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SOMACLONAL VARIABILITY IN CALLUS CULTURE OF *Lycopersicon hirsutum* f. *typicum* AND *Lycopersicon chilense*

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Abstract. The aim of the study was to induce somaclonal variability in the callus culture of *L. hirsutum* f. *typicum* and *L. chilense* and to characterize them in respect to tolerance to salinity. Callus was initiated on the cotyledon fragments grown on the medium supplemented with NAA and BAP. The tolerance of callus to salt was tested on MS media containing NaCl in concentration 25, 50, 75, 100, 200 mM. Callus tolerant to 100 mM NaCl was next regenerated by ten weeks on the media with different doses of growth regulators. After this time genetic differences between selected fragments and control (MS medium containing no growth regulators) were determining using ISSR-PCR method. The results show that NaCl concentration significantly affects the regeneration of callus in *L. hirsutum* f. *typicum* and *L. chilense*. The dose 200 mM NaCl of the medium results, in both species, in callus dying. Comparing the genetic similarity of examined callus samples with the control ones in both species, it may be stated that the differences in their response to NaCl and applied growth regulators were generally in the range 2–30%.

Key words: Callus, tomato, wild species, tolerance, salinity, growth regulators, DNA, variability

INTRODUCTION

Cultivar genetic homogeneity, not only in tomato, is one of the major causes of breeding progress deceleration and the shortage of initial breeding material [Kochieva et al. 2002, Rzepka-Plevneš et al. 2007a, b]. It is especially true for resistance breeding, which has been growing in importance in recent years. Therefore, the search for the sources of genetic variability as far as the traits of economic significance are concerned, is a necessity.

The most important for plant breeding group of genetic variability constitute natural populations of wild species [Enmilio et al. 1998, Rzepka-Plevnes 1990, Kochieva et al.

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2002]. Among them there are genotypes resistant to common diseases and unfavorable environmental conditions, and their occurrence frequency may be increased by the induction of mutation and somaclonal variability *in vitro* [Larkin and Scowcroft 1983, Mc Coy 1987, Karp 1991, 1995]

In this work, we report the procedure of initiation of somaclonal variability in callus culture of two wild tomato species – *L. hirsutum* f. *typicum* and *L. chilense*.

MATERIALS AND METHODS

Two wild species of tomato, *L. hirsutum* and *L. chilense*, were used in the study. Their seeds were obtained from the Tomato Genetics Research Center, University of California, Davis (origin – Peru). *L. hirsutum* f. *typicum* was classified as the subgenus – *Eriopersicum*, *L. chilense* – as *Eulopersicon*. [Kochieva et al. 2002]. The above mentioned species are characterised by different reproductive systems. *L. hirsutum* f. *typicum* belongs to autogamous species (self-polinator), *L. chilense* to allogamous (cross-polinator).

The experiment was conducted in four stages. During the first one callus was initiated, during the second one the tolerance of callus fragments to salt was obtained, during the third one they were regenerated and during the fourth one genetic differences between selected fragments of callus were characterised by ISSR-PCR method.

