

Influence of Grafting on the Copper Concentration in Tomato Fruits under Elevated Soil Salinity

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Abstract

Salinity is one of the most important ecological problems that affect irrigated agriculture in the world. Increased soil salinity inhibits plant growth through osmotic and ionic stress, but can also decrease availability of certain micronutrients. Copper is an essential metal for normal plant growth and development that participates in numerous biochemical and physiological processes and it is an essential cofactor for many metalloproteins. Copper concentration in two commercial tomato cultivars (grafted and non-grafted) was investigated under different levels of elevated soil salinity. Soil with EC 9.1 dS m⁻¹ led to the highest copper deficiency, approximately by 37% in the non-grafted and 25% in the grafted tomato plants. The effect of a grafting technique is considered as an environmentally friendly tool for overcoming soil salinity problem.

Key words: tomato, salinity, grafting, copper

Introduction

The limited supply of appropriate quality water in many arid and semi-arid regions necessitates the use of saline water, where available, for crop production (Evans & Sadler, 2008). Besides that, new areas of saline soils are being added every year because of secondary salinization (Glick et al. 2007). Disability of soil leakage in greenhouses leads to the accumulation of osmotic active particles, which negatively influence the plant growth and development (Marchese et al., 2008).

Tomato is classified as being "moderately sensitive" to salinity and the reduction of its growth starts at soil electrical conductivity (EC) over 3 dS m^{-1} (Maas, 1986). Elevated salinity conditions diminish plant growth through disturbance of osmotic and ionic balance (Meloni et al., 2001). The reduction in growth is a consequence of several biochemical and physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behaviour and photosynthetic efficiency (Chartzoulakis, 2005). Salt accumulation in root zone causes disruption of cell ion homeostasis by inducing inhibition in the uptake of macronutrients (Rahman et al., 1993). Studies on micronutrient levels required for plant growth have shown that salinity is an important factor in determining micronutrient availability (Richards, 1954). Copper is classified as one of the essential micronutrients in plant nutrition, which acts as a cofactor of numerous enzymes and has a vital role in cellular functions (Printz et al. 2016). As a transition metal Cu in plant cells appears in two forms: the reduced Cu^+ state and the oxidized Cu^{2+} state. The reduced form preferentially binds sulphur-containing compounds, whereas the oxidized form coordinates mainly with oxygen or imidazole nitrogen group (Cohu & Pilon, 2010).

The most abundant Cu protein is a photosynthesis-related protein plastocyanin that is involved in the transfer of electrons in chloroplasts (Abdel-Ghany & Pilon, 2008). In addition, copper is involved in the control of the redox state in mitochondria, since it acts as a cofactor in the Cu-Zn superoxide dismutase (Tapken et al. 2012). Copper deficiency usually induces impairment in the photosynthetic transport chain because of lack of plastocyanin (Abdel-Ghany & Pilon, 2008). It has been reported that elevated soil salinity decreases Cu uptake by the root system and leads to its deficiency (Rahman et al. 1993). It has been proven that grafting could be used as an environmentally friendly tool for overcoming problems in plant cultivation under saline conditions (Oztekin & Tuzel, 2011).

The rootstock of grafted plants has better water use efficiency in elevated soil salinity (Koleška et al. 2017), but whether grafting could prevent micronutrient loss in these conditions has not been investigated enough.

The aim of this research was to investigate if grafting of tomato plants can reduce copper deficiency under different levels of elevated soil salinity.

Material and Methods

Two commercial tomato cultivars (Buran F1 and Berberana F1, Enza Zaden, Enkhuizen, The Netherlands) were used as scions and the genotype "Maxifort" (De Ruiter Seeds, Bergshenhoek, The Netherlands) was selected as rootstock for grafting. Sixty-day-old seedlings were planted in plastic pots and filled with 3 kg of plant substrate TS3 (Klasmann-Deilmann GmbH, Geeste, Germany). Pots were placed in a greenhouse with controlled conditions: average temperature 28.5 °C, relative air humidity 65% and photoperiod 12/12 h. Both grafted and non-grafted variants were divided into control and three levels of salinity and 1.5 L of NaCl solution per pot was added to the substrate in the concentrations of 0 M, 0.5 M, 1 M and 1.5 M. In the suspension of soil and distilled water 1:5 (v:v) final EC was measured using InoLabCond 720 with TetraCon 325 electrode and values were 1.7 dS m⁻¹ (control), 3.80 dS m⁻¹ (S1), 6.95 dS m⁻¹ (S2) and 9.12 dS m⁻¹ (S3), respectively. After twelve weeks fruits from the first fruit-bearing branch were sampled and used for further analyses.

Determination of copper concentration in the fruits

To extract copper, 1 g of dry fruit tissue was mixed with 10 mL HNO₃ and 4 mL H₂SO₄ and incubated for 16 hours at a room temperature. After that samples were heated at 95 °C for thirty minutes and after cooling the solutions were filtered through quantitative filter paper. The absorbance was measured by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan).

Statistical data processing

Statistical analyses were performed using SPSS Statistics 23 (2013). The data was analysed by fitting the General Linear Models (GLM) with subsequent *post-hoc* analysis by LSD test. Differences were declared significant at $P < 0.05$ probability level.

