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RESPONSE OF TOMATO GENOTYPES TO SALINITY STRESS ASSESSED AT THE SEEDLINGS STAGE

M. Smolik[1], P. Kram[2], M. Krupa-Małkiewicz[1], B. Smolik[3], K. Malinowska[4]

[1] *Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin*

[2] *13th General Secondary School, Szczecin: Biology&Chemistry,*

[3] *Department of Biochemistry West Pomeranian University of Technology in Szczecin,*

[4] *Department of Plant Physiology West Pomeranian University of Technology in Szczecin*

ABSTRACT

The aim of the study was to characterize phenotype response to salt stress under *in vitro* conditions of four tomato genotypes: 'Malinowy Ożarowski', 'Pokusa', 'Awizo' F_1 and *Lycopersicon chmielewskii* LA2678. Tomato seeds were cultured with 0 (control), 50, 75, 100 and 125 mmol·dm^{-3} NaCl in nutrient solutions. The effect of the stress applied on the morphological traits was evaluated in 14 day-old seedlings. The contents of proline in tomato shoots was also examined. Statistical correlations were found in shoot and root length as well as in the number of roots, with the exception of wild form *L. chmielewskii*. The analysis of variance showed that the highest shoots were grown on 50 and 100 mmol·dm^{-3} NaCl solution. The shortest shoots were observed in the control. Longer roots (8.6 cm) were developed by the plants from the solutions containing 100 mmol·dm^{-3} NaCl, the shortest (6.6 cm) – 75 mmol·dm^{-3} NaCl. NaCl concentration in the medium significantly affected the number of tomato roots. However, the number of roots developed by the wild form of tomato *L. chmielewskii* was significantly lower in comparison with the other examined cultivars (mean 38-47%). The highest accumulation of proline in tomato cultivars was observed at 125 mmol·dm^{-3} NaCl in nutrient solution and in the wild form at 50 mmol·dm^{-3} NaCl.

Key words: *Solanum*, genotypes, response, salinity, *in vitro*, seedling stage, proline

INTRODUCTION

Tomatoes (*Solanum lycopersicum*) are one of the most important vegetable crops. However, their production is seriously limited because of abiotic stress, such as drought or salinity [Cuartero et al., 2006]. High salt concentration in the root zone hinders plants growth and development. To overcome this problem, plants have developed the mechanisms of physiological adaptation, such as development of the root system to acquire water or accumulation of osmoprotectants. Proline is one of well-known osmoprotectants and its accumulation is widely observed in various organisms under salt stress. The amino-acid may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes. In tomatoes, proline accounts for only a small fraction of the total concentration of osmotically active solutes [Lutts et al., 1996; Viègas and Gomes da Silveira, 1999; Alian et al., 2000; Ueda et al., 2007; Trovato et al., 2008; Szabados and Savourè, 2009].

The first tests made to receive salt-tolerant tomatoes consisted in traditional cross-breeding of salt-tolerant wild forms with tomato cultivars. However, traditional methods are time-consuming and ineffective due to the time of breeding and a problem with the selection of criteria suitable for breeding purposes. One of the methods which can significantly shorten the breeding cycle and test a greater number of plants in laboratory stress conditions is *in vitro* culture. Additionally, it is also an effective method of the selection of genotypes tolerant to abiotic stress. According to some authors [Rzepka-Plevneš et al., 2008; Goel et al., 2010], tolerance to abiotic stress at the seedling stage is positively correlated with tolerance to abiotic stress in mature plants.

The aim of the study was to characterize the phenotype response to salt stress in *in vitro* culture of 14 day-old seedlings of four tomato genotypes and to indicate differences in proline accumulation in tomato shoots.

