

## CONTENT OF SOME ANTIOXIDANTS IN INTERCROPPED MAIZE AND SOYBEAN GRAIN

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**Abstract:** Intercropping, as a combination of different crops at the same time and the same field, enables interaction of their roots, improving plant growth and stress tolerance, thus improving nutritional quality of produced grains. The investigation was aimed to examine the effect of different cropping systems: intercropping in combination with alternating rows and alternating strips of maize and soybean, as well as single cropping, combined with different fertilization regimes (conventional, application of organic fertilizer, bio-fertilizer and control) on the antioxidant content (glutathione [GSH], phenolics and yellow pigment [YP]) in red maize and black soybean grain. Black soybean is richer in antioxidants than red maize. Season expressed the highest influence on the level of GSH, phenolics and YP in maize and soybean, while cropping system and fertilization regime influenced GSH and phenolics. The antioxidant level in grains with greater weight corresponded with an increased GSH level for maize, as well as an increased GSH and phenolic level for soybean, while smaller grains were characterised by the increased YP content. Generally, antioxidant content was increased mainly by alternating strips in maize grain and by alternating rows in soybean grain. Bio-fertilizer had the highest impact on an increase in GSH in maize grain and YP in soybean grain, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain.

**Key words:** antioxidant content, intercropping, red maize, black soybean.

### Introduction

Sustainable agriculture combines various measures aimed to produce high quality and healthy crops, together with preservation of an agro-ecosystem. Produced crop yields are more nutritious and free from agrochemicals and their

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residues. This type of agriculture includes a combination of different crops at the same field, application of organic fertilizers and bio-fertilizers, facilitating better utilization of time, space and nutrients, i.e. soil potential, with minimal inputs.

Intercropping, as a combination of different crops at the same time and the same field, enables interaction of their roots, by the root exudates, and interaction with soil micro-organisms (Zhang et al. 2013). Roots of intercrops have greater root development, going deeper than roots of sole crops (Yang et al., 2010). Also, intercropping enables increased resistance to various diseases and pests. For instance, in soybean intercropped with maize, resistance to red crown rot was increased, by enhancing of phenolic acid concentration in root exudates, which constrain *C. parasiticum* growth (Gao et al., 2014). Root exudates create a specific environment for development of soil microorganisms, improving soil chemical and microbial properties. Thus, when pepper was intercropped with green garlic, growth of actinomycetes was improved, with inhibition of fungi growth, together with increased activity of invertase, alkaline phosphatase and catalase (Ahmad et al., 2013). Moreover, some biochemical pathways of intercrops could be altered, like phenylpropanoid and organosulfur biosynthesis in Chinese onion, when it is intercropped with cucumber (Yang et al., 2013).

Application of bio-fertilizers which contain promoting microorganisms could enhance crop growth and stress tolerance. Aroca and Ruiz-Lozano (2009) found that pulses, inoculated with rhizobial bacteria had improved growth during drought conditions, due to the bacteria induced regulation of plant hormones, like abscisic acid and ethylene. Pandey et al. (2016) also confirmed a positive impact of rhizobacteria on crop growth and stress tolerance, by increasing of the antioxidant defence and nutrient absorption.

The additional health effect on humans could be achieved by consumption of food produced from specific genotypes, such as red grain maize or black soybean. Red maize is characterised by about 20% higher protein content than white or yellow genotypes, with an increased anthocyanin and flavonoid level (Žilić et al., 2011a), while black soybean contains two times higher phenolic level in grain than yellow grain genotypes (Žilić et al., 2011b). Some other important antioxidants for plants and human health include glutathione and yellow pigments (mainly  $\beta$ -carotene), which play an important role in the antioxidative defence and stress signalling (Foyer and Noctor, 2005; Grodstein et al., 2007).

The aim of experiment was to examine effects of different cropping systems: intercropping and single cropping and fertilization regimes (conventional, application of organic fertilizer and bio-fertilizer) on the antioxidant content (glutathione, phenolics and yellow pigment) in maize and soybean grain.

