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The Effect of Grafting on Calcium Influx in Tomato Fruits under Salt Stress Conditions

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Abstract

Two commercial tomato cultivars were used to determine whether grafting could prevent decrease of Ca^{2+} concentration under salt stress conditions. The cultivars Buran F1 and Berberana F1 were grafted onto rootstock "Maxifort" and grown under three levels of the elevated soil salinity (S1 EC 3.80 dS m^{-1}, S2 6.95 dS m^{-1} and S3 9.12 dS m^{-1}). Ca^{2+} concentration of non-grafted plants of both examined hybrids was lower at all salinity levels in comparison to the control. In the fruits of grafted plants salt stress significantly decreased Ca^{2+} concentration only at the third salinity level (EC 9.12 dS m^{-1}). The possibility of grafting tomato plants to improve influx of Ca^{2+} under salt stress conditions is discussed.

Key words: Calcium, salt stress, grafting, tomato

Introduction

Calcium has a vital role in the regulation of a wide range of biochemical and physiological processes in plants (White & Broadley, 1993).

The role of calcium is essential in processes that preserve the structural and functional integrity of plant cell membranes (Tuna et al. 2007), stabilize cell wall structures (Neves-Piestun & Bernstein, 2001), regulate ion transport, selectivity and ion-exchange behavior as well as cell wall enzyme activities (Ashraf et al. 2004).

Calcium ion (Ca^{2+}) is one of the most important ubiquitous intracellular second messenger molecules involved in many signal transduction pathways in the response to environmental stresses (Tuteja & Mahajan, 2007). Changes in the plant's calcium concentrations caused by different types of stress have been reported by many researches (Knight et al. 1996; Pandey et al. 2000; Ranty et al. 2016). Similarly, water stress and heat induced changes in calcium levels were also reported (Takano et al. 1997; Gong et al. 1998). It was found that calcium may be involved even in long-term processing of signals in plants in response to abiotic stresses (Verdus et al. 2007). It has been shown that Ca^{2+} is an important determinant for plant salt tolerance and confers protective effects on plants grown in sodic soils (Hadi & Karimi, 2012). On the other hand, Ca^{2+} deficiency can occur in some species at high Na^{+}/Ca^{2+} ratio in the external solution, although the plant response will vary with genotype. At high soil salinity decreased Ca^{2+} uptake by roots and its transport via xylem to the fruits have been identified as main causes of physiological disorders in tomato (Ehret & Ho, 1986). Blossom-end rot is a local deficiency of Ca^{2+} in tomato fruit. The uptake of Ca^{2+} is reduced by osmotic stress (Ehret and Ho, 1986) or by cation competition (Raleigh and Chucka, 1944) in the root zone. As Ca^{2+} movement in tomato is virtually confined to the xylem, transport of the absorbed Ca^{2+} to the shoot is inhibited by salinity (Ho, 1989). While the concentration of Ca^{2+} in the fruit is intrinsically low (about one tenth of that in the leaves), the transport of Ca^{2+} within the fruit to the distal half is very poor and is restricted further by high salinity (Ehret and Ho, 1986).

While K^{+} and Ca^{2+} play key roles in several physiological processes, Na^{+} does not function as a macro-nutrient, and thus the substitution of K^{+} by Na^{+} and decrease in Ca^{2+} concentration may lead to nutritional imbalances. The control of Na^{+} accumulation, by exclusion strategy, and high shoot K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios may enhance salt tolerance in tomato (Al-Karaki, 2000). Increasing the Na^{+}/Ca^{2+} ratio in the external solution has been reported to alleviate the effects of salinity on depolarization and selectivity of the plasma membrane (Rinaldelli & Mancuso, 1996). In addition, reduction of Ca^{2+} content by salinity has also been detected in leaves of lettuce (Lazof & Läuchli, 1991).

Tomato (*Lycopersicon esculentum* Mill.) is considered as moderately sensitive or moderately tolerant to salinity depending on a cultivar or a growth stage, but calcium deficiency in salt stress conditions has been confirmed in some researches (Juan et al. 2005; Parvin et al. 2016). Nowadays, significant attempts for dealing with salinity problem include the generation of more resistant transgenic plants (Yamaguchi & Blumwald, 2005). From the ecological point of view, it is important to develop more conventional, environmentally friendly methods which will enable high crop production under saline conditions in the near future.

One such method consists of grafting salt-sensitive species onto more tolerant rootstocks. It has been confirmed that grafted tomato plants better absorb water and have potential to exclude saline ions. However, the grafting effect on the calcium content in these conditions is still unknown.

In this research, it has been investigated whether a grafting technique could prevent calcium deficiency in tomato plants grown under elevated soil salinity.