MATERIAL AND METHODS

Seeds of three tomato cultivars *Solanum lycopersicum*: 'Malinowy Ożarowski', 'Awizo' F_1 and 'Pokusa', obtained from W. Legutko Breeding and Seed Company (Smolice), and wild genotype of *Lycopersicum chmielewskii* LA2678, obtained from the Tomato Genetics Resource Center, Department of Vegetable Crops, University of California, were used in this study. The experiment was conducted in glass pots of 250 cm^3 capacity in a phytotron. As a selection factor, nutrient solution containing 0 (control), 50, 75, 100 and 125 $mmol \cdot dm^{-3}$ NaCl was used. Seeds – 60 per each tomato genotype, were germinated on three layers of filter paper moistened with 12 cm^3 of nutrient solutions and deionized water. Seeds were previously soaked in 0.1% NaOCl solution for 70 min. Then rinsed with sterile water for 30 min and soaked in 5 $mmol \cdot dm^{-3}$ $CaSO_4$ solution for 1 min and transferred to a proper medium in test pots (4 seeds in each test pot). The experiment was replicated three times. The test pots were held for 14 days in the phytotron at 25°C under cool white light (intensity of 40 PAR $\mu Em^{-2}s^{-1}$) with a 16 h photoperiod and 75% RH. After 14 days, shoot and root length (cm) and root number were evaluated. Proline concentration was determined according to the Bates method (1973), and the measurements were replicated three times for each combination of tomato genotype and nutrient solutions used in the experiment. Morphological traits were statistically validated by the analysis of variance. The significance of differences between means was determined with the use of the Tukey's test at $\alpha = 0.05$ and 0.01.

RESULTS

The examined tomato cultivars and the wild form differed significantly in terms of shoot and root length and root number of 14-day-old seedlings tolerant to salt under laboratory conditions (Table 1). The analysis of variance showed that the highest shoots were observed at 50 $mmol \cdot dm^{-3}$ NaCl solution (ranging from from 3.2 cm – *L. chmielewskii* to 4.2 cm – 'Awizo F_1') and 100 $mmol \cdot dm^{-3}$ (from 2.8 cm – *L. chmielewskii* to 3.9 cm – 'Awizo F_1') (Figure 1). The shortest shoots were observed in the control. Their mean length ranged from 2.4 cm for cultivar 'Pokusa' to 3.4 cm for 'Awizo' F_1. No statistically significant influence of various concentrations of NaCl on shoot length in *L. chmielewskii* was demonstrated. However, an increase in the length of tomato shoots of cultivar 'Malinowy Ożarowski' was observed in a higher concentration of NaCl in the solution, except for the solution containing 125 $mmol \cdot dm^{-3}$ NaCl, where shoots had the same length as the control (2.81 cm). Irrespective of nutrient solution, shoots of cultivar 'Awizo F_1' were noted to be 38% higher than those of *L. chmielewskii* and 19% higher than those of 'Malinowy Ożarowski'.

Table 1. Two-way analysis of variance of tomato seedlings traits

Morphological traits	Source of variability	Variance
Root length	genotype	11.21**
	salinity	3.07*
	genotype × salinity	2.14*
Root number	genotype	36.26**
	salinity	3.68*
	genotype × salinity	1.80
Shoot length	genotype	8.30**
	salinity	0.91
	genotype × salinity	0.48

