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GENOTYPIC DIFFERENCES BETWEEN TOMATO CULTIVARS DIFFERING IN THEIR RESPONSE TO SALINITY STRESS

RÓŻNICE GENOTYPOWE POMIĘDZY ODMIANAMI POMIDORA O RÓŻNEJ ODPOWIEDZI NA STRES SOLNY

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Streszczenie. Celem pracy było określenie różnic genotypowych między trzema odmianami uprawnymi pomidora [*Lycopersicon esculentum* (Mill.)] – Zorza, Paw i Złoty Ożarowski, poddanych działaniu stresu solnego w warunkach laboratoryjnych. Tolerancję na zasolenie określono za pomocą testu szalkowego na płytkach Petriego, stosując trzy stężenia soli: 25 mM · dm^{-3} NaCl + 25 mM · dm^{-3} KCl, 25 mM · dm^{-3} NaCl + 50mM · dm^{-3} KCl oraz 50 mM · dm^{-3} NaCl. Kontrolę w doświadczeniu stanowiła woda sterylna. Na podstawie przeprowadzonych pomiarów biometrycznych (zdolność kiełkowania, długość siewki i korzeni), badane genotypy podzielono na trzy klasy fenotypowe według ich cech morfologicznych. Siewki uznane za tolerancyjne przydzielono do pierwszej klasy fenotypowej, średniotolerancyjne do drugiej, a wrażliwe do klasy trzeciej. Różnice genotypowe pomiędzy badanymi obiektami określono, stosując technikę ISSR-PCR. Zaobserwowano różnice w długości amplifikowanych sekwencji miedzymikrosatelitarnych pomiędzy badanymi odmiana pomidora, jak i siewkami należącymi do różnych klas fenotypowych. Podobieństwo genetyczne między odmianą Paw i Złoty Ożarowski wynosiło 45,2%, a między odmianą Zorza i Paw – 54,8%.

Key words: genotypes, ISSR-PCR, *Lycopersicon esculentum*, response, salinity.
Słowa kluczowe: genotypy, ISSR-PCR, *Lycopersicon esculentum*, reakcja, zasolenie.

INTRODUCTION

Salinity is one of the main abiotic stress, which considerably reduces plant productivity worldwide (Foolad and Chen 1998). High salt concentrations in plant root areas significantly hinder their growth and development. To minimise the harmful effect of soil salinity, researches have been conducted on selection of genotypes tolerating salinity and on using them in breeding programmes as source components for tolerant cultivars (Alian et al. 2000; Cuartero et al. 2006; Rzepka-Plevneš et al. 2007).

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Proper assessment of the plant response to salinity and selection of tolerant genotypes from cultivated plants, including tomato, depends on the availability of verified methods and access to diverse genetic resource, i.e. sources of genes of tolerance (Lammerts van Bueren et al. 2010) and wild tolerant genotypes (Aslan et al. 2011). Currently, on the one hand such research is intensively supported by MAS (Marker Assisted Selection) techniques, and on the other hand, it is used as research material for the identification of new types of molecular markers. ISSR (Inter Simple Sequence Repeats) is one of the screening technique very often used in genotyping, such as in MAS (Ziętkiewicz et al. 1994; Kochieva et al. 2002; Rzepka-Plevneš et al. 2004; Cuartero et al. 2006; Muchero et al. 2010). This technique was used by Kochieva et al. (2002) for screening cultivated and wild accessions of the tomato *Lycopersicon* (Tourn) Mill., while Rzepka-Plevneš et al. (2007) determined the genetic differences within *Lycopersicon peruvianum* somaclones, for which selection has been investigated according selection criteria presented in previous paper (Rzepka-Plevneš 1996).

The aim of the study was to determine genotypic differences between three tomato cultivars of *Lycopersicon esculentum* (Mill.): Paw, Zorza and Złoty Ożarowski differing in response to salinity stress.

MATERIAL AND METHODS

The research material consisted of seeds of three tomato [*Lycopersicon esculentum* (Mill.)] cultivars: Zorza, Paw and Złoty Ożarowski.