Material and Methods

Sixty-day old tomato (*Lycopersicon esculentium* Mill.) cultivars (Buran F1 and Berberana F1) produced in “Adria Histhil d.o.o. company” were selected for the experiment. The seedlings were grafted onto the “Maxifort” rootstock (De Ruiter Seeds, Bergshenhoek, the Netherlands) and after that planted in a greenhouse with controlled conditions: 28.5 ^{0}C air temperature, relative air humidity 65% and 12/12 h light/darkness photo regime. TS3 Klasmann-Deilmann (GmbH, Geeste, Germany) was used as substrate and after acclimatization all plants (grafted and non-grafted) were submitted to three different soil salinity regimes, with a control group. During the experiment average electrical conductivity (EC) of plant groups was as follows: 1.7 dS m^{-1} in control groups, 3.8 dS m^{-1} at the first salinity level (S1), 6.95 dS m^{-1} at the second (S2) level and 9.12 dS m^{-1} at the final (S3) salinity level. After 12 weeks, all fruits from the first fruit-bearing branch were collected and forwarded to a laboratory for further analyses.

Determination of Ca^{2+} concentration

Calcium was extracted from 1 g of dry fruit mass adding 10 mL HNO_3 and 4 mL H_2SO_4. After 16 hours of incubation at a room temperature homogenized mass was heated at 95 ^{0}C for thirty minutes and filtered through filter paper after cooling. The absorbance of each sample was measured by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan).

Statistical data analysis

All results were presented as average value of three replicates ± standard deviation. Data were analyzed by fitting the General Linear Models (GLM) with subsequent *post-hoc* analysis by the LSD test ($p<0.05$) using SPSS Statistics 23 (2013).

Results and Discussion

The obtained results indicate that elevated soil salinity did not decrease calcium content with the same intensity comparing the grafted and non-grafted plants, both in Buran F1 and Berberana F1 (Figures 1 and 2). In the non-grafted variant of both cultivars salt stress affected Ca^{2+} starting already at S2, but in the grafted variants only at S3. The average loss in both non-grafted hybrids at S2 was by 20% and at S3 by 30 % in comparison to the control. On the other side, Ca^{2+} content in the grafted plants in both variants at S3 approximately decreased by 18% in comparison to the control.

It is interesting that the grafted plants of both hybrids had significantly higher Ca^{2+} content in comparison with the non-grafted counterparts at all salinity levels, including the control. Grafting is regarded as a promising tool to broaden the salinity optimum for tomato cultivars (Estan et al., 2005; Koleška et al., 2018).The root system of grafted plants is stronger and more efficient in water and nutrients uptake which indirectly improves yield and fruit quality (Miskovic et al., 2005).

Also, grafting a commercial tomato cultivar onto rootstocks has the potential to exclude saline ions and reduce the concentration of both Na+ and Cl− in the leaves of the scion (Martinez-Rodriguez et al., 2008). Some researchers have proven that grafted tomato plants have a higher yield and larger fruits (Flores et al., 2010; Turhan et al., 2011; Koleška et al, 2018).

Many researchers investigated Na^{+}/Ca^{2+} interactions in plants (Epstein, 1961; Cramer, 2002; Cabot et al. 2009).

It has been shown that salinity can increase the incidence of calcium-related physiological disorders either by competition between Na^{+} and Ca^{2+} during uptake, or by decreasing the soil water potential and thus root pressure (Sonnenveld & van den Ende, 1975). Calcium availability could be seriously reduced under salinity, especially at low Ca^{2+}/Na^{+} ratio since Na^{+} readily displaces Ca^{2+} from its extracellular binding sites (Cramer, 2002). In addition, the decreased uptake of Ca^{2+} under salt stress might be due to its precipitation and the increase in ionic strength that reduces its activity.

Calcium deficiency, in general, can impair the selectivity and the integrity of the cell membrane and permit the passive accumulation of Na^{+} in plant tissues. It has been proven that grafting a commercial tomato hybrid onto rootstocks has the potential to exclude saline ions reducing the concentration of Na^{+} cations (Martinez-Rodriguez et al. 2008).

Also, the exposure of grafted tomato cultivars to high NaCl concentrations resulted in lower Cl^{-} and Na^{+} concentrations as compared with non-grafted variants (Fernandez-Garcia et al. 2002).