Results and Discussion

The results of fruit copper concentration of both variants of examined tomato cultivars were presented in the Table 1. The obtained results show that elevated soil salinity in both examined cultivars significantly decreased copper concentration at S2 and S3 in comparison with control, both in grafted and non-grafted plants. Grafting somewhat prevented average copper loss since at S2 level in non-grafted plants of both cultivars Cu content approximately decreased by 25% in comparison with the control, whereas in the grafted one by 20%, respectively. At S3 level Cu concentration in the non-grafted variant of both cultivars approximately decreased by 37% in comparison with the control, whereas in the grafted plants 25% lower Cu content in comparison with the control was detected.

It is interesting that the non-grafted plants of both examined cultivars had significantly lower copper content in comparison with the grafted one at all salinity levels, including the control. In that sense, the non-grafted Buran F1 cultivar had lower copper content by 16 % at control, by 19% at S1, by 20% at S2 and by 37% at S3 in comparison with the grafted counterparts. In the Berberana F1 cultivar the non-grafted variants also had lower copper content in comparison with the grafted ones at all salinity levels (by 22 % at the control, by 16% at S1, by 27% at S2 and by 29% at S3, respectively).

Tab. 1. The copper concentration (ppm) in the fruits of non-grafted (NG) and grafted (G) plants of the Buran F1 and the Berberana F1 tomato cultivars 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).

	Buran F1		Berberana F1	
	NG	G	NG	G
	AV \pm SD	AV \pm SD	AV \pm SD	AV \pm SD
C	4.10 ^a \pm 0.69 *	4.89 ^a \pm 0.32	4.25 ^a \pm 0.96 *	5.45 ^a \pm 0.25
S1	3.77 ^a \pm 0.31 *	4.65 ^a \pm 0.35	4.62 ^a \pm 0.72 *	5.51 ^a \pm 0.22
S2	3.05 ^b \pm 0.26 *	3.77 ^b \pm 0.22	3.26 ^b \pm 0.54 *	4.48 ^b \pm 0.18
S3	2.27 ^c \pm 0.19 *	3.59 ^c \pm 0.49	2.92 ^c \pm 0.019 *	4.10 ^c \pm 0.2

* indicates statistical significance between NG and G plants of both cultivars according to LSD test; ^{abc} indicate statistical significance between different salinity levels ($p < 0.05$).

Elevated salinity can differentially affect micronutrient concentrations in plants, depending upon the crop species and the salinity level (Oertli, 1991). Generally, elevated salinity leads to decline of water and nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Hu & Schmidhalter, 2005). The dominant cation in soils with high EC is Na^+ which can cause ionic imbalance and affect $\text{Na}^+ : \text{Ca}^{2+}$ and $\text{Na}^+ : \text{K}^+$ ratio as well as $\text{Ca}^{2+} : \text{Mg}^{2+}$ ratio indirectly. In addition, saline and sodic soils have micronutrient deficiencies as a result of high pH and osmotic potential (Rengasamy, 2006). Generally, very little attention has been directed towards the effect of salinity on the Cu uptake and accumulation in horticultural crops. According to some researches, Cu accumulation in salt-stressed maize decreased (Rahman et al., 1993; Izzo et al., 1991).

Eskandari et al (2014) have also found that high soil salinity had an inhibitory effect on Cu uptake. Harter (1993) reported that copper solubility was strongly influenced by the soil pH and explained that its availability for plants was very low at $\text{pH} > 7.5$. Some data suggest that there may be an important link between ionic aspects of salinity stress and transition metal homeostasis (Maathuis, 2006). This author indicates that the uptake of transition metals like Cu^{2+} , Fe^{2+} and Zn^{2+} is reduced during salinity stress whereas their active extrusion is promoted, even physiological relevance of this is still unclear. However, through substitution of Ca^{2+} by Na^+ , salinity compromises membrane integrity and leads to an augmented influx of heavy metals, such as Cu^{2+} . Alternatively, salinity-induced perturbation of cellular and intracellular Na^+ , K^+ , and Ca^{2+} fluxes may affect general cation homeostasis, including that of copper. It has been proved that grafting of tomato cultivars onto rootstocks can exclude saline ions and reduce the concentration of both Na^+ and Cl^- in the scion (Martinez-Rodriguez et al. 2008). A vigorous root system of a rootstock is often capable of absorbing water and nutrients more efficiently than scion roots, and our results suggest this also influenced the copper content.

Conclusion

The results of our research proved that grafting prevented copper deficiency in tomato plants grown under elevated soil salinity and improved their resistance. The influence of grafting on the relations between copper nutrition and salinity is not simple, and for better understanding of this problem, the future research should be focused on molecular and biochemical mechanisms of this phenomenon.

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Утицај калемљења на концентрацију бабра у плодовима парадајза гајеног у условима повишеног салинитета земљишта

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Сажетак

Салинитет представља један од најважнијих еколошких проблема који утичу на наводњавање у пољопривреди у свијету. Повећан салинитет земљишта инхибира раст биљака путем осмотског и јонског стреса, али такође може умањити доступност одређених микронутријената. Бакар је есенцијални метал за нормалан раст и развој биљака, који учествује у бројним биохемијским и физиолошким процесима и битан је кофактор многих металопотеина. У овом раду је испитивана концентрација бабра у двије комерцијалне сорта парадајза (калемљена и некалемљена) под различитим нивоима повишеног салинитета земљишта. Земљиште са ЕС 9.1 dS m⁻¹ условило је највећи недостатак бабра, приближно за 37% код некалемљених и за 25% код калемљених биљака парадајза. Калемљење биљака парадајза сматра се еколошки веома прихватљивим поступком за превазилажење проблема салинитета земљишта.

Кључне ријечи: парадајз, салинитет, калемљење, бакар

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