The callus was initiated on the cotyledon fragments grown on MS medium supplemented with auxin NAA (5 $mg \cdot dm^{-3}$) and cytokinin BAP (5 $mg \cdot dm^{-3}$). The tolerance of callus to salt was tested on MS media containing NaCL at the concentration 25, 50, 75, 100, 200 mM NaCl (tab. 1). The callus growing on the medium without NaCl was treated as control. Fragments of callus tolerant to 100 mM NaCl in the medium of both examined species were regenerated on the media with different doses of auxins and cytokinins (tab. 2). After four weeks of culture under controlled lighting and temperature conditions phenotypic differences within the selected *L. hirsutum* f. *typicum* and *L. chilense* callus fragments were determined. On the DNA level the differences between selected callus fragments and the control were determined using ISSR primers.DNA was isolated from callus, using the Genomic DNA Prep Plus (A&ABiotechnology) kit. Sequences of genomic DNA between microsatellite sequences, characterized by a different length and different composition of the repeated motif, were subjected to a molecular analysis. The DNA amplification was conducted according to the method described by Zietkiewicz et al. [1994]. The amplification products were separated one hour on a Sub Gel GT (Bio-Rad) apparatus, on 2% agarose, under a constant current of 65V.The obtained products were visualised on a transluminator UV 21 (Fotodyne) in the presence of ethidium bromide (5 $mg \cdot dm^{-3}$) and documented (camera Polaroid DS 34). For the analysis of the ISSR amplified products "Diversity one" ed.1.3. (Pharmacia LKB) computer software was used. The overall number of ISSR products was determined including the number of mono-, polymorphic products (tab. 3, 4). A comparison was made between the products specific for the amplification of tolerant to salt fragments of callus *L. hirsutum* f. *typicum* and *L. chilense* and for the control callus fragments and plants (*L. hirsutum* f. *typicum*). Genetic similarity between callus fragments was expressed in percentages (tab. 5, 6).

RESULTS

The examined tomato species *L. hirsutum* f. *typicum* and *L. chilense* differed in their callus initiation capacity depending on the applied at this stage medium hormonal composition. *L. hirsutum* f. *typicum* produced most callus on the medium containing 5 mg·dm^{-3} NAA and 2 mg·dm^{-3} BAP (0.23 g), *L. chilense* – on 5 mg·dm^{-3} NAA and 5 mg·dm^{-3} BAP (0.52 g). With the lack of growth regulators there was no callus initiation in *L. hirsutum* f. *typicum* and in *L. chilense* no significant effect on the amount of produced callus tissue was observed (tab. 1). The callus of *L. hirsutum* f. *typicum* was characterised by a light green colour with white coating and brown fragments (Photo 1a) *L. chilense* – light green colour and loose structure (Photo 1c).

