodatku NaCl. Badane linie zareagowały w różny sposób na dodatek soli do pożywki. Dodatek NaCl do pożywki w stężeniu od 25 do 75 mM NaCl wpłynął korzystnie na wysokość roślin *L. peruvianum*, liczbę wykształconych liści, masę badanych roślin i kalusa linii LA0462. W przypadku linii LA1692 różnice we wzroście były mniejsze (nieistotne statystycznie), choć dodatek NaCl działał korzystnie na rozwój roślin i przyrost tkanki kalusowej. Wartości wszystkich cech morfologicznych roślin i kalusa trzeciej z badanych linii – LA 4316 – rosnących na pożywkach z dodatkiem NaCl, były niższe od kontroli.

Słowa kluczowe: kalus, selekcja, tolerancja na zasolenie.

Key words: callus, salt tolerant, selection.

INTRODUCTION

The tolerance of plants to salt has attracted much attention in recent years because of the theoretical and practical importance of this problem (Cano et al. 1998, Hernandez et al. 2000, Xiong et al. 2002, Kavi-Kishor et al. 2005).

Salt tolerance is the inborn plant ability to grow and produce satisfactory yield in saline environment. There are two strategies used by plants providing protection from negative effects of salt excess. The first one is removing salt excess from the organism without interrupting protoplasm functions. The mechanism that helps to avoid the negative result of salt stress is regulation of the ion transport. The second strategy is tolerating toxic and osmotic results of excessive salt content conditions. There are two types of plant response to high salt concentration: synthesis of compatible compounds and synthesis of salinity stress proteins. Compatible compounds are amino acids like: proline, alanin, glycinobetain,

glutamin, asparagin, sugars and polyols – mannitol and sorbitol (Tal and Shannon 1983). Many research demonstrate that salinity has an impact on almost every physiological and biochemical aspect in plants, for example in *Lycopersicon esculentum* Mill. High salt stress results in the inhibition of plant growth (Philis et al. 1979, Papadopoulos and Rendig 1983, Tal and Shannon, 1983, Al-Rawahy et al. 1992, Atta-Aly et al. 1992, Ho et al. 1992, Knight et al. 1992, Minimide and Ho 1993). Wild tomatoes exhibit great differences in salt tolerance and they are good materials to improve the resistance of cultivated tomatoes to unfavorable environmental conditions (Tal and Shannon 1983, Perez-Alfocea et al. 1994, Rzepka-Plevnes et al. 2007a, b). A useful and easy method of testing plants for salt tolerance is *in vitro* culture. Using this method, there is no impact of environmental factors on the plant material. This method also gives a possibility of screening thousands of plants in a relatively short time. In research on *in vitro* cultures of *Lycopersicon esculentum* Mill, a positive correlation between the growth of salt-stressed callus cultures and whole plants has been observed (Tal et al. 1978, Dracup 1991, Pérez-Alfocea et al. 1994). Another kind of culture that has a big advantage because of the genetic stability of plants and is useful for salinity tolerance selection is the meristem culture (Martinez et al. 1996). In this work, an attempt has been made to test three wild tomato lines *L. peruvianum* for salt tolerance using *in vitro* callus culture and meristem culture.

MATERIALS AND METHODS

Plant material. In the presented experiment, three inbred tomato of the *L. peruvianum* lines: LA0462 (Sobraya, Tarupaca, Chile), LA1692 (Putrima, Lima, Peru), LA4316 (Kuntur Wasi, Cajamarca, Peru) were used. Twelve seeds of every tomato line, obtained from the Tomato Genetics Resources Center of Davis University in California, were sterilized for 5 minutes in 70% ethanol, washed twice in sterile distilled water and germinated on hormone-free MS medium (Murashige and Skoog 1962). Seeds were placed into Erlenmeyer flasks containing a 20 ml medium (one per jar). After four weeks, 1-cm long shoot apexes were isolated and transferred on MS medium with $2 \text{ mg} \cdot \text{dm}^{-3}$ KIN to obtain the appropriate number of plants for establishing two experiments.