Response to salt stress. The first stage of the research involved the determination of response to salt stress on the basis of a pan test on Petri dishes. To obtain a salinity effect, three combinations of salt solutions were used: 25 mM · dm^{-3} NaCl + 25 mM · dm^{-3} KCl, 25 mM · dm^{-3} NaCl + 50 mM · dm^{-3} KCl, and 50 mM · dm^{-3} of NaCl, with a pH of 6.0. Sterile water was used in as the control. The seeds were sterilised in 0.1% NaOCl solution for 70 minutes, rinsed in sterile water for 30 minutes and next immersed in 5.0 mM · dm^{-3} $CaSO_4$ solution for 1 minute and after they were placed on Petri dishes lined with filter paper and moistened with 6.0 dm^{-3} of salt solution. 25 seeds were used per treatment, including control, for each cultivar. The experiments were repeated 3 times. The Petri dishes with the seeds were stored in a growth chamber at a constant temperature of 25ºC and 16-hour lighting. After 3 days energy of germination were assessed. After 10 days, biometric measurements were performed, in which the following parameters were determined: the number of germinating seeds, seedling's height (cm) and the length of roots (cm). On the basis of the obtained results, seedlings of each cultivars were divided into three phenotypic classes according to Rzepka-Plevneš (1996). According to Rzepka-Plevneš (1996) opinion the tolerant cultivar of tomato is characterized by high frequency of seedlings belongs to the first phenotypic class.

ISSR-PCR. ISSR-PCR technique was used to screen genotypic differences between the tomato cultivars being under analysis and between selected seedlings differing in the response to salinity stress. In the analysis seedlings from the control (sterile water) have been included.

DNA was prepared from fresh leaves using liquid nitrogen and Genomic DNA Prep Plus kit (A&A Biotechnology). To determine the quantity and purity of each DNA sample, a GeneQuant RNA/DNA Calculator (Pharmacia LKB) spectrophotometer was used. PCR mixtures (25 μl) contained: 1.5 mM $MgCl_2$, 100 mM KCl, 20 mM Tris-HCl, pH 8.3, 0.1% Triton X-100, 0.2 μM primer, 0.2 mM of each dNTPs, 1.0 unit of *Taq* DNA polymerase (Thermo Scientific) and 50 ng template genomic DNA. Ten ISSR primers were used (UBC – Canada). Amplifications were performed according to Ziętkiewicz et al. (1994) protocol. The PCR products were electrophoresed in 2% agarose gel in presence of ethidium bromide (5mg · dm^{-3}), then UV visualized, and photographed using a MiniBis Pro DNR camera (Bio-Imaging System Ltd, Israel). O'RangeRuler 200bp DNA Ladder (Thermo Scientific) was used as a size marker (3000–200 bp). The relative mobility position of all bands for each analyzed samples was calculated and transformed in a data matrix in which the character "1" mean the presence of a specific band and "0" represent its absence. Gel Quant (Bio-Imaging System Ltd, Israel) and Diversity One 1.3 (Pharmacia LKB) softwares were used to construct dendrogram by the UPGMA (unweighted pair group with arithmetic mean) method.

RESULTS

Phenotypic variability. The research results obtained showed that the genotype, treatments and salt concentrations influenced on the germination ability of the tomato cultivars (Table 1). Cultivar Zorza seeds were characterized by the lowest germination ability, which are germinated on average after 3 and 10 days. After 3 days of testing, germinating seed was observed in the media with salt concentrations 50 mM · dm^{-3} of NaCl and 25 + 50 mM · dm^{-3} of NaCl + KCl. Considering salt solution NaCl + KCl 25 + 25 mM · dm^{-3}, the percentage of germinated seeds was low and it amounted to 12% for cultivar Paw, 8% for cultivar Zorza and 4% for cultivar Złoty Ożarowski. The seeds of all cultivars tested in the study germinated in 50 mM · dm^{-3} of NaCl and 25 + 50 mM · dm^{-3} of NaCl + KCl solutions only after 10 days. Out of the selected tomato cultivars, cultivar Zorza was characterised on average by the lowest germination ability in the testing media.

