VARIABILITY OF TOCOPHEROLS IN SWEET MAIZE GRAIN INDUCED BY HERBICIDES

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Abstract

Maize with specific traits, such as sweet maize, is widely used in the human diet, especially due to its sweetness and flavor. Tocopherols, subgroup of vitamin E, are biologically active compounds. Herbicides in maize crop are used for weed control in order to create and ensure optimal conditions for the plant growth. The aim of this study was to examine the herbicides effects on the nutritive quality, i.e. tocopherols content in sweet maize kernel. This study included three different sweet maize hybrids (ZP1, ZP2 and ZP3) and two herbicides, belonging to the different groups, applied in the recommended doses. The application of herbicides has shown different reduction in content of tocopherols (α-, β + γ-, δ-) in kernel samples compared to the control (no herbicide application).

Key words: *nutritional quality, nicosulfuron, mesotrione, corn, α- tocopherol*

1. INTRODUCTION

Maize (*Zea mays* L.) is the third planted and the most productive cereal in the world after wheat and rice (FAOSTAT, 2014). It is widely used in human and animal nutrition as well as in the industry. For human consumption, the most attractive is maize with specific properties such as popcorn and sweet maize. Large usage of sweet maize in human diet due to its sweetness, taste and aroma requires a higher nutritional quality attention. One of the parameters for nutritional quality is the vitamin content. Tocopherols (*α-, β-, γ-, δ-*), the subgroup of vitamin E, are biologically active compounds (Zingg, 2007; Bramley et al., 2000). The most important for human health, from all tocopherols, is α-tocopherol, but lately, a many studies reported a high antioxidant activity of γ- tocopherol (Jiang et al., 2001). It is known that maize grain is important source of vitamin E, especially of γ- tocopherol (Rocheford et al., 2002). Vitamin E has a very high impact on seed longevity as well as prevention of the lipid peroxidation during germination (Sattler et al., 2004).

Using of sweet maize for human consumption is constantly growing which leads to higher production demand. One of the most important parts of maize production, in order to enable optimal plant growing and high yield, is weed control. Weed control options for sweet maize are more limited compared to the standard maize hybrids due to its higher sensitivity (O'Sullivan et al., 2000). Weeds presence in the crops leads to the competition for resources (water, nutrient, space) which can result in a yield decrease (Carvalho et al., 2009). Therefore, the application of appropriate herbicides in sweet maize crop is necessary in order to create and ensure optimal conditions for the plant growth.

Mesotrione is the 2,4-disubstituted benzoylcyclohexane-1,3-dione and belongs to the triketone herbicides (Beaudegnies et al., 2009). This herbicide is widely used for weed control in maize crop and act as the inhibitor of the p-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme which catalyzes the conversion of tyrosine to plastoquinone and α- tocopherol (Mitchell et al., 2001). In the sensitive species, the consequence of the HPPD inhibition is decreased biosynthesis of carotenoids and pigments bleaching.

Nicosulfuron is a member of sulfonylurea herbicides, used for post-emergence weed control in maize crop. According to the chemical structure, nicosulfuron differs from the other sulfonylurea herbicides due to the replacement of the benzene with pyridine ring on the left side of the sulfonylurea bridge and the existence of a carboxamide groups on the pyridine ring (Gonzalez et al., 1996) Nicosulfuron inhibits acetolactate synthase (ALS), the first enzyme in biosynthesis of the essential branched-chain amino acids: leucine, valine, and isoleucine (Babczinski et al., 1991).

The applicability of the herbicide in the crops depends on its selectivity as well as the response (sensitivity) of the crop where it is used. Many studies have been reported a different maize sensitivity to the herbicides depending to: herbicides selectivity; the amount of the applied herbicide; environmental conditions and maize genotype (Brankov et al., 2015; Soltani et al., 2007; Stevanovic et al., 2010; Williams II et al., 2010). Selectivity can be defined as the capacity of a certain herbicide to eliminate weeds in a crop, without the influence on the yield or quality of the final product (Carvalho et al., 2009). Response of maize hybrid to the herbicide can be described by measuring: visual injures, plant height, cob size or yield (Brankov et al., 2015; O'Sullivan et al., 2000; Soltani et al., 2007)

According to that the aim of this study was to examine the influence of nicosulfuron and mesotrione on nutritional quality *i.e.* the tocopherols content in the maize kernel of the three sweet maize hybrids as well as the sensitivity of these hybrids by measuring the kernel yield.