differences significant at: *p = 0.05; ** p = 0.01

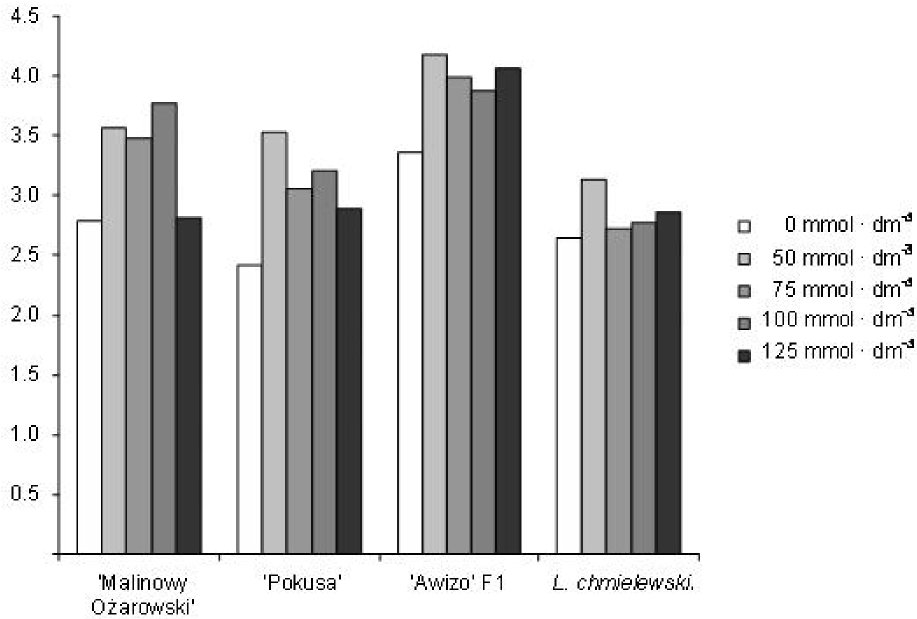

Fig. 1. Effect of salinity on shoot length of four tomatoes genotypes after two weeks of exposure to 0, 50, 75, 100 and 125 mmol · dm^{-3} NaCl.

A significant influence of NaCl solution on root length was found in all the examined tomato seedlings (Table 1). Longer roots (8.6 cm) were developed by the plants growing in solutions containing 100 mmol·dm^{-3} NaCl, while the shortest (6.6 cm) – 75 mmol·dm^{-3} NaCl (Figure 2). Greater root length of seedlings was obtained for the cultivars 'Pokusa' at 100 and 125 mmol·dm^{-3} NaCl solution, respectively: 12.1 and 9.1 cm. In cultivar 'Awizo' F_1, the longest roots were developed by the seedlings tested in the control medium, i.e. water (11.1 cm), whereas the roots of the seedlings tested in different solutions of NaCl were significantly shorter (Figure 2).

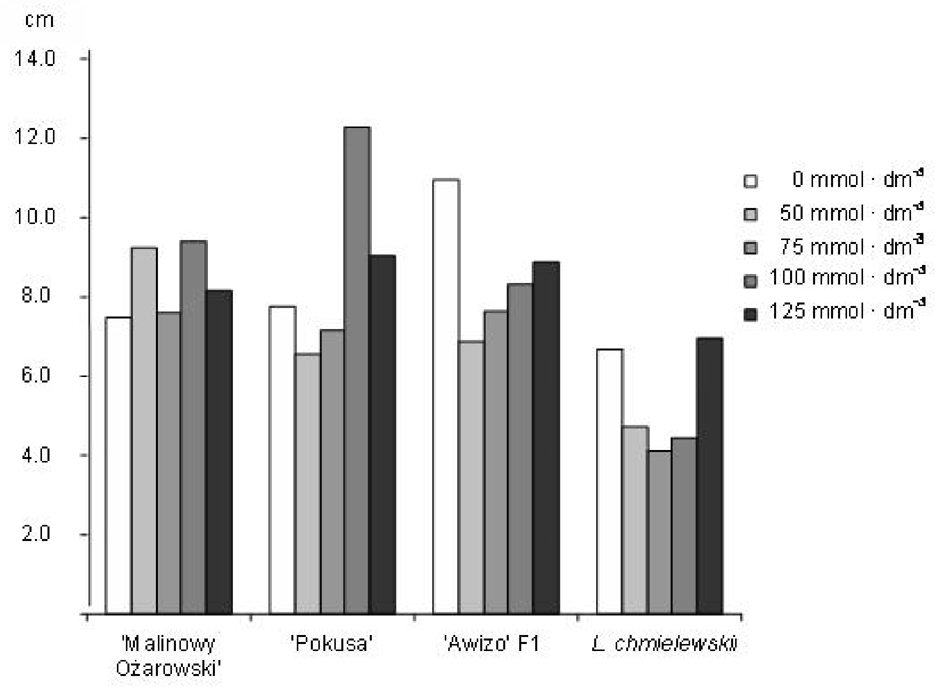

Fig. 2. Effect of salinity on root length of four tomatoes genotypes after two weeks of exposure to 0, 50, 75, 100 and 125 mmol · dm^{-3} NaCl.