Table 1. Energy and seed germination (%) of *L. esculentum* cultivars evaluated after 3 and 10 days of salt treatment

Tabela 1. Energia i zdolności kiełkowania nasion (%) *L. esculentum* po 3 i 10 dniach traktowania roztworem soli

Cultivar Odmiana	Energy germination (%) Energia kiełkowania (%) after 3 days – po 3 dniach				Seed germination (%) Kiełkowanie nasion (%) after 10 days - po 10 dniach			
	control kontrola	NaCl (mM · dm^{-3}) 50	NaCl + KCl (mM · dm^{-3}) 25 + 25	 25 + 50	control kontrola	50 mM · dm^{-3} NaCl	NaCl + KCl (mM · dm^{-3}) 25 + 25	 25 + 50
Paw	68	0	12	0	84	84	88	72
Zorza	40	0	8	0	84	64	64	60
Złoty Ożarowski	80	0	4	0	92	96	80	56
Average Średnia	62.6	0	8	0	86.7	81.3	77.3	62.7

The cultivars selected for the test differed in terms of percentage of seedlings tolerant to the salinity treatments applied (Table 2).

Table 2. Mean values for seedling height and roots length of tomato cultivars
Tabela 2. Średnia wysokość i długość korzeni siewek pomidora

Cultivar Odmiana	Seedling height – Wysokość siewek												Root length – Długość korzeni								
	I phenotypic class I klasa fenotypowa				II phenotypic class II klasa fenotypowa				III phenotypic class III klasa fenotypowa				I phenotypic class I klasa fenotypowa			II phenotypic class II klasa fenotypowa			III phenotypic class III klasa fenotypowa		
	cm	SD	%	VC%	cm	SD	%	VC%	cm	SD	%	VC%	cm	SD	VC%	cm	SD	VC%	cm	SD	VC%
											Paw										
Control – Kontrola	2.4												6.2								
NaCl (50 mM · dm^{-3})	4.20	0.07	65	2	2.94	0.26	22	9	1.42	0.41	15	29	7.95	1.48	19	8.14	2.02	25	4.14	2.55	62
NaCl + KCl (25 + 25 mM · dm^{-3})	4.42	0.33	63	7	3.28	0.30	22	9	1.53	0.47	14	39	9.16	2.16	24	7.24	2.86	40	2.53	0.65	26
NaCl + KCl (25 + 50mM · dm^{-3})	4.12	0.11	28	3	3.26	0.56	45	17	1.20	0.58	29	41	8.42	4.60	14	8.93	2.50	28	3.46	1.94	56
											Zorza										
Control – Kontrola	2.3												7.8								
NaCl (50 mM · dm^{-3})	4.10	0.00	12	0	2.84	0.28	85	10	1.25	0.78	12	62	7.05	2.19	31	8.01	2.47	31	1.75	0.78	44
NaCl + KCl (25 + 25 mM · dm^{-3})	4.35	0.26	26	6	3.07	0.51	69	17	0.90	0.00	7	0	11.53	2.77	24	7.95	3.65	46	2.10	0.00	0
NaCl + KCl (25 + 50 mM · dm^{-3})	4.10	0.00	20	0	3.48	0.42	54	12	1.10	0.64	28	58	6.50	1.32	14	5.28	2.82	53	1.90	1.70	89
											Złoty Ożarowski										
Control – Kontrola	3.1												9.1								
NaCl (50 mM · dm^{-3})	4.22	0.16	46	4	3.19	0.48	40	15	1.75	0.07	15	4	9.24	2.72	29	7.75	2.31	30	2.55	0.64	25
NaCl + KCl (25 + 25 mM · dm^{-3})	4.57	0.50	46	11	2.94	0.58	40	20	1.27	0.45	15	36	9.00	2.24	25	5.23	3.08	59	3.53	1.22	35
NaCl + KCl (25 + 50 mM · dm^{-3})	4.40	0.35	36	8	3.63	0.28	58	17	1.90	0.00	7	0	7.00	1.89	27	5.60	0.94	17	2.10	0.00	0

*SD – standard deviation,
SD – odchylenie standardowe,
*% – frequency,
% – częstotliwość,
*VC% – coefficient of variability,
VC% – współczynnik zmienności.

