EFFECT OF GENOTYPE AND SELENIUM BIOFORTIFICATION ON CONTENT OF IMPORTANT BIOACTIVE SUBSTANCES IN TOMATO (LYCOPERSICON ESCULENTUM MILL.) FRUITS

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Abstract

The effect of genotype and selenium foliar biofortification in the form of an aqueous solution of sodium selenate on the content of total carotenoids, vitamin C and total polyphenols content in the tomato fruits was studied. Field experiment was held in the Botanical garden of the Slovak University of Agriculture in 2014. Two determinant varieties of tomato (Brixol F1 and Uno rosso F1) in the three variants were observed. Foliar application of selenium had a positive effect on the increase of total polyphenol content. The influence of Se biofortification on the content of vitamin C and carotenoids was not detected. Within the followed varieties significant effect of genotype on observed characteristic wasn’t found. Selenium foliar fertilization in dosage 150 g/ha is suitable way of tomato fruits enriching in polyphenols, without negative effect on other antioxidants content.

Key words: tomato, selenium, biologically active substances, biofortification

1. INTRODUCTION

Global production of tomato fruits (Lycopersicon esculentum Mill.) reaches at present time historically the highest value exceeding 163 million tons per year; thereby the tomato ranks on the first place in term of global vegetables production. At the present the biggest tomato producers are China, India and USA. In Slovak Republic, according to last statistical outputs from the 2014, tomatoes were the second most cultivated vegetables (Valšíková 2015). They are consumed like a functional food all over the world because of health promoting compounds in its fruit. The main reason is due to the presence of different antioxidant molecules such as carotenoids, ascorbic acid, vitamin E and polyphenol compounds such as flavanones, flavonols (Vallverdú-Queralt 2011), flavonoids (Frusciante 2007) and other. Lycopene is the main carotenoid in tomato (Yoshida 2011). According to various authors, the total carotenoids content as well as lycopene in fresh tomato fruits depends mainly on genotypes, as well as on the maturity degree, growing conditions and agricultural technologies (Mendelová 2013, 2015, Carli 2011, Uher 2009). Latest research highlights the relationship between consuming tomato and its products with reduced risk of various maladies like obesity, hyperglycemic and hypercholesterolemic attributes, cardiovascular disorders and cancer insurgences (Perveen 2015).

Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidant (Pandey 2009). The free radical scavenging, in which the polyphenols can break the free radical chain reaction, as well as suppression of the free radical formation by regulation of enzyme activity or chelating metal ions involved in free radical production are reported to be the most important mechanisms of their antioxidant activity (Knezevic 2012). The content of polyphenols was more dependent on year and cultivar than on cultivation conditions (Anton 2014). Phenolic content of tomato fruits was significantly affected by the spectral quality of ambient solar UV radiation available (Luthria 2006).

Ascorbic acid (AA) also named vitamin C is an essential micronutrient soluble in water. It is occurring in almost all living organisms. The people, as well as a number of other animals cannot synthesize the vitamin C in their bodies and therefore it can be taken only through the diet (Klimešová 2013). Vitamin C is an important antioxidant – it captures free radicals, promotes iron absorption necessary for blood formation, it is necessary for the formation of collagen and fibrous tissues (Zittlau 2008). The tomato is a very rich source of AA (ascorbic acid), a very sensitive vitamin. However, processing
Agriculture & Food
ISSN 1314-8591, Volume 4, 2016

causes a negative effect on AA content (Gümüsay 2015). In the surface layer is three times more of vitamin C than in the flesh and juice (Rop 2005). Following Pinela (2012) ascorbic acid was the most abundant antioxidant in all tomato samples (following vitamins, carotenoids and phenolics). Together with phenolic compounds, ascorbic acid represents the main water-soluble antioxidant in tomatoes and contributes to the antioxidant activity of the water-soluble fraction (Guil-Guerrero 2009).