2. EXPERIMENTAL

2.1. Plant material and chemicals

In this study, during 2016, three hybrids (ZP1, ZP2 and ZP3) were sowed, on a slightly calcareous chernozem soil type in three replications, in the experimental field of the Maize Research Institute "Zemun Polje", Serbia. There were three treatments: control (without herbicide application); mesotrione (herbicide from triketone group) and nicosulfuron (herbicide from sulfonylurea group). Herbicides were applied in the recommended doses: mesotrione – 120 g a.i./ha and nicosulfuron – 45 g a.i./ ha, in 15-16 leaf stage of maize according to the BBCH scale. Seeds were collected 21 days after pollination (silking) and stored at -21ºC until analysis. For the analysis, only undamaged kernels were taken and frozen.

Acetonitrile, methanol, 2-propanol as well as tocopherols standards (α-, γ- and δ-) were purchased from Sigma Aldrich (Germany). All applied chemicals were of HPLC grade.

2.2. Extraction of tocopherols

Method used for extraction of tocopherols was modificated method proposed by Gliszczyńska-Świgło et al., 2004. Prior to analysis, defrost maize kernels (approximately 0.6 g) were carefully chopped, transferred with all lactic juice in the erlenmeyer, mixed with 10 mL of 2-propanol and homogenized in the ultrasonic bath for 30 min at ambient temperature. The extracts were then centrifuged at 3000 rpm for 5 min, filtered through a 0.45 μm nylon syringe filter (Thermo Scientific) and clear extract was injected into the Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific, Germany) equipped with fluorescence detector (FLD–3100).

2.3. HPLC method

High performance liquid chromatography (HPLC) is one of the most powerful and most used analytical technique for tocopherols determination (Abidi, 2009). Chromatographic separation of tested tocopherols was achieved on analytical column, Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 μm) thermostatted at 30 ºC. Mobile phase consisted of acetonitrile and methanol (1:1, v/v) was used at the flow rate of 1 mL/min. Injection volume was 15 μL. Wavelengths for excitation and emission were set at 290 nm and 325 nm, respectively.

Tocopherols have been identified by comparing the retention time of the standard. The calibration curve of the appropriate standards, obtained by plotting the peak areas (detector response) versus the standard concentration was used for quantitative calculations. The content of tocopherols was expressed as μg per g of fresh matter (FW).

3. RESULT AND DISCUSSION

3.1. Maize kernel yield

In order to determinate the sensitivity of tested sweet maize hybrids to the applied herbicides the kernel yield was measured. Yield data were reported as the mean values from three replications for each hybrid. Obtained yield values (Fig. 1) were compared between treatments (control, mesotrione and nicosulfuron).

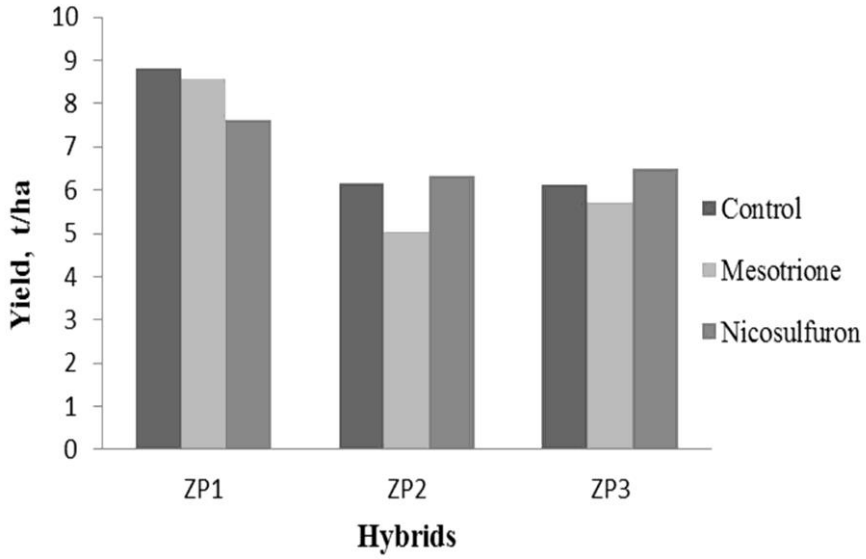

Figure 1. Kernel yield of different sweet maize hybrids (ZP1-ZP3) treated with mesotrione and nicosulfuron.