Seedlings were considered as tolerant or medium-tolerant when displayed mean values higher than the control in the majority of the cases. For seedlings tolerant to salinity, the tallest seedlings (4.57 cm) were observed in cultivar Złoty Ożarowski on the medium containing 25 + 25 mM · dm^{-3} of NaCl + KCl salt while the shortest (4.10 cm) seedlings were observed in cultivar Zorza on the media containing 25 + 25 mM · dm^{-3} of NaCl + KCl and 25 + 50 mM · dm^{-3} of NaCl + KCl, respectively (Table 2). The response of tolerant seedlings to the salinity stress manifest in an increase in the root length. The length of seedlings root of cultivars Paw, which was considered to be salinity-tolerant, ranged from 7.95 to 9.16 cm, Zorza – from 6.50 to 11.53 cm, and Złoty Ożarowski from 7.0 to 9.24 cm (Table 2). The particularly longer roots were characterized for seedlings of cultivars Paw and Zorza obtained from treatment 25 + 25 mM · dm^{-3} of NaCl + KCl and Złoty Ożarowski obtained from treatment 25 + 25 mM · dm^{-3} of NaCl + KCl and 50 mM · dm^{-3} of NaCl + KCl. The length of the roots was significantly correlated with the seedling height (data not shown).

Genetic differences. Out of 10 ISSR primers used for DNA amplification, it was possible to obtain visible PCR profiles for 5 of them (Table 3). Altogether, 36 loci were amplified, out of which 11 were described as polymorphic (Table 4), 20 were monomorphic and 5 were described as genotype-specific (Table 5). The largest number of polymorphic loci (4) was obtained when 809 and 848 primers were used for amplification. On the one hand the use of three primers (809, 817 and 848) resulted in obtaining genotype-specific loci (Table 5), which in detail were listed in the table 5. The loci in length of 1900 bp (809), 1400, 880 and 450 bp (817) were specific for Paw, but 400 bp in length (848) for Zorza. On the other hand no significant genotypic differences were found between seedlings tolerant and susceptible to salinity stress, selected from the investigated cultivars. Despite this, is has been shown that the presence of genotype-specific loci may be used in research for identification of the cultivars of *Lycopersicon esculentum*.

Table 3. Characteristic of ISSR-PCR products obtained for tomato cultivars
Tabela 3. Charakterystyka produktów amplifikacji otrzymanych w reakcji ISSR-PCR dla badanych odmian pomidora

Primer number Numer startera	Fragment size range (bp) Długość produktów (pz)	Cultivar Odmiana			Loci Loci			
		Paw	Zorza	Złoty Ożarowski	Polymorphic Polimorficzne	Monomorphic Monomorficzne	Genotype-specific Genotypowo-specyficzne	Total Razem
809	320 – 1900	7	7	7	4	4	1	9
817	450 – 1600	8	3	5	2	3	3	8
818	600 – 1400	5	5	5	0	5	0	5
820	800 – 1700	4	5	5	1	4	0	5
848	400– 2000	6	8	6	4	4	1	9
Total Razem	320 – 2000	29	27	27	11	20	5	36

Table 4. Polymorphic loci produced by ISSR primers
Tabela 4. Produkty polimorficzne otrzymane przy użyciu markerów ISSR

Cultivar Odmiana	Primer number and length [bp] of ISSR products Starter i długość [pz] generowanych produktów
Paw	$809_{[1500, 1100]}$; $817_{[1600, 600]}$; $848_{[2000, 1800]}$;
Zorza	$809_{[1200, 1100, 950]}$; $820_{[800]}$; $848_{[1800, 1500,800]}$;
Złoty Ożarowski	$809_{[1500, 1200, 950]}$; $817_{[1600, 600]}$; $820_{[800]}$; $848_{[2000, 1500]}$;