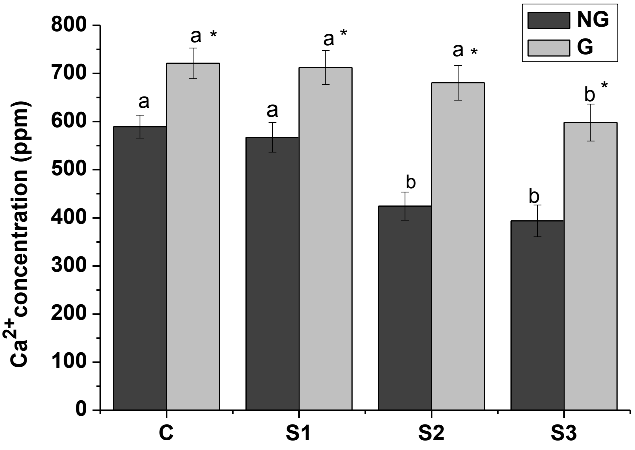

Fig. 1. The concentration of Ca^{2+} (ppm) in the fruits of non-grafted (NG) and grafted (G) plants of the Buran F1 tomato cultivar at different soil salinity levels: control 1.7 dS m^{-1} (C), 3.8 dS m^{-1} (S1), 6.9 dS m^{-1} (S2) and 9.1 dS m^{-1} (S3). * indicates statistical significance between the NG and G plants according to the LSD test; [ab] indicates statistical significance between different salinity levels ($p < 0.05$).

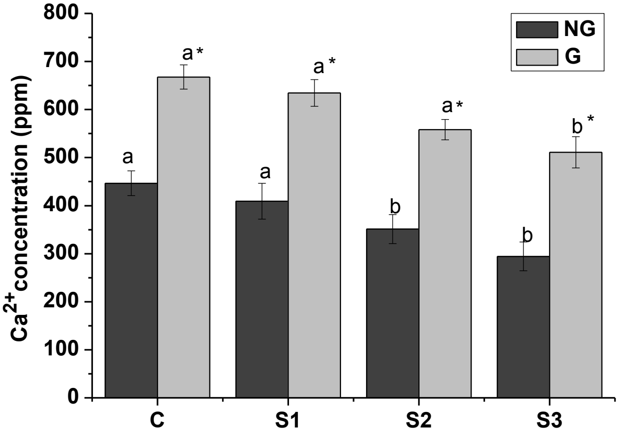

Fig. 2. The concentration of Ca^{2+} (ppm) in the fruits of the non-grafted (NG) and grafted (G) plants of the Berberana F1 tomato cultivar at different soil salinity levels: control 1.7 dS m^{-1} (C), 3.8 dS m^{-1} (S1), 6.9 dS m^{-1} (S2) and 9.1 dS m^{-1} (S3). * indicates statistical significance between the NG and G plants according to the LSD test; [ab] indicates statistical significance between different salinity levels ($p < 0.05$).

The exclusion of chloride from the shoot seems to be controlled primarily by the ability of cell membranes to restrict the movement of chloride across the root to the vascular tissue, and by the degree of chloride accumulation in the roots (Walker & Douglas 1983). The mechanism of Na^+ exclusion in a rootstock seems to depend on removing Na^+ from the xylem. This process involves an exchange of Na^+ for K^+, which thus enters the xylem sap (Walker 1986). The most probable explanation for better Ca^{2+} uptake by grafted plants is Na^+ exclusion by rootstock or improved water potential which could increase its absorption.

Conclusion

Grafted tomato plants which have higher potential of rootstock to absorb water and nutrients adapted better to saline conditions than their non-grafted counterparts, thus contributing to lower calcium content reduction in both examined tomato cultivars under salt stress conditions. Based on the obtained results it can be said that grafting improved calcium uptake in tomato plants. The exact mechanism of ion homeostasis and relations between Ca^{2+} and Na^+ in the absorption process by grafted tomato should be the subject of future research.

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Утицај калемљења на доступност калцијума у плодовима парадајза гајеног у условима соног стреса

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Сажетак

Испитиване су двије комерцијалне сорте парадајза да би се одредило да ли калемљење може спријечити смањење концентрације Ca^{2+} јона у условима соног стреса. Сорте Buran F1 и Berberana F1 су калемљене на подлогу "Maxifort" и узгајане под три нивоа повишеног салинитета тла (S1 EC 3,80 dS m^{-1}, S2 6,95 dS m^{-1} и S3 9,12 dS m^{-1}). Концентрација Ca^{2+} јона некалемљених биљака оба испитивана хибрида била је нижа на свим нивоима салинитета у односу на контролу. У плодовима калемљених биљака, сони стрес је значајно смањио концентрацију Ca^{2+} јона само у трећем нивоу салинитета (EC 9,12 dS m^{-1}). Разматрана је могућност калемљења биљака парадајза у циљу побољшања доступности Ca^{2+} јона у условима соног стреса.

Кључне ријечи: калцијум, сони стрес, калемљење, парадајз

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