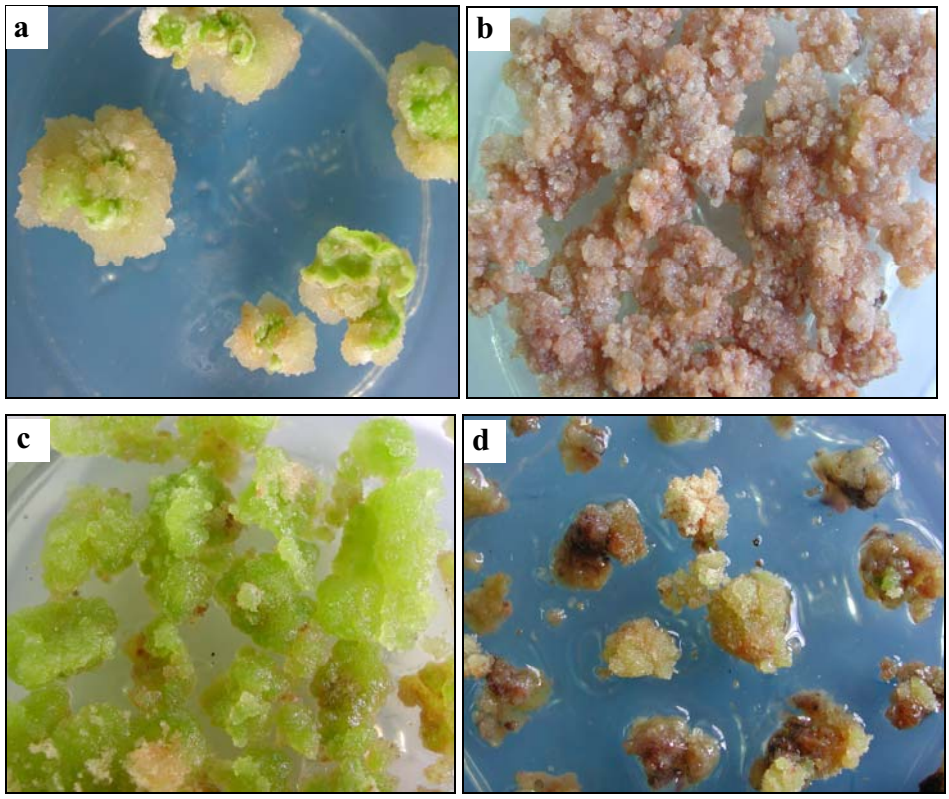

Fig. 1. The influence of NaCl for multiplication of tomato callus (a – control of *Lycopersicum hirsutum*, b – medium with 100 mM NaCl, c – control of *Lycopersicum chilense*, d – medium with 100 mM NaCl

Fig. 1. Wpływ NaCl na multiplikacje kalusa pomidora (a – kontrola z *Lycopersicum hirsutum*, b – średnio z 100 mM NaCl, c – kontrola z *Lycopersicum chilense*, d – średnio z 100 mM NaCl

The effects of NaCl content in the medium on callus growth were significant in both the species (tab. 1). In *L. hirsutm* f. *typicum* the least callus was obtained on the media supplemented with 200, 150, 100 mM NaCl: 0.13, 0.46, 0.93 g respectively. In other experimental combinations its weight did not depend on NaCl amount and was at the same level as the control. In *L. chilense* a significantly lower amount of callus was obtained on the media with 200, 150 and 75 mM NaCl. Corresponding callus weight amounted to: 9.22 g, 19.92 g and 27.92 g whereas on the remaining ones ranged from 28.56 (control) to 39.66 g. Like at the stage of callus initiation, its weight was significantly the highest in *L. chilense*, irrespective of the combination (tab. 1, Photo 1a, b, c, d).

Table 1. Mean weight of callus (g) *L. hirsutum* f. *typicum* and *L. chilense* after proliferation on the media with different concentration of NaCl

Tabela 1. Średnia masa kalusa (g) *L. hirsutum* f. *typicum* i *L. chilense* po namnażaniu na pożywkach o różnym stężeniu NaCl

Species Gatunek	Control Kontrol	Concentration of NaCl, mM Stężenie NaCl, mM					
		25	50	75	100	150	200
L. hirsutum f. *typicum*	1.19 a*	1.09 a	1.08 a	1.02 a	0.93 b	0.46 c	0.13 d
Percent of control Procent kontroli	100	91	90	86	78	39	11
L. chilense	28.56 a	32.02 a	30.66 a	27.22 b	31.13 a	19.92 c	9.22 c
Percent of control Procent kontroli	100	112	107	95	109	66	32
Differences between species Różnica pomiędzy gatunkami	27.37	30.93	29.58	26.20	30.20	18.46	9.09

* a,b,c … – Means in columns differ significantly (p = 0.05), if they are not marked with the same letters
* a, b, c …. – Średnie różnią się istotnie (p = 0,05), jeśli nie są oznaczone tymi samymi literami

Study results revealed a significant influence of growth regulators on the regeneration of tolerant to salinity (100 mM NaCl) callus fragments in both tomato species (tab. 2). In the case of *L. hirsutum* f. *typicum* significantly highest amounts were obtained on the media with NAA, BAP and IBA at the following proportions: 7.5 mg·dm^{-3} NAA : 5 mg·dm^{-3} BAP (14.8 g), 2 mg·dm^{-3} IBA: 5 mg·dm^{-3} BAP (13.8) and 3 mg·dm^{-3} NAA: 1 mg·dm^{-3} BAP (14.5 g), in *L. chilense* – on the media containing: 1 mg·dm^{-3}, 2 mg·dm^{-3} BAP (30.0 g), 3 mg·dm^{-3} NAA, 3 mg·dm^{-3} BAP (29.90 g) and 5 mg·dm^{-3} NAA, 5 mg·dm^{-3} BAP (26.71). The callus weight of *L. chilense* significantly exceeded the weight of *L. hirsutum* f. *typicum* callus, regardless of the applied growth regulators.In order to determine the genetic variability of *L. hirsutum* f. *typicum* salt – tolerant callus 18 ISSR primers were used and 10 primers for *L. chilense*. Five and three of them, for *L. hirsutum* f. *typicum* and *L. chilense*, respectively, generated visible on electrophoregrams DNA bands (tab. 3). ISSR *loci* specific for the control and *L. hirsutum* f. *typicum* callus fragments k_3, k_4, k_5, k_6, k_7 were obtained using the primers 801, 808, 812 and 818. The locus of 1157bp (primer 818) was specific for the control, the *loci* of 1650bp and 606bp (primer 808) for k_3, 745bp (primer 801) for k_4, 1639bp (primer 812) for k_5, 838 and 1783bp (primer 818) – for k_6 and 504bp (primer 801) for k_7.