Table 5. Genotype-specific amplified products generated for two genotypes of *L. esculentum*
Tabela 5. Produkty genotypowo-specyficzne generowane w reakcji ISSR-PCR dla trzech badanych odmian pomidora *L. esculentum*

Cultivar Odmiana	Primer number and length [bp]of ISSR products Starter i długość [pz] generowanych produktów
Paw	$809_{[1900]}$; $817_{[1400, 880, 450]}$;
Zorza	$848_{[400]}$;

Based on the dendrogram topology, it has been found that similarity between tomato cultivars ranged from 45.2 to 54.8% (Fig. 1). The Zorza and Złoty Ożarowski cultivar seedlings were the most similar to each other (54.8%). The lowest genetic similarity was observed between Paw and Złoty Ożarowski.

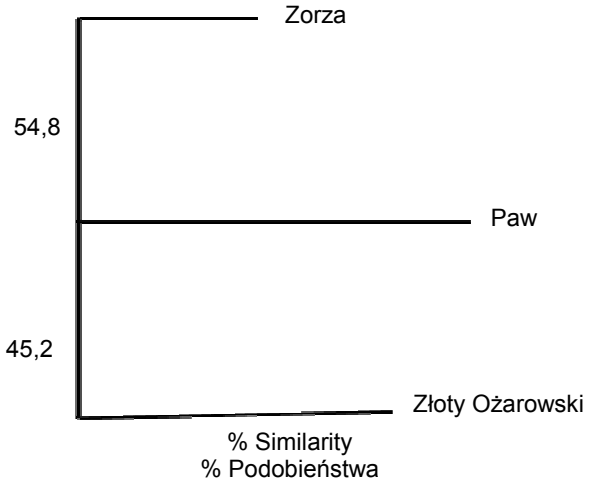

Fig. 1. UPGMA dendrogram of the *L. esculentum* cultivars
Rys. 1. Drzewo podobieństwa genetycznego badanych odmian pomidora *L. esculentum* otrzymane metodą UPGMA

DISCUSSION

Environmental stresses are one of the main factors seriously reducing plant production (Flowers 2004). Out of these, salinity is one of the most important factors, to which numerous studies have been devoted (Wei Sun et al. 2010; Aslan et al. 2011). There are numerous reports in the scientific literature on attempts at improving cultivated plants which would

tolerate salinity stress using classical methods (Borsani et al. 2001, 2003; Queirós et al. 2007; Rzepka-Plevneš et al. 2010). However, success in this area is very limited as the tolerance to high salinity is very complex trait both in genetic and physiological terms (Flowers 2004). Even, if there exist halophytic species within a genus, just as in the case of the tomato, the creation of salinity-tolerant cultivars is very time-consuming (Cuartero and Fernandez-Muñoz 1999).

So far, numerous studies have described a search for a genetic source of tolerance to salinity stress. Such studies have focused on *Pinus elliottii* Englem (Zhang et al. 1997), *Pinus silvestris* L. (Rzepka-Plevneš et al. 2006); Sugar cane (Wahid et al. 1997), tomato (Rzepka--Plevneš and Furmanek 1997; Cuartero et al. 2006; Rzepka-Plevneš et al. 2007) and other species. The presented authors have used a number of various methods, including field and greenhouse tests in describing plant response at various stages of the plant growth (Rzepka--Plevneš and Furmanek 1997), studies using callus cultures (Rzepka-Plevneš et al. 2010), or genetically modified tomatoes (Zhang and Blumwald 2001). However, the mechanisms of tolerance to salinity has only recently to be elucidated.