Tomatoes contain minerals such as calcium, magnesium, iron and potassium, as well as microelements such a copper, zinc and manganese (Kopec 2010). Moreover, the tomatoes are ranged in to the vegetables with the highest concentration of selenium (0.034 mg kg⁻¹). Selenium (Se) is ultramikroelement, essential trace mineral and antioxidant. It is essential for the growth of animals and humans. Studies have shown that selenium is also part of enzyme systems. The functional forms are biologically active selenoproteins - enzymes glutathione peroxidase, iodine-5-deiodinasis and others. These enzymes protect cells from oxidation. The boundary between the positive and negative impacts of selenium is narrow; the higher doses of this element are toxic for the plants, but also for humans. As a consequence, the limit values are defined in the soil (Kováčik, Skawiński 2012). In recent years, selenium research attracts great interest because its activity plays an important role in protection against oxidative stress, initiated by redundant reactive oxygen forms (ROS) and reactive nitrogen forms (NOS). The increased risk of diseases such as cancer and heart disease, and also civilization diseases are associated with low income of selenium (Ujang Tinggi 2008).

Plants receive selenium from the growing medium in the form of inorganic compounds and they subsequently incorporate it thanks to selenocysteine in to its proteins (Hegedus 2005). Foliar application was significantly several times more effective than the application of fertilizers (ASPI 2005). Important factor at foliar application is the concentration of applied solution. At foliar application the selenium ions diffuse from the surface of leaves to epidermal cells. There is strong correlation between solution concentration on the leaf surface and ions absorption rate, anyway, too high concentration can damage the leaf surface (Wójcik 2004). Absorption rate is limited with damage of ectodesmata (Ježek 2012). Concentration of solution at foliar application of selenium should be chosen with care, based on recommendations concerning the nutrition with microelements (Nawaz 2012).

Increasing of carotenoids, vitamin C and other antioxidants in vegetables is eligible, and their content is one of the important parameter monitored in breeding of new varieties, also in testing of new cultivation and fertilization technologies without exception of biofortification. The aim of the work was to monitor the effect of genotype and selenium foliar biofortification in the form of an aqueous solution of sodium selenate on the content of total carotenoids, vitamin C and total polyphenols content in the tomato fruits.

2. METHODS

The trial establishment

An experiment was founded in 2014 in Botanical Garden of Slovak University of Agriculture (below BG SUA) in field conditions. Area is situated in very warm agro-climatic region, very dry sub-region. The mean annual temperature was 10 °C. Meteorological measurements were carried out by the help of meteorological station in the area of botanical garden, SUA in Nitra. The mean monthly air temperature and average rainfall for the year 2014 were evaluated by the climate normal 1961-1990 (Table 1, 2).
Table 1. Evaluation of the mean monthly air temperature in 2 m in the selected months in 2014, according to climatology normal 1961-1990

<table>
<thead>
<tr>
<th>Year</th>
<th>t [ºC]</th>
<th>Normal 1961-90</th>
<th>Δt [ºC]</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.</td>
<td>15.2</td>
<td>15.1</td>
<td>0.1</td>
<td>Normal</td>
</tr>
<tr>
<td>VI.</td>
<td>19.3</td>
<td>18.0</td>
<td>1.3</td>
<td>Warm</td>
</tr>
<tr>
<td>VII.</td>
<td>21.8</td>
<td>19.8</td>
<td>3.0</td>
<td>Warm</td>
</tr>
<tr>
<td>VIII.</td>
<td>18.9</td>
<td>19.3</td>
<td>2.6</td>
<td>Normal</td>
</tr>
<tr>
<td>IX.</td>
<td>16.8</td>
<td>15.6</td>
<td>-0.9</td>
<td>Warm</td>
</tr>
</tbody>
</table>

Table 2. Evaluation of monthly total rainfalls in selected months in 2014, according to climatology normal 1961-1990