Sweet maize hybrid ZP1 had shown a different response to the applied herbicides, when compared to the other two hybrids. For ZP1 hybrid, kernel yield was higher, when the herbicide mesotrione was applied comparing to the treatment with the nicosulfuron. Obtained average yield values of the ZP1 hybrid for the control, mesotrione and nicosulfuron were 8.80 t/ha, 8.58 t/ha and 7.61 t/ha, respectively, which is in the agreement with the O'Sullivan et al., 2002, who found that the sweet maize hybrid is more tolerant to the mesotrione than to the sulfonylurea herbicides. They also suggest pre-emergence application rather than post-emergence application of the mesotrione.

Sweet maize hybrids ZP2 and ZP3 had shown lower values for yield in the treatment with the mesotrione. Obtained average yield values of the ZP2 hybrid for the control, mesotrione and nicosulfuron were 6.15 t/ha, 5.04 t/ha and 6.32 t/ha, respectively. Obtained average yield values of the ZP3 hybrid for the control, mesotrione and nicosulfuron were 6.12 t/ha, 5.70 t/ha and 6.49 t/ha, respectively. This result is in an agreement with Robinson et al., 1993, who found that the sweet maize is tolerant to the nicosulfuron applied at the concentration of 35 g/ha. They also found that the response of the sweet maize cultivars can be influenced by the plant stage.

Authors Grey et al., 2000 and O'Sullivan et al., 2000, in their studies found that the tolerant sweet maize hybrids had no response to the nicosulfuron while the sensitive ones respond by yield reduction. They suggest that nicosulfuron can be used for weed control, but only for tolerant sweet maize hybrids.

3.2. Tocopherols content

In this study, in order to examine the herbicides impact on nutritive value, the tocopherols content in fresh sweet maize kernel was determined by using HPLC with fluorescent detector. Chromatographic separation of analyzed tocopherols (*α*-, *β*+*γ*- and *δ*-) was performed with total separation time of 20 minutes. Obtained chromatogram for tested tocopherols is shown in Figure 2.

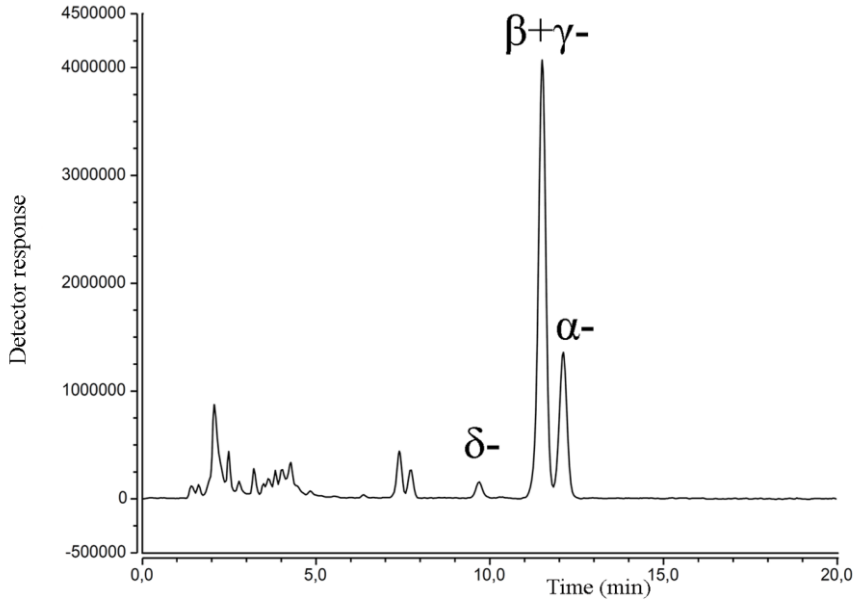

Figure 2. Recorded chromatogram of tocopherols in sweet maize kernel.

In the reverse phase system, which was employed in this study, there is no possibility to separate *β*- and *γ*- tocopherol, so their content is expressed as their sum (Abidi, 2009; Gliszczyńska-Świgło et al., 2004). Since the content of *β*- tocopherol in the maize kernel is very small, its separation from the *γ*-tocopherol is unnecessarily (Abidi, 2009; Gliszczyńska-Świgło et al., 2004; Panfili et al., 2003)

Average values obtained for *α*-, *β*+*γ*- and *δ*- tocopherol content was higher, when compared to those found in apples, avocados, bananas, pears, strawberries, cranberries, carrot, broccoli, cabbage, potato (Chun et al., 2006). Average values of *α*-, *β*+*γ*- and *δ*- tocopherol, in ZP1 hybrid for control, mesotrione and nicosulfuron treatment was 1.70 μg/g FW, 6.94 μg/g FW and 3.97 μg/g FW, respectively, in the ZP2 hybrid average concentration of *α*-, *β*+*γ*- and *δ*- tocopherol was 0.60 μg/g FW, 6.92 μg/g FW and 1.09 μg/g FW, respectively, and in ZP3 the average amount of *α*- , *β*+*γ*- and *δ*-tocopherols was 0.52 μg/g FW, 9.21 μg/g FW and 2.42 μg/g FW, respectively. This result indicate that the sweet maize is a valuable source of tocopherols and poses a very a high nutritive value.