Table 2. Mean weight of callus (g) *L. hirsutum* f. *typicum* and *L. chilense* tolerance of NaCl after proliferation on the media with different doses of growth plant hormones ($mg \cdot dm^{-3}$)

Tabela 2. Średnia masa kalusa (g) *L. hirsutum* f. *typicum* i *L. chilense* tolerancyjnego na NaCl po namnażaniu na pożywkach o zróżnicowanej zawartości roślinnych regulatorów wzrostu ($mg \cdot dm^{-3}$)

Species Gatunek	Medium – Pożywka										
	control kontrol MS	7,5 NAA 5 BAP	2 IBA 5 BAP	3 NAA 1 BAP	1 NAA 2 BAP	0,02 NAA 2 kinetin	5 NAA 5 BAP	3 NAA 3 BAP	3 NAA 2 BAP	5 NAA 2 BAP	x
L. hirsutum f. *typicum*	11.5 b	14.1 a	13.8 a	14.5 a	10.1 b	9.8 b	8.2 c	7.8 c	5.8 d	5.6 d	9.0
Percent of control Procent kontroli	100	123	120	126	88	85	71	68	50	49	78
L. chilense	16.5 d	–	–	19.8 c	30.0 a	18.9 d	26.7 a	29.9	20.7 c	23.4 b	21.2
Percent of control Procent kontroli	100			120	182	115	162	181	126	142	128
Difference between species Różnica między gatunkami	5.0	–	–	5.3	19.9	9.1	18.5	22.1	14.9	17.8	15.6

* a, b, c …. – means in columns differ significant (p = 0.05) if they are not marked with the same letters
* a, b, c …. – średnia różniące się istotnie (p = 0,05), jeśli nie są oznaczone tymi samymi literami

Table 3. Products of ISSR-PCR amplification of the analyzed tomato species

Tabela 3. Produkty reakcji ISSR-PCR analizowanych gatunków pomidora

Species Gatunki	Primer no Nr startera	Sequence Sekwencja (5' – 3')	Max – min length Długość max – min (bp)	Total number of DNA fragments Liczba fragmentów DNA	Number of products Liczba produktów		
					monomorphic monomorficzne	polymorphic polimorficzne	specyfic specyficzne
L. hirsutum f. *typicum*	801	$(AG)_8G$	431–1892	12	0	11	1
	808	$(AG)_8C$	606–2000	20	10	8	2
	809	$(GA)_8YG$	216–1868	41	10	30	1
	812	$(GA)_8C$	549–2328	31	0	30	1
	818	$(CA)_8G$	838–1866	16	0	13	3
Total – Suma				120	20	92	8
L. chilense	818	$(CA)_8G$	645–3672	22	0	20	2
	831	$(AC)_8C$	383–3000	71	22	49	0
	834	$(AG)_8GC$	62–3000	45	0	43	2
Total – Suma				138	22	112	4

The ISSR loci specific for *L. chilense* were obtained for three callus fragments – control of 2000bp (primer 818), k8 – 1259bp (primer 834) and k5 – 3672bp (primer 818) and 1096bp (primer 834).