<table>
<thead>
<tr>
<th>Year</th>
<th>Z[mm]</th>
<th>Normal 1961-90</th>
<th>% of normal</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.</td>
<td>58</td>
<td>58</td>
<td>99</td>
<td>Normal</td>
</tr>
<tr>
<td>VI.</td>
<td>53</td>
<td>66</td>
<td>80</td>
<td>Normal</td>
</tr>
<tr>
<td>VII.</td>
<td>64</td>
<td>52</td>
<td>123</td>
<td>Normal</td>
</tr>
<tr>
<td>VIII.</td>
<td>56</td>
<td>61</td>
<td>92</td>
<td>Normal</td>
</tr>
<tr>
<td>IX.</td>
<td>122</td>
<td>40</td>
<td>305</td>
<td>Extra wet</td>
</tr>
</tbody>
</table>

There were included 2 tomato determinate varieties: Brixol F1 a Uno Rosso F1. Sowing was carried out on March 13th, 2014 in a heated greenhouse BZ SUA. According to the techniques of tomato cultivation it was done preparing of the land. According to agrochemical analysis of the soil (Table 3) in case of all three variants there was applied nitrogen fertilizer DASA®26 / 13 - Ammonium nitrate with sulfur (26% N and 13% of S) in dosage 190 kg /ha (60 % of recommended normative).

Table 3. Agrochemical characteristics of the soil before the foundation of the experiment in 2014

<table>
<thead>
<tr>
<th>humus</th>
<th>pH/KCl</th>
<th>Nutrients content in mg.kg⁻¹ of the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>3.46</td>
<td>6.47 Sl. A</td>
<td>19.5 M</td>
</tr>
</tbody>
</table>

Explanatory notes: Nutrient content: L – low content, M – medium content, G – good content, H – high content, VH- very high content, pH: pH - Sl. A - Slightly acidic

The field experiment was established by block method in three replications, plants were planted in uniform plant spacing 0.7 x 0.3 m. Planting of pre-growing planting was carried out on May 5th, 2014. According to necessity the supplementary irrigation was applied, as well as loosening and weeding of the crop. The application of selenium was done on June 26th, 2014 at the growth stage of fruits creation on the first inflorescences and second inflorescence establishment. Selenium was foliar applied in solution Na₂SeO₄. During the growing season the crop is in the process of setting up third-fourth inflorescence fertilization with nitrogen fertilizers (40% of the recommended normative). In the second half of vegetation protection of the plants against fungal diseases was carried out. Collection was realized in August 28th, 2014 in full botanical ripeness of the fruits.
In the small plot experiment there were observed the following options:
1st variant (control) – without applying of selenium
2nd variant (SeI) – selenium was applied in dosage of 150 g/ha
3rd variant (SeII) – selenium was applied in dosage of 300 g/ha

Qualitative characteristics
Following quantity in chosen tomato varieties, the qualitative characteristics were estimated in laboratory of Department of vegetable growing, SUA, in Nitra involving:

Total carotenoids estimation - Carotenoids were estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on spectrophotometer PHARO 100 at 445 nm wavelengths. As a dissolution reagent there was used acetone.

Ascorbic acid estimation (AA) - HPLC method of vitamin C content estimation was used for its quantity estimation by the help of liquid chromatograph with UV detector (HPLC fý VARIAN).

Total polyphenols content estimation (TPC) - total polyphenols were determined by the method of Lachman (2011) and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 L of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at 765 nm of wavelength against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Statistical analysis
The analysis of variance (ANOVA), the multifactor analysis of variance and the multiple Range test were done using the Statgraphic Centurion XVII (StatPoint Inc. USA).

3. RESULTS AND DISCUSSION
Carotenoids content
The carotenoids content moved in range from 16.65 to 20.71 mg /100 g FM (fresh matter) for Brixol and from 17.23 to 24.34 mg/100 g FM for Uno Rosso variety. In case of Uno Rosso the values were higher, but differences between varieties wasn’t statistically significant (Figure 1). The influence of selenium biofortification on carotenoids content wasn’t statistically confirmed (Table 4).