In our research, applied herbicides expressed a various influence on the content of different tocopherols in analyzed hybrids. In ZP1 hybrid, content of *δ*- tocopherol compared to the control was increased by mesotrione for 143.64% (Fig. 3). Opposite, the treatment with nicosulfuron had shown the decreasing trend in *δ*- tocopherol content for 7.27%.

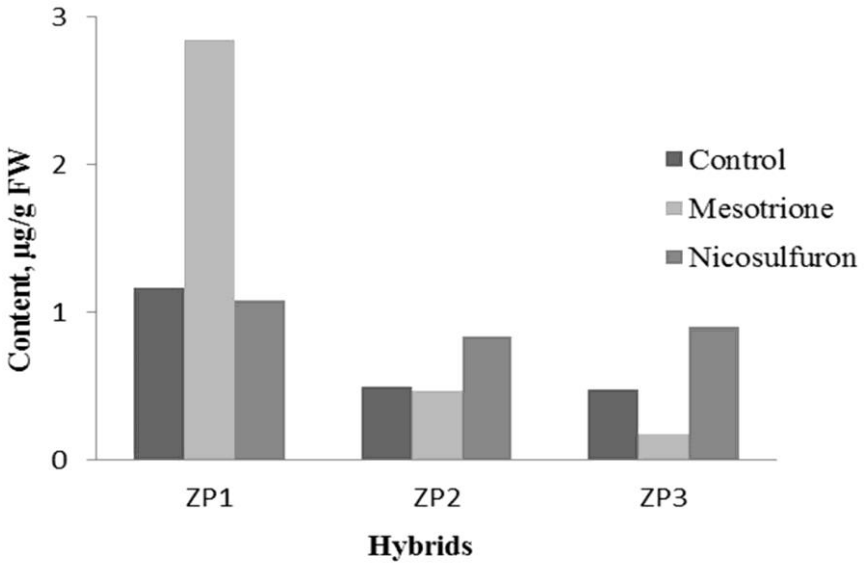

Figure 3. The content of *δ*- tocopherol in different sweet maize hybrids kernel treated with mesotrione and nicosulfuron.

Amount of *δ*- tocopherol in ZP2 and ZP3 hybrids was lower in the treatment with mesotrione compared to control and was reduced by 5.75% and 62.72%, respectively (Fig. 3). Contrary, treatment with nicosulfuron showed an increase in amount of *δ*- tocopherol and it was 66.14% and 88.58%, respectively for ZP2 and ZP3 hybrids.

Compared to the control, in the ZP1 and ZP2 hybrids concentration of *β*+*γ*- tocopherol was lower in both herbicides treatments (Fig. 4). However, nicosulfuron expressed a smaller impact on reduce of *β*+*γ*- tocopherol content and it was 28.93% and 2.95 %, respectively. Reduction of *β*+*γ*- tocopherol content in ZP1 and ZP2 hybrids when mesotrione was applied was 54.24 % and 28.85%, respectively.