Two control forms marked with c and k_1 in table 5 were used for estimating genetic similarity between the examined fragments of *L. hirsutum* f. *typicum*. The control marked with c consisted of the plants obtained from the direct regeneration on MS medium, k_1 – callus initiated on MS medium. The obtained results indicate great genetic

differences between those control forms. There was 40% similarity between them and from 0 (k_2) to 33.3 % (k_4, k_5) similarity between the control plants and salt-tolerant callus parts, whereas the genetic similarity of salt-tolerant callus to control plants (k_1) ranged from 37.5 (k_2) to 72% (k_7) and from 37.5 (k_2) to 87% (k_9) between salt-tolerant callus fragments.

Table 4. Accession-specific products revealed trough ISSR fingerprinting
Tabela 4. Produkty specyficzne uzyskane dzięki reakcji ISSR

Number of callus fragments Liczba fragmentów kalusa	Primer no. and products length (bp) Numer startera i długość produktu (bp)	
	L. hirsutum f. *typicum*	*L. chilense*
K3	808 (1650), 808 (606)	–
K4	801 (745)	–
K5	812 ((1639	818 (3672), 834 (1096)
K6	818 (838, 1783)	–
K7	801 (504)	–
K8	–	834 (1259)
Control – Kontrola	818 (1157)	818 (2000)

Table 5. Similarity (%) between control and callus fragments of *L. hirsutum* f. *typicum* with tolerance to 100 mM NaCl in medium.
Tabela 5. Podobieństwo (%) między kontrolą a fragmentami kalusa *L. hirsutum* f. *typicum* tolerancyjnymi na zasolenie 100 mM NaCl w pożywce

Callus trial (no.) Próba Kalusa (nr)	c*	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}
c*	100.0	40.0	0.0	30.0	33.3	33.3	14.3	22.2	25.0	26.7	21.1
k_1	40.0	100.0	37.5	51.9	52.6	56.0	38.1	72.0	69.6	63.6	69.2
k_2	0.0	37.5	100.0	28.6	30.8	21.1	40.0	42.1	35.3	25.0	30.0
k_3	30.0	51.9	28.6	100.0	50.0	60.0	53.8	60.0	64.3	51.9	64.5
k_4	33.3	52.6	30.8	50.0	100.0	45.5	33.3	45.5	50.0	31.6	43.5
k_5	33.3	56.0	21.1	60.0	45.5	100.0	33.3	57.1	61.5	56.0	75.9
k_6	14.3	38.1	40.0	53.8	33.3	33.3	100.0	41.7	45.5	38.1	40.0
k_7	22.2	72.0	42.1	60.0	45.5	57.1	41.7	100.0	84.6	80.0	82.8
k_8	25.0	69.6	35.3	64.3	50.0	61.5	45.5	84.6	100.0	87.0	81.5
k_9	26.7	63.6	25.0	51.9	31.6	56.0	38.1	80.0	87.0	100.0	76.9
k_{10}	21.1	69.2	30.0	64.5	43.5	75.9	40.0	82.8	81.5	76.9	100.0

*Control – Kontrol
C – plants from directly regeneration on the MS medium – rośliny uzyskane w wyniku bezpośredniej regeneracji na pożywce MS
k_1 – callus fragments from MS medium K1 – fragment kalusa z pożywki MS
Callus with tolerance for salt from media – Kalus tolerancyjny na zasolenie z pożywki:
k_2 – 1 NAA + 2 BAP mg·dm^{-3},
k_3 – 0,02 NAA + 2 KIN mg·dm^{-3},
k_4 – 5 BAP + 2 IBA mg·dm^{-3}
k_5 – 3 NAA + 3 BAP mg·dm^{-3}
k_6 – 3 NAA + 2 BAP mg·dm^{-3}
k_7 – 5 NAA + 2 BAP mg·dm^{-3}
k_8 – 3 NAA + 1 BAP mg·dm^{-3}
k_9 – 5 NAA + 5 BAP mg·dm^{-3}
k_{10} – 7,5 NAA + 5 BAP mg·dm^{-3}

Table 6. Similarity (%) between control and callus fragments of *L. chilense* with tolerance to 100 mM NaCl in medium.