Different NaCl solution concentrations significantly affected the number of tomato roots (Table 1). In the solutions containing 50 and 100 mmol·dm^{-3} NaCl, the root number were higher (mean 3.4) than in the control (mean 2.8) (Fig. 3). A root number significantly higher than that of the control was recorded for 'Pokusa' in the solution containing 50 mmol·dm^{-3} (139% of the control) and for 'Awizo' F_1 in the liquid with 100 mmol·dm^{-3} NaCl (151% of the control). However, the number of roots developed by *L. chmielewskii* was significantly lower in comparison with the other examined cultivars (ranging from 38 to 47%).

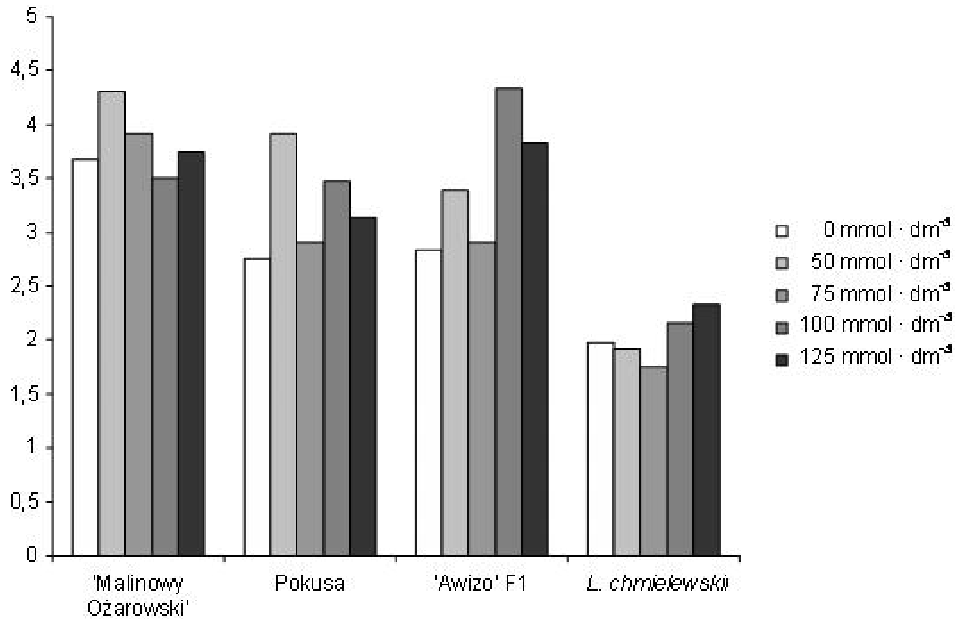

Fig. 3. Effect of salinity on roots number of four tomatoes genotypes after two weeks of exposure to 0, 50, 75, 100 and 125 mmol · dm^{-3} NaCl.

It was observed that concentration of proline in seedling shoots was positively correlated with NaCl concentration in nutrient solution (Table 2). The highest proline concentration was observed in shoots of three tomato cultivars tested in the solution containing 125 mmol·dm^{-3} NaCl (mean 0.142 µg·g^{-1} f.m.). *L. chmielewskii* was an exception – the highest proline concentration in the shoots was found in the solution containing 50 mmol·dm^{-3} NaCl (mean 0.235 µg·g^{-1} f.m.). In the other combinations of the experiment, proline concentration was different depending on both accessions and used NaCl solution and ranged from 0.013 μg·g^{-1} f.m. (‘Malinowy Ożarowski’ – 50 mmol·dm^{-3} NaCl) to 0.120 μg·g^{-1} f.m. (*L. chmielewskii* – the control) (Table 2).