## Materials and Methods

The experiment was conducted during the 2011 and 2012 vegetative period, at Zemun Polje (44°52'N 20°20'E), on a slightly calcareous chernozem, with 53.0% sand, 30.0% silt, 17.0 % clay, 3.3% organic matter, 7.0 pH KCl and 7.17 pH H<sub>2</sub>O. Chemical analysis showed that soil contained 37.45 ppm N, 10.70 ppm P, 107.40 ppm K, 327.95 ppm Mg, 0.65 ppm Fe and < 0.02 ppm Zn in the 0–30 cm layer. Red grain maize (variety Rumenka) and black grain soybean (variety Dukat) were grown in three different cropping systems: as a single crop (SC), in alternating rows of both crops (AR) and in alternating strips (3 rows of each species – AS), with a 70-cm inter-row distance, as well as with a 22- and 4-cm intra-row distance for maize and soybean, respectively. The crops were sown on the 11th May 2011 and 2012. The elementary plot encompassed 4 × 4.2 m in a completely randomized block design with 4 replications. The effect of fertilization regimes included the incorporation of bio-fertilizer Uniker (11 l ha<sup>-1</sup>), organic fertilizer Ofert (3 t ha<sup>-1</sup>), urea (163 kg ha<sup>-1</sup>) and control – without fertilization. Bio-fertilizer Uniker contains the following bacteria: *Bacillus megaterium*, *B. licheniformis* and *B. subtilis*. Ofert contains a minimum of 2.2% N, 4.8% P<sub>2</sub>O<sub>5</sub>, 2.8 % K<sub>2</sub>O, 60% organic matter, C/N 12.17, and 1.08% Mg.

After harvesting, grain weight was measured from 4x10 grains and expressed in g per grain. Then grains were milled and the content of total glutathione (GSH) was determined by the method of Sari Gorla et al. (1993), water soluble phenolics were determined by the method of Simić et al. (2004) and expressed in µg of *3-hydroxy-4-methoxycinnamic acid* g<sup>-1</sup> and yellow pigment (YP) was determined by the American Association of Cereal Chemists Method (AACC, 1995) and expressed in µg of β-carotene g<sup>-1</sup>.

Significant differences between treatment means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) using a two-factorial RCB design. Differences with p<0.05 were considered as significant. The interdependences between the grain weight of maize and soybean and examined antioxidants were processed by regression analysis.

Meteorological conditions: The vegetative period of 2012 could be considered as unfavourable with an unequal distribution of precipitation (the lowest value was observed in August), followed by the high average temperatures in July and August (Table 1), indicating drought stress during the grain filling period. Oppositely, 2011 could be considered as a relatively moderate year, with lower average temperatures and higher amounts of monthly precipitation.

Table 1. Average monthly air temperatures and precipitation sums for the vegetative period (April–September) of 2011 and 2012 at Zemun Polje.

	Month	IV	V	VI	VII	VIII	IX	Aver./Σ
T aver.	2011	13.4	16.8	21.5	23.3	23.9	21.6	20.1
	2012	14.5	17.9	24.6	27.1	26.2	22.1	22.1
Σ precip.	2011	14.9	89.6	26.2	44.0	66.0	32.6	273.3
	2012	64.2	66.4	17.5	30.7	5.8	26.0	210.6

### Results and Discussion

According to the results presented in Table 2, a significant variation in GSH and phenolic content was induced by the all examined factors (cropping system, fertilization and year) and their interactions, while a significant variation in YP was present under the influence of year and its interaction with the other two factors in grain of both crops.

Table 2. Analysis of variance for the effect of cropping system, fertilization and year on contents of glutathione (GSH), phenolics and yellow pigment (YP) in maize and soybean grain.

Source of variation	df	GSH (nmol g <sup>-1</sup> )		Phenolics (μg g <sup>-1</sup> )		YP (μg g <sup>-1</sup> )		
		MS	LSD <sub>0.05</sub>	MS	LSD <sub>0.05</sub>	MS	LSD <sub>0.05</sub>	
Maize grain	Replications	3						
	CS	2	17971*	103.6	69507*	256.2	1.26	1.582
	F	3	30025*	97.35	155560*	231.2	0.01	1.617
	Y	1	182040*	85.55	1604530*	179.8	58.83*	1.106
	CS x F	11	13558*	101.3	70821*	253.5	0.28	1.783
	CS x Y	5	45839*	89.41	190247*	132.4	812682*	77.92
	F x Y	7	51930*	62.29	32997*	139.8	11.447*	0.935
	CS x F x Y	23	20961*	39.05	131688*	51.4	4.256*	0.905
	CV (%)		19.01		28.02		18.25	
	Average		552.53		915.51		8.58	
	Min		337.55		625.62		5.82	
	Max		798.34		1518.07		11.60	
	Soybean grain	Replications	3					
CS		2	107441*	191.7	1478188*	606.8	29.9	8.465
F		3	19297*	203.1	342795*	638.7	12.9	8.595
Y		1	772211*	154.5	7849883*	503.7	2595.3*	3.871
CS x F		11	53879*	188.3	625479*	592.6	17.5	9.267
CS x Y		5	58088*	86.82	9159254*	276.0	768821*	77.98
F x Y		7	145032*	146.1	1409855*	491.3	402.0*	3.429
CS x F x Y		23	76714*	66.1	841822*	81.9	137.77*	2.196
CV (%)			28.65		25.00		32.03	
Average			695.96		2577.91		26.10	
Min			291.46		1082.35		15.69	
Max			1181.54		3392.04		49.36	

\*Significant at the 5% probability level; df: degrees of freedom; MS: mean squares; LSD<sub>0.05</sub>: least significant difference at the 0.05 probability level; CS: cropping system; F: fertilization; Y: year; CV: coefficient of variation.