Tabela 6. Podobieństwo (%) między kontrolą a fragmentami kalusa *L. chilense* tolerancyjnymi na zasolenie 100 mM NaCl w pożywce

Callus trial Próba kalusa (no.)	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}	k_{11}
k_1	100	42.1	43.5	40.0*	76.9	55.6	33.3	33.3	38.5	38.1	44.4
k_2	42.1	100	78.6	64.0	44.4	52.2	60.7	23.5	71.0	76.9	60.9
k_3	43.5	78.6	100	75.9	45.5	59.3	51.9	47.6	85.7	73.3	66.7
k_4	40.0*	64.0	75.9	100	52.6	33.3	75.0	44.4	68.8	59.2	66.7
k_5	76.9	44.4	45.5	52.6	100	58.8	35.3	54.5	40.0	30.0	58.8
k_6	55.6	52.2	59.3	33.3	58.8	100	18.2	50.0	60.0	56.0	45.5
k_7	33.3	60.9	51.9	75.0	35.3	18.2	100	12.5	53.3	56.0	63.6
k_8	33.3	23.5	47.6	44.4	54.5	50.0	12.5	100	33.3	21.1	50.0
k_9	38.5	71.0	85.7	68.8	40.0	60.0	53.3	33.3	100	72.7	60.0
k_{10}	38.1	76.9	73.3	59.3	30.0	56.0	56.0	21.1	72.7	100	48.0
k_{11}	44.4	60.9	66.7	66.7	58.8	45.5	63.6	50.0	60.0	48.0	100

*Control – Kontrol

k_1 – callus fragments from MS medium – fragment kalusa z pożywki MS

Callus with tolerance for salt from media – Kalus tolerancyjny na zasolenie z pożywki:

k_2 – 1 NAA + 2 BAP mg·dm^{-3},
k_3 – 0,02 NAA + 2 KIN mg·dm^{-3},
k_4– 2 NAA + 2 BAP mg·dm^{-3}
k_5 – 3 NAA + 3 BAP mg·dm^{-3}
k_6 – 3 NAA + 2 BAP mg·dm^{-3}
k_7 – 5 NAA + 2 BAP mg·dm^{-3}
k_8 – 3 NAA + 1 BAP mg·dm^{-3}
k_9 – 5 NAA + 5 BAP mg·dm^{-3}
k_{10} – 5 NAA + 3 BAP mg·dm^{-3}
k_{11} – 2 NAA + 5 BAP mg·dm^{-3}

Isolated from *L. chilense* callus tissue fragments, tolerant to NaCl, differed from the control at DNA level (tab. 6). Their genetic similarity was within the range 33.3% (for k_7, k_8) – 76.9% (for k_5). The most distant genetically were found to be the callus fragments represented by k_8 and k_7 (12.5% similarity) and the closest ones – k_3 and k_2 (78.6% similarity).

Comparing the genetic similarity of examined callus samples with the control ones (k_1) in both the species, it may be stated that the differences in their response to NaCl and applied growth regulators were generally in the range 2–30 % (tab. 5, 6). Samples k_2 may be a good example, 40% similarity between k_2 and k1 in *L. hirsutum* f. *typicum* and 42.1% in *L. chilense*. In turn, there was 51.9% similarity between k_3 and k_1 in *L. hirsutum* f. *typicum* and 78.6% in *L. chilense*; 56% likeness between k_2 and k_5 in *L. hirsutum* f. *typicum* and 45.5% in *L. chilense*. Similar differences may be observed between other DNA samples of NaCl-tolerant callus samples, regenerated on the media with the same amount of growth regulators (tab. 5, 6) .