Table 2. Mean values of proline concentration in tomato shoots after two weeks of exposure to 0, 50, 75, 100 and 125 mmol·dm^{-3} NaCl

Genotype	Control (μg·g-1 f.m.)	50 mM (μg·g-1 f.m.)	75 mM (μg·g-1 f.m.)	100 mM (μg·g-1 f.m.)	125 mM (μg·g-1 f.m.)
‘Malinowy Ożarowski’	0.098	0.013	–	0.102	0.170
‘Pokusa’	0.085	0.076	0.018	0.039	0.132
‘Awizo’ F1	0.074	0.020	0.029	0.057	0.123
L. chmielewskii	0.120	0.235	0.064	0.073	0.079

DISCUSSION

The Earth is a salty planet with most of its water containing about 30 g of sodium chloride per litre. This salt solution has been affecting the land on which crops are or might be grown [Flowers, 2004]. It seems that a salt-tolerant cultivar in different stages of plant development should be introduced into agriculture. The mechanism of salt tolerance depends on the capacity for osmotic adjustment which allows growth to continue under saline conditions. Besides, plant response to salinity is different in their life cycles [Rzepka-Plevneš et al., 2008]. According to most authors [Alian et al., 2000; Cuartero and Fernandez-Munoz, 1999; Rzepka-Plevneš et al., 2004], salt stress is more dangerous for young plant seedlings.

The data presented in this paper shows that among four tomato genotypes only the wild form did not show significant differences in shoot and root length and number of roots under these stress conditions in comparison with the control.

Similar data was obtained by Cano et al. [1998], who analyzed salt tolerance of *S. lycopersicon* and the wild form of *L. pennellii* in *in vitro* culture. Seeds of the both species were exposed to different levels of salt in the solutions: 25, 70, 105, 140, 175 and 210 mmol·dm^{-3} NaCl. The reduction of *S. lycopersicon* root growth was greater than in the case of *L. pennellii*.

Cuartero and Fernandez-Munoz [1999] suggested that exposure of plants to salt stress usually begins with exposure of the roots. Salt stress leads to changes in growth, morphology and physiology of the roots that will in turn change water and ion uptake and the production of signals (hormones) that can transfer information to the shoot. Then the whole plant is affected when the roots are growing in a salty medium.

The identification of one or more physiological parameters as indices of drought and salt resistance has been a subject of many research works [Alian et al., 2000; Bahaji et al., 2002; Rzepka-Plevneš et al., 2007, 2008]. The present experiment showed statistically significant differences in shoot and root length and root number of the examined tomato cultivars with the exception of the wild form of tomato (*L. chmielewskii*). The highest shoots were observed in the solution containing 50 mmol·dm^{-3} NaCl. However, the shoots of cultivar ‘Malinowy Ożarowski’ were lower at 125 mmol·dm^{-3} concentration of NaCl in the solution.

According to Rzepka-Plevneš et al. [2008], the ability of plants to avoid unfavorable effect of environmental stress is most probably directly proportional to the level of root development. In the present studies it was especially noticeable in the seedling of cultivar ‘Pokusa’, where the longest roots were observed in a higher concentration of NaCl in the solution. Among the forms analyzed in the present experiment, ‘Pokusa' demonstrated higher tolerance to salinity at the seedling stage. However, there is no significant difference in root length in the wild form *L. chmielewskii*. Their roots were shorter by 36% in comparison with all the other examined seedlings.