Test for salt tolerance in meristem culture. In the first stage of the studies fragments of shoot apex, 1 cm long, were put on the Murashige and Skoog medium (1962) with the addition of: 25, 50, 75, 100 and 150 mM NaCl. The plants on the medium without NaCl were treated as the control. Each jar filled with 30 ml of medium contained 6 explants. After four weeks of culture, the number of leaves, height and weight of plants were determined.

Test for salt tolerance in callus culture. In the second experiment, salt tolerance of the examined forms was determined in the callus cultures. Callus cultures were induced using the leaf fragments placed on the MS medium supplemented with $2 \text{ mg} \cdot \text{dm}^{-3}$ NAA and $5 \text{ mg} \cdot \text{dm}^{-3}$ BAP. The initiated callus was propagated twice on the medium given above. After propagation, it was placed on MS with $10,7 \mu\text{M}$ NAA and $22,2 \mu\text{M}$ BAP and NaCl at concentrations of 75, 100, 125, 150 mM NaCl. Approximately 1 g callus fragments were transferred to glass jars with 30 ml of medium. Four weeks later, the callus weight was determined.

Conditions of culture, pH and autoclaving conditions were the same in all stages. The medium was adjusted to pH 5.7 before autoclaving at 120°C for 20 min. Cultures were conducted at temperature 25°C , under 16-h photoperiod, in fluorescent lighting $40 \text{ PAR} (\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})$.

In both experiment was set in a two-factor completely randomised design, each combination consisted of 10 replications – one jar per replication. The significance of differences was assessed by analysis of variance and the Tukey test at $\alpha = 0.05$. Homogeneous groups between plants of each line growing on media with different NaCl content were marked in the table column with successive letters of alphabet.

RESULTS AND DISCUSSION

Hassanein (2004) in research on *L. esculentum* Mill. states that selection for salt tolerance using indirect organogenesis is much more restrictive than conduct them only in the stage of meristem cultures. Development of *L. esculentum* Mill. plants in meristem cultures was entirely inhibited when the concentration of NaCl in the medium was 129 mM and the regeneration of plants from the callus was inhibited when the NaCl concentration in medium was 43 mM. Even though the meristem cultures characterize lower the genetic variability than callus cultures, which is favorable to maintenance traits, the object of the selection, so it makes the selection more effective than in the case of callus cultures (Hu and Wang 1983). Moreover, getting fully developed plants is easier and quicker.

The analysis of variance has shown a highly significant NaCl effect on the height of all *L. peruvianum* plants, number of leaves and weight of plants. Highly significant were also differences between tested lines and the interaction between lines and culture medium in all above-mentioned morphological traits.

For the LA0462 line, the biggest growth of plants was measured in the medium with 25 mM NaCl (6.03) and 50 mM NaCl (5.0 cm) (Table 1).

Table 1. Values of morphological traits of *Lycopersicum peruvianum* L. lines regenerated on media with various NaCl content in meristem cultures

Tabela 1. Wartości cech morfologicznych roślin *Lycopersicum peruvianum* L. lines regenerowanych na pożywkach o zróżnicowanej zawartości NaCl w kulturach merystemów wierzchołkowych

NaCl concentration Koncentracja NaCl (mM) (A)	Height of plants (cm) Wysokość roślin (cm)			Number of leaves Liczba liści			Weight of plants (g) Masa roślin (g)		
	<i>Lycopersicum</i> lines – Linie <i>Lycopersicum</i> (B)								
	LA0462	LA1692	LA4316	LA0462	LA1692	LA4316	LA0462	LA1692	LA4316
0 – control	1.6 c	2.0 a	4.3 a	4.6 abc	5.7 ab	5.5 a	1.1 ab	1.0 ab	0.5 a
25	6.0 a	2.2 a	1.4 ab	3.7 abc	6.1 ab	3.2 ab	1.1 ab	1.2 ab	0.4 a
50	5.0 ab	2.5 a	1.1 b	6.9 a	5.2 ab	2.3 ab	1.2 a	1.0 ab	0.3 a
75	3.0 abc	3.2 a	1.6 b	5.0 ab	7.8 a	3.1 ab	0.9 ab	1.6 a	0.3 a
100	2.2 bc	2.7 a	1.1 b	1.2 c	3.4 b	2.4 ab	0.5 b	1.2 ab	0.3 a
150	1.6 c	0.8 a	0.6 b	2.0 bc	3.2 b	0.9 b	0.4 b	0.6 b	0.2 a

* Values, in the same column, followed by the same letter are not significantly different at the $\alpha = 0.05$ level according to Tukey's test.

* Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie według testu Tukeya na poziomie istotności $\alpha = 0,05$.

Plants from the media with the addition of NaCl were higher than the plants from MS medium. Only plants from the medium with the biggest sodium chloride concentration (150 mM) had the same height as control plants (1.6 cm). The number of leaves and weight of plant of this line was the greatest in the medium supplemented with 25, 50 and 75 mM NaCl and was greater than on the control medium, but the differences wasn't statistically significant.

In the case of line LA1692 differences in tested morphological traits were lower (not statistically significant), although the addition of NaCl acted favorably on the development of plant.

All morphological traits: height of the plants, number of leaves and weight of plants line LA 4316, growing on media with the addition of NaCl was significantly reduced in comparison to plant from the control medium.

In studies on tomato Rahman and Kaul (1989) observed that NaCl concentration greater than 80 mM causes the lowering of fresh and dry matter of plants stems and roots. So far, the research conducted on *L. esculentum* Mill have confirmed that there is a positive correlation between salt tolerance of callus and tolerance of fully developed plant (Tal et al. 1978, Perez-Alfocea et al. 1994). In research of Alfocea et al. (1993), it was found that the callus initiated from leaves of *L. esculentum* Mill. accumulated more Na⁺ kations than leaves of fully developed plants. Cano et al. (1998) compared salt tolerance of *L. esculentum* Mill. and its wild relative *L. pennellii* at the stage of meristem cultures and callus cultures. Both kinds of cultures had the same NaCl concentration in the medium: 0, 35, 70, 105, 140, 175 and 210 mM. *L. esculentum* Mill plants did not develop roots even in the media with poor NaCl concentrations and *L. pennellii* plants developed roots even in the media with the highest NaCl concentration. Authors observed that plant height had no effect on the determination of salt tolerance in plants. But growth of callus was a good indicator for salt tolerance since the results regarding callus growth were similar to results obtained for the length of roots. Callus of *L. pennellii* in the medium with NaCl supplementation had greater growth than callus of *L. esculentum* Mill. Rzepka-Plevneš et al. (2007a) in studies on salt tolerance of two wild tomatoes *L. peruvianum* f. *glandulosum* and *L. pennellii* observed that the weight of callus of both species decreased in the medium supplemented with 150 to 200 mM NaCl.

In our study NaCl has a highly significant effect on the weight of callus in all *L. peruvianum* tested lines (Table 2).

Table 2. Weight of callus (g) propagated on the media with different concentration of NaCl
Tabela 2. Masa kalusa (g) namnażanego na pożywkach o zróżnicowanej zawartości NaCl

NaCl concentration Koncentracja NaCl (mM)	<i>Lycopersicum peruvianum</i> lines Linie <i>Lycopersicum peruvianum</i>		
	LA 0462	LA 1692	LA 4316
0 – control	2.2 b	1.6 a	1.0 a
75	11.2 a	3.4 a	1.7 a
100	11.7 a	2.7 a	1.1 a
125	8.4 a	1.1 a	0.9 a
150	3.9 b	1.0 a	0.5 a

* Values, in the same column, followed by the same letter are not significantly different at the $\alpha = 0.05$ level according to Tukey's test.

* Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie według testu Tukeya na poziomie istotności $\alpha = 0,05$.

Highly significant were the differences between the tested lines and the interaction between lines and culture medium. In the medium with the highest 150 mM NaCl concentration, the weight of callus was the biggest for LA0462 line – 3.9 g and it was greater than the control – 2.2 g. This result suggests that NaCl supplementation has a positive impact on the weight of callus. The callus of LA0462 line had the greatest weight on the medium with 100 mM supplementation – 11.7 g. For the callus of LA1692 line, the greatest weight of callus was observed on the medium supplemented with 75 mM NaCl – 3.4 g. The lowest weight of callus of all tested lines was observed for the callus of LA4316 line, the greatest weight was measured on the medium supplemented with 75 mM NaCl – 1.7 g.

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