DISCUSSION

Environmental stresses belong to the factors limiting plant productivity. Salt stress is one of the most important from them [Borsani et al. 2001, 2003, Queirós et al. 2007]. High salt concentration in root zone hinders their growth and development. The study

results of some authors [Hulisz 2007] demonstrate that even a slight soil salinity may lower the fecundity of sensitive plants whereas strong salinity results in high yields only in the crops tolerant to salinity. According to Domin [2003] the majority of field crops cannot complete their life cycle under conditions of salinity (NaCl) exceeding 100 mM. In the case of two experimental species of wild tomato *L. hirsutum* f. *typicum* and *L. chilense* the tolerance to NaCl stress at callus level was high. In *L. hirsutum* f. *typicum* significantly lower amounts than the control ones were obtained on the media with the addition of 200, 150, and 100 mM NaCl, in *L. chilense* – on the media with 200, 150, 75 mM NaCl.

Genetic sources of salt tolerance have been searched for among: *Pinus elliottii* Englem [Zhang et al. 1997], *Quercus robur* L. [Aloui-Sosse et. al. 1995], *Pinus silvestris* [Rzepka-Plevneš et al. 2006], sugar cane [Wahid et al. 1997], *Solanum tuberosum* L. [Queirós et al. 2007], tomato [Rzepka-Plevneš and Furmanek 1997, Rzepka-Plevneš et al. 2007a, Rzepka-Plevneš et al. 2007b], bermudagrass – *Cynodon transvaalensis* × *C. dactylon* [Lu et al. 2007], Persian clover – *Trifolium resupinatum* var. *major* Boiss [Ates and Tekeli 2007], strawberry – *Fragaria* × *ananassa* Duch. [Dziadczyk et al. 2003].

There are many methods of estimating the tolerance of breeding materials to salinity. They may be used in the field and greenhouse in different stages of plant development, at seedling stage [Rzepka-Plevneš and Furmanek 1997], at the stage of callus and regenerated from it plants [Pollard and Walker 1990, Rzepka-Plevneš et al. 2007 b]. In each case obtaining initial breeding material is a difficult task since the genetic mechanism determining this trait has not been recognized.

In the opinion of Monforte et al. [1997] the advances in breeding salt-tolerant tomato cultivars are not spectacular. The reason is the lack of appropriate sources of genetic tolerance to salinity. In wild tomato species that have been used so far it was generally correlated positively with low productivity. Their improvement in this respect requires many breeding practices and backcrossing with cultivated cultivars [Rzepka-Plevneš 1990].

L. hirsutum f. *typicum* and *L. chilense* selected for our studies are characterized, like *L. pennelli* [Cano et al. 1996] by a high level of salt (NaCl) tolerance. However, their use for breeding may be limited not only because of positive correlation with undesirable traits but also problems with callus regeneration both towards morphogenesis and somatic embryogenesis.

Among the studies on tomato tolerance to salinity the papers of Jia et al. [2002] concerning its transformation by gene BADH (betaine aldehyde dehydrogenese) deserve attention. The studies showed that tomato sensitivity to salt stress results from the lack of glicynobetaine (cell osmoprotectant from salt stress) accumulation capacity. BADH gene was isolated from the salt-tolerant *Atriplex hortensis*. Transgenic plants with this gene were tolerant to NaCl, even to 120 mM of the medium. Promising outcome of producing transgenic tomatoes with the gene controlling salt-tolerance, derived from other genera, do not preclude the scientists from further quest within the genus *Lycopersicon*.

Borsani et al. [2001] used for this purpose mutagenesis resulting in recessive genes within two gene loci TSS1 and TSS2 sensitive to salinity. Retarded growth of mutants tss1 and tss2 was caused by excessive amount of Na+ and Li+ ions in the medium. Mutant tss1 was incapable of growing on the medium with low concentration of K+ ions.