Under stress conditions, high concentrations of polyamines, free amino-acids and ammonium ion occur in plant tissue which, in turn, favors proline synthesis, particularly under reduced demand for amino acids protein synthesis and growth in stressed plants [Hare and Cress, 1997; Trovato et al., 2008]. Studies conducted by Viégas et al. [1999] on young cashew plant tolerance to NaCl (0.5 or 100 mol·dm^{-3}) demonstrated that in plants growing with 100 mol·dm^{-3} NaCl, the total leaf GS (glutmine synthetase) activity increased significantly in comparison with the activity assessed in the roots. Viégas et al. [1999] indicated that root proline content was not changed in response to salinity, while in leaves it was about 19 times increased in plants growing with 100 mol·dm^{-3} NaCl. Viégas et al. [1999] concluded that it is probable that an increase of this order of magnitude was not only dependent on the higher availability of ammonia and glutamate from leaf protein catabolism but also on photorespiration induced by NaCl salinity. Thus, it is important to emphasize that root was not able to accumulate proline in response to salt stress. However, Petrus and Winicov [1997] noted that only salt-tolerant alfalfa cell line from roots showed a doubling in proline concentration within 24 h after exposure to salt. In contrast, salt-sensitive plant roots showed no change in proline concentration during this time.

In the present experiment on the solution with 50 mmol·dm^{-3} NaCl, shoot proline content of wild form *L. chmielewskii* was two times greater in comparison with the control, while in the other solutions shoot proline content was reduced by 50-60% when compared to the control. In the case of tomato cultivars it was observed that shoot proline content increased by about 55-73% in comparison with the control in the plants growing with 125 mmol·dm^{-3} NaCl.

The data presented in the *in vitro* experiment showed that salt tolerance of the examined tomato plants is dependent on a cultivar. The examined cultivars were classified as moderately salt-tolerant. The ability of plants to avoid unfavorable effects of environmental stress is most probably directly proportional to the level of root development. It is generally assumed that roots are the first to suffer from exposure to environmental stress, then they are followed by an injury. Thus, even a slight root damage permits a large flux of ions to the shoot. As a result, a comparison of species or genotypes is more reliable if ion accumulation is determined in the leaves [Alian et al., 2000]. Therefore the level of root development may be one of the criteria evaluating the resistance of plants to salinity or drought in laboratory conditions.

CONCLUSION

The tolerance of the tomato to salinity is a complex trait. On the basis of the height of seedlings, root length and number, wild form *L. chmielewskii* was identified as tolerant to salinity.

Among tomato cultivars used in the study, the highest shoots were grown on 50 and 100 mmol·dm^{-3} NaCl solution. The shortest shoots were observed in the control. NaCl concentration significantly affects the root number and length. Seedlings from the medium containing 100 mmol·dm^{-3} NaCl solution developed the longest roots. However, the wild form of tomato had a lower root number in comparison with the other examined cultivars. The relationship between proline concentration and tolerance to salinity was also analyzed in this study. The highest concentration of NaCl in a medium significantly affects accumulation of proline of tomato cultivars. However, the highest proline concentration in the wild tomato form was observed at 50 mmol·dm^{-3} NaCl. Presumably the tolerance to salinity observed in the both wild and cultivated tomatoes in most of the cases is a matter of genetic variability.

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M. Smolik
Department of Plant Genetics, Breeding and Biotechnology
West Pomeranian University of Technology in Szczecin
msmolik@zut.edu.pl, phone: +48-91-449-63-03
Słowackiego St. 17, 71-434 Szczecin, Poland

M. Krupa-Małkiewicz
Department of Plant Genetics, Breeding and Biotechnology
West Pomeranian University of Technology in Szczecin
mkrupa@zut.edu.pl
Słowackiego St. 17, 71-434 Szczecin, Poland

B. Smolik
Department of Biochemistry
West Pomeranian University of Technology in Szczecin
bsmolik@zut.edu.pl
Słowackiego St. 17, 71-434 Szczecin, Poland

K. Malinowska
Department of Plant Physiology, Breeding and Biotechnology
West Pomeranian University of Technology in Szczecin
kmalinowska@zut.edu.pl
Słowackiego St. 17, 71-434 Szczecin, Poland

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