As it can be concluded from the scientific literature young seedlings are the most sensitive to the effect of salt solution (Cuartero and Fernandez-Muñoz 1999; Alian et al. 2000; Rzepka-Plevneš et al. 2004). Hence, the aim of the study was to determine phenotypic and genotypic differences between seedlings of three tomato cultivars Paw, Zorza and Złoty Ożarowski differ in response to salinity stress under laboratory conditions. On the basis of the results obtained, it was found that concentration 25 + 50 mM · dm^{-3} of NaCl + KCl have had the most inhibiting influence on the ability of seed germination. Those results were consistent with the results obtained by Jones (1986), who conducted research using 13 wild and 20 cultivated tomato accessions. He showed that an increase in the salt concentration up to 100 mM · dm^{-3} in the medium has a distinctly inhibitory effect on the germination ability of tomato seeds. A similar relationship was observed by Cuartero and Fernandez-Muñoz (1999), who found that salt stress in this plant has a negative effect on the seed germination ability, the growth of the seedlings and the root biomass. Cano et al. (1998) in their research on response to tolerance to salinity within cultivated *L. esculentum* variety and the wild genotypes of *L. pennellii in vitro*, used media containing NaCl solution in concentrations: 35, 70, 105, 140, 175 and 210 mM · dm^{-3}, respectively. On the basis of the measurements of biometric and biochemical characteristics, authors have shown, that the majority of *L. esculentum* explantates did not develop roots on media containing higher salt concentrations, while *L. pennellii* explantates formed roots regardless of the NaCl concentration. Such a relationship was also observed in the author's own research, where it was found that a high salt concentration has a stimulating efffect on the root growth of the *Lycopersicon esculentum* cultivars being under analysis.

According to Kochieva et al. (2002), the cultivated tomato is characterized by low genetic variability. Therefore, it is not an easy task to obtain a new cultivar of this plant, which is better than the existing ones. The ISSR-PCR technique was used in the author's own research to define genetic differences between the cultivated tomato varieties. Seedlings differ in the level of tolerance to salinity were characterized by a low level of genetic variability. Electrophoretic patterns, which were obtained for both groups of seedlings, constitute evidence. They differed in the number and length of polymorphic ISSR loci. The

largest number of loci (4) was observed for cultivar Paw, while their lack was found only in electrophoretic patterns of cultivar Złoty Ożarowski. The genetic similarity between tolerant and susceptible tomato cultivars ranged from 45.2 to 54.8%.

It is possible to conclude that genotypic differences in the tomato response to a high salinity stress in the medium observed at the seedling stage might result from interactions between genetic and epigenetic factors.

CONCLUSIONS

The seedlings of the three cultivars of tomato *Lycopersicon esculentum* were characterized by low tolerance to NaCl + KCl treatment and the higher to NaCl. The results of the research have shown that it is possible to select genotypes tolerant to salinity stress. The genetic similarity between the tomato cultivars assessed using ISSR ranged from 45.2 to 54.8%. Zorza and Złoty Ożarowski were the most similar to each other, while the lowest similarity was described for cultivars Paw and Złoty Ożarowski.

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Abstract. The aim of the study was to determine genotypic differences between three tomato [*Lycopersicon esculentum* (Mill.)] cultivars – Zorza, Paw and Złoty Ożarowski which were subjected to salinity stress under laboratory conditions. The plant response to salinity was determined by means of a pan test on Petri dishes, using three concentrations of salt: 25 mM · dm^{-3} NaCl + 25 mM · dm^{-3} KCl, 25 mM · dm^{-3} NaCl + 50 mM · dm^{-3} KCl and 50 mM · dm^{-3} NaCl. Sterile water was used as the control. On the basis of the biometric measurements (the number of germinating seeds, seedling's height, and the length of seedling's roots), each genotypes were divided into three phenotypic classes. The tolerant seedlings were classified to the first phenotypic class, the medium-tolerant to the second one, and the susceptible to the third class.The genotypic differences between cultivars investigated were described using the ISSR-PCR technique. It has been found that not only tomato cultivars, but also seedlings from different classes differed in respect to length of sequences amplified located in the inter simple sequence repeats regions of tomato genomes, and genetic similarity described for cultivars Paw and Złoty Ożarowski was 45.2%, and for cultivars Zorza and Paw – 54.8%.