In our studies, genotypes tolerant to salinity were searched for among *in vitro* culture regenerates. It appeared that inducing somaclonal variability *in vitro* does not require special treatment. In *L. hirsutum* f. *typicum* for instance, merely placing the explants on the medium resulted in the genotype alteration of induced callus. The callus genetic similarity to the plants obtained by direct organogenesis was only 40%, which is in agreement with the findings of other authors concerning *in vitro* initiation of somaclonal variability [Larkin and Scowcroft 1981, Peschke and Philips 1992, Lutts et al. 1998]. In the both species under study the influence of applied growth regulators for the regeneration of callus tolerant to 100 mM NaCl was similar. The differences between chosen DNA fragments were within the range 2–30%. There was: 12.5–78.6% similarity between *L. chilense* callus samples and 33.3–76.9% between them and the control (callus on MS), 37.5–72% similarity between *L. hirsutum* f. *typicum* and control samples, 37.5–87% between *L. hirsutum* f. *typicum* samples.

The conducted studies on tomato tolerance to salinity show that understanding salt-tolerant plant adaptation mechanisms requires further examinations. They should focus on the identification of metabolic processes and genes, whose activity is regulated by salt stress, as well as related with them gene markers [Borsani et al. 2003]. Of no lesser importance for breeding is a continuous search for tolerant forms – potential sources of salt-tolerant genes.

CONCLUSIONS

Among two tomato species used in the studies *L. chilense* has a higher callus initiation capacity than *L. hirsutum* f. *typicum*, with the highest weight obtained on the medium supplemented with 5 mg·dm^{-3} NAA and 5 mg·dm^{-3} BAP. The highest weight of *L. hirsutum* f. *typicum* was obtained on the medium with 5 mg·dm^{-3} NAA and 2 mg·dm^{-3} BAP. These species are difficult study objects due to their weak callus regeneration capacity both by somatic embryo initiation and morphogenesis.

NaCl concentration significantly affects the regeneration of callus in *L. hirsutum* f. *typicum* and *L. chilense*. The weight of *L. hirsutum* f. *typicum* did not differ from the control at the concentration: 25, 50, 75 mM NaCl, in *L. chilense* – at 25, 50, 75 and 100 mM. The dose 200 mM NaCl of the medium results, in both species, in callus dying.

Observed in both species callus tolerance to NaCl salinity is probably in the majority of cases a genetic variability as it can be seen from the found differences at DNA level between salt-tolerant callus fragments and their genetic distance in relation to the control.

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ZMIENNOŚĆ SOMAKLONALNA W KULTURACH KALUSOWYCH *Lycopersicon hirsutum* f. *typicum* I *Lycopersicon chilense*

Streszczenie. Celem pracy była próba indukowania zmienności somaklonalnej w kulturach kalusowych *L. hirsutum* f. *typicum* i *L. chilense* oraz charakterystyka ich tolerancji na zasolenie. Kalus inicjowano na fragmentach liścieni wyłożonych na pożywkę uzupełnioną NAA i BAP. Tolerancję na zasolenie badano, wykładając fragmenty kalusa na pożywkę uzupełnioną 25, 50, 75, 100, 200 mM NaCl. Kalus uznany za tolerancyjny był regenerowany na pożywkach uzupełnionych różnymi kombinacjami roślinnych regulatorów wzrostu. Następnie metodą ISSR-PCR określono różnice genetyczne pomiędzy wybranymi fragmentami kalusa a kontrolą. Na podstawie otrzymanych wyników badań stwierdzono, że stężenie NaCl w sposób istotny wpływa na regenerację w przypadku obu badanych gatunków. Stężenie 200 mM NaCl powoduje zamieranie tkanki kalusowej. Obserwowana u obu gatunków pomidora na poziomie kalusa tolerancja na zasolenie NaCl jest prawdopodobnie w większości przypadków zmiennością dziedziczną.

Słowa kluczowe: kalus, pomidor, dzikie gatunki, tolerancja, zasolenie, regulatory wzrostu, DNA, zmienność

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