The values of carotenoids content from similar trials moved from 2.63 to 6.55 mg/100g for 8 tomato varieties estimation according to Mendelova (2013), from 4.71 to 13.07 mg / 100 g in case of seven Serbian tomato varieties evaluation in Hegedűsová (2015) and from 3.63 to 17.5 mg/100g for in 7 tomato parental lines and 8 tomato hybrids, where also significant differences were found among genotypes for all parameters analyzed. From the results of statistical analysis of Kotíková (2011) emerged, that there is a significant difference in the ability of tomatoes to synthesize carotenoids in relation to variety. At the mature phase of fruits an average content of carotenoids was determined as 552 μg/g dry matter (39 μg/g fw) in 2008. The year effect was significant for total carotenoids, lycopene and b-carotene content Carli (2011). The carotenoid composition of the varieties strongly depends on the degree of maturation in which each variety is usually consumed (Guil-Guerrero 2009). They tested 8 tomatoes varieties, with the range of the results from 133 ± 15 to 583 ± 60 μg/g
dry wt., with the significant differences between the varieties according to Duncan’s Multiple Range Test.

Table 4. Carotenoids content in estimated varieties, Nitra, 2014

<table>
<thead>
<tr>
<th>Carotenoids content (mg/100g FM)</th>
<th>Variant</th>
<th>Brixol</th>
<th>Uno Rosso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.71 ± 1.57a</td>
<td>17.23 ± 2.31A</td>
<td></td>
</tr>
<tr>
<td>Se I</td>
<td>18.73 ± 2.22a</td>
<td>24.19 ± 6.00A</td>
<td></td>
</tr>
<tr>
<td>Se II</td>
<td>16.65 ± 3.47a</td>
<td>25.34 ± 6.59A</td>
<td></td>
</tr>
</tbody>
</table>

* Different lowercase letters in superscript denote significantly different at P < 0.05 by LSD in ANOVA (Statgraphic Centurion XVII)

Figure 1. Graphical analyses of variety influence on carotenoids content, Nitra, 2014 (Statgraphic Centurion XVII)

Ascorbic acid (AA)

Ascorbic acid content was ranged in interval from 11.51 mg/100g (‘Tornado F1’) to 18.72 mg/100g (‘Uragan SVF F1’) in evaluation of 7 Serbian tomato varieties grown in Slovak Republic conditions (Andrejová 2015). According to Guil-Guerrero (2009) all their values were higher than those habitually mentioned in the literature. Rambo (263 mg), Racimo (174 mg) and Pera (164 mg) on kg fresh weight, were the best sources of this vitamin. In study of Kotíková (2011) in eight tomato varieties an ascorbic acid content ranged from 3095 μg/g (217 μg/g fw) in Orkado variety to 3684 μg/g (258 μg/g fw) in Tornado variety with an average value 3495 μg/g (245 μg/g fw) of dry matter. George (2004) indicate the content of ascorbic acid in tomato pulp from 8.4 to 32.4 mg/100 g. Substantial amounts of ascorbic acid were also detected in peels (9.0–56.0 mg/100 g fw and 104–462 mg/100 g dwb). When recommended daily allowance of vitamin C is 60 mg per person (Guil-Guerrero 2009) and in fruits of Brixol there is in average 16.68 mg / 100 g of AA, than 60 mg of AA is in 36 g of fresh fruits. When one fruit of Brixol has in average 68 g, than one person should consume 5.3 fresh fruits of Brixol variety to cover daily intake. In case of Uno Rosso the situation is
similar. The ability of ascorbic acid to regenerate lycopene in two-compound mixtures was found by Liu (2008).