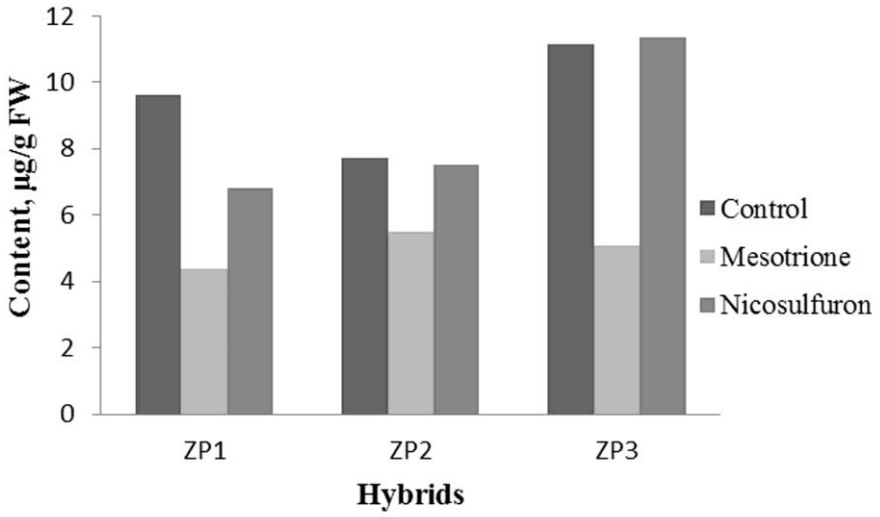

Figure 4. The content of *β*+*γ*- tocopherol in different sweet maize hybrids kernel treated with mesotrione and nicosulfuron.

Similarly, in the ZP3 hybrid, content of $\beta+\gamma$- tocopherol was lower in the treatment with mesotrione, for 54.40% compared to the control (Fig. 4). Opposite, in the treatment with nicosulfuron, the concentration of the $\beta+\gamma$- tocopherol was higher (for 1.82%) compared to the control.

In all tested hybrids, the concentration of α- tocopherol was lower in both treatments, when compared to the control (Fig. 5). Treatment with nicosulfuron had shown the lower impact on the decreasing of α-tocopherol content in comparison to mesotrione and it was 33.36%, 34.63% and 58.76%, respectively for ZP1, ZP2 and ZP3. This result could be explained by the fact that the consequence of mesotrione mode of action is preventing the conversion of tyrosine to α- tocopherol (Mitchell et al., 2001).

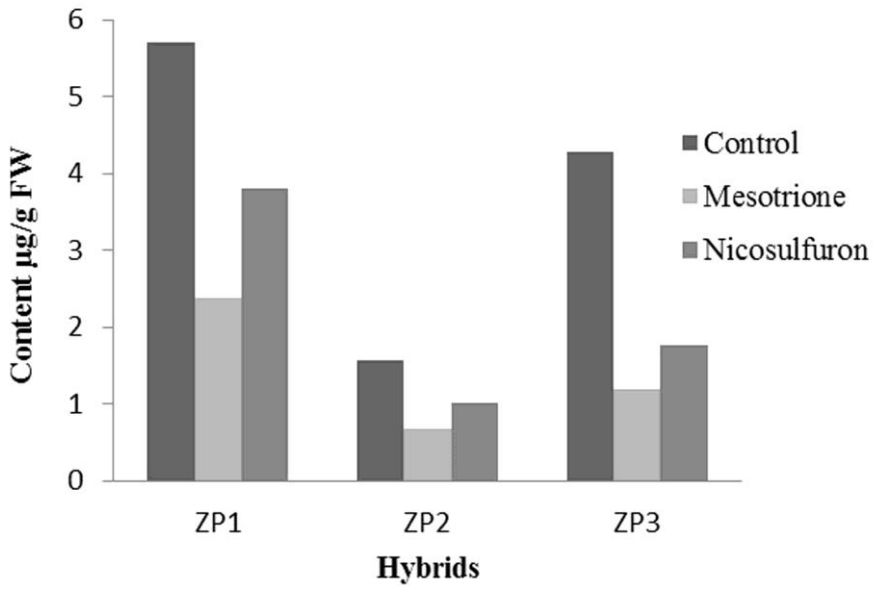

Figure 5. The content of α- tocopherol in different sweet maize hybrids kernel treated with mesotrione and nicosulfuron.

Reduction of α- tocopherol content in ZP1, ZP2 and ZP3 hybrids in the treatment with mesotrione was 58.24 %, 56.55% and 72.10%, respectively.

4. CONCLUSION

Obtained yield values indicate that the hybrid ZP1 is more tolerant to the mesotrione, but more sensitive to the nicosulfuron, contrary to the ZP2 and ZP3 hybrids. Depending on the applied herbicide, different reduction in the content of α-, $\beta+\gamma$- and δ- tocopherol has been noted. Simultaneously, in all three hybrids treated with nicosulfuron the trend of smaller reduction in α-tocopherol content was noticeable, when compared to the mesotrione treatment.

According to the obtained results, variability in the nutritional quality of sweet maize, based on tocopherol concentration in kernel is related to different sensitivity of tested hybrids on applied herbicides. Therefore, in order to ensure and obtain the better nutritional quality of sweet maize, in terms of tocopherols content, more attention should be given to herbicides selection.

ACKNOWLEDGMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects TR31068 and TR31037). The authors are thankful to Biljana Noro for assistance during the experiment.

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