Table 5. Ascorbic acid content in estimated varieties, Nitra, 2014

<table>
<thead>
<tr>
<th>Variant</th>
<th>Brixol</th>
<th>Uno Rosso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.21 ± 6.82a</td>
<td>14.73 ± 3.34A</td>
</tr>
<tr>
<td>Se I</td>
<td>13.53 ± 1.88a</td>
<td>16.73 ± 2.35A</td>
</tr>
<tr>
<td>Se II</td>
<td>17.30 ± 2.42a</td>
<td>17.57 ± 0.78A</td>
</tr>
</tbody>
</table>

* Different lowercase letters in superscript denote significantly different at P < 0.05 by LSD in ANOVA (Statgraphic Centurion XVII)

Figure 2. Graphical analyses of variety influence on ascorbic acid content, Nitra, 2014 (Statgraphic Centurion XVII)

**Total polyphenol content (TPC)**

The total polyphenols content was the highest in case of selenium applied in dosage of 150 g/ha (Se I), where the value for Brixol reached 10285.13 mg GAE/kg and for Uno Rosso 10009.80 mg GAE/kg. In case of both varieties, the values were statistically different in comparison with lower values in control and double dose of selenium (table 6). As there was found the significant influence of biofortification on total polyphenol content, the results are in contrary with Anton (2014), where generally, the cultivation system had minor impact on polyphenols content, and only a few compounds were influenced by the mode of cultivation in all tested cultivars. The differences in total polyphenol content between Brixol and Uno Rosso weren’t statistically significant (Figure 3). The quantification data allowed Gómez-Romero (2010) to compare the three tomato cultivars selected. Most phenolic compounds could be detected in all three varieties, although their quantities differed considerably. The highest levels of phenolic were detected in the Daniela cultivar. Four lots of tomatoes of each cultivar and each UV treatment were assayed for total phenolic (TP) content in trial of Luthria (2006), where they estimated the values in range from 34 mg GAE/g to 44 mg GAE/g for total phenolics.
Table 6. Total polyphenol content in estimated varieties, Nitra, 2014

<table>
<thead>
<tr>
<th>Variant</th>
<th>Total polyphenol content (mg GAE/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brixol</td>
</tr>
<tr>
<td>Control</td>
<td>9190.33 ± 387.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Se I</td>
<td>10285.13 ± 592.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Se II</td>
<td>8832.00 ± 293.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Different lowercase letters in superscript denote significantly different at P < 0.05 by LSD in ANOVA (Statgraphic Centurion XVII)

Figure 3. Graphical analyses of variety influence on total polyphenol content (TPC), Nitra, 2014 (Statgraphic Centurion XVII)

Before the trial establishment following the soil composition there was applied the nitrogen fertilizer DASA®26/13 with sulfur. Selenium concentrations in plants may, further more, be reduced following application of sulphate as a result of competition between sulphate and selenate for transporters in plant roots (Lyons 2004). Selenium and sulphur (S) compete with each other in the biochemical pathways, leading to synthesis of selenomethionine (Se-met) and methionine in plant cells. The concentration of Se-met in plant seeds must therefore be expected to depend strongly on the ratio of Se uptake to S uptake in the roots (Haugh 2007). That is the possible reason of results distortion in case of selenium influence on observed characteristics.

The ability of ascorbic acid to regenerate lycopene in two-compound mixtures was found by Liu (2008). As the lycopene is the major carotenoid in total carotenoids in tomatoes, there was done correlation analyzes between the AA and total carotenoids (Figure 4), as well as between the other estimated compounds. According to correlation analysis there was found no significant relationship between all the tested antioxidants, which means that the selenium foliar application will not affect the coherences between them.
CONCLUSIONS

Selenium biofortification has influence on total polyphenol content in dosage of 150 g/ha, when the content was significantly increased. This way it is possible to enrich the tomatoes in polyphenols, which are antioxidants and to enhance healthy effects of the fruits. Other meaning antioxidants such a vitamin C and carotenoids weren’t negatively influenced by selenium foliar spraying, their content remained unchanged. According to competition between Se and S in re-building to the plant tissues, there is strong recommendation of using suitable form of fertilizer in case of selenium application. The influence of genotype in case of Brixol and Uno Rosso on all selected characteristics wasn’t found.

ACKNOWLEDGMENTS

The work was supported by VEGA project No. 1/0105/14 and KEGA project 038SPU-4/2014.
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