It was already mentioned that GSH and phenolics play an important role in soybean nutritional quality (Dragičević et al., 2010). The highest variation and the highest average values of examined antioxidants were noticed in soybean grain compared to maize grain. This could be related to the high antioxidant level, particularly phenolics, present in the black grain soybean (Astadi et al., 2009; Žilić et al., 2011b).

Among fertilization regimes, the bio-fertilizer had the highest impact on an increase in GSH in maize grain (Figure 1) and YP in soybean grain (Figure 2), with the highest average values of 608.90 nmol g<sup>-1</sup> and 28.43 μg g<sup>-1</sup>, respectively, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain (average values of 744.31 nmol g<sup>-1</sup> and 2861.84 μg g<sup>-1</sup>, respectively). Taie et al. (2008) also acquired the highest phenolic content in soybean fertilized with compost and bio-fertilizer, compared to fertilization with compost only and conventional fertilization. Dragičević et al. (2013) obtained the elevated β-carotene content in intercropped soybean and maize grain under the influence of bio-fertilizer.

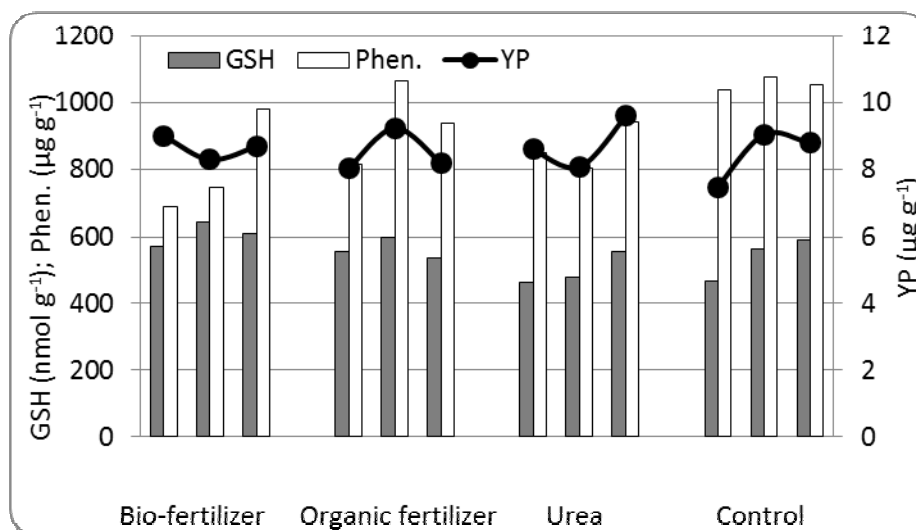


Figure 1. The effect of different cropping systems (SC – single crop, AR – alternating rows, AS – alternating strips) and fertilizer types on variation in glutathione (GSH), soluble phenolics (Phen.) and yellow pigment (YP) in maize grain (average for 2011–2012).

Rhizobacteria provide cross protection against numerous stresses by increasing of the antioxidant defence, nutrient absorption, etc., what is of particular importance during drought conditions (Pandey et al., 2016) which were

present during 2012. This could be one of the reasons for the increased antioxidant content in grain of maize and soybean from bio-fertilizer treatment. Urea and control treatments were characterised by higher YP and phenolic content in maize grain ( $8.75 \mu\text{g g}^{-1}$  and  $1055.13 \mu\text{g g}^{-1}$ , respectively). When cropping systems were compared, alternating strips mainly had the highest impact on the increase of GSH (urea and control) and phenolics (bio-fertilizer), while alternating rows were important for GSH increase (bio-fertilizer and organic fertilizer), as well as phenolics and YP (organic fertilizer and control) in maize grain (Figure 1). Alternating rows had the highest effect on the increase of GSH (organic fertilizer, urea and control), phenolics (bio-fertilizer, organic fertilizer and control) and YP (all four cropping systems), while a single crop increased mainly GSH (bio-fertilizer) and phenolics (urea) in soybean grain (Figure 2). Dragičević et al. (2013) also emphasized alternating rows as an important intercropping system for GSH and phenolic increase. Other than that, the highest values of GSH content in maize grain and YP in soybean grain were noticed in AR + bio-fertilizer combination. The highest GSH and phenolic content in soy grain was noticed in AR + organic fertilizer treatment combination, and in maize grain the highest YP was noted in AS + urea combination. Similarly, the highest phenolic content was observed in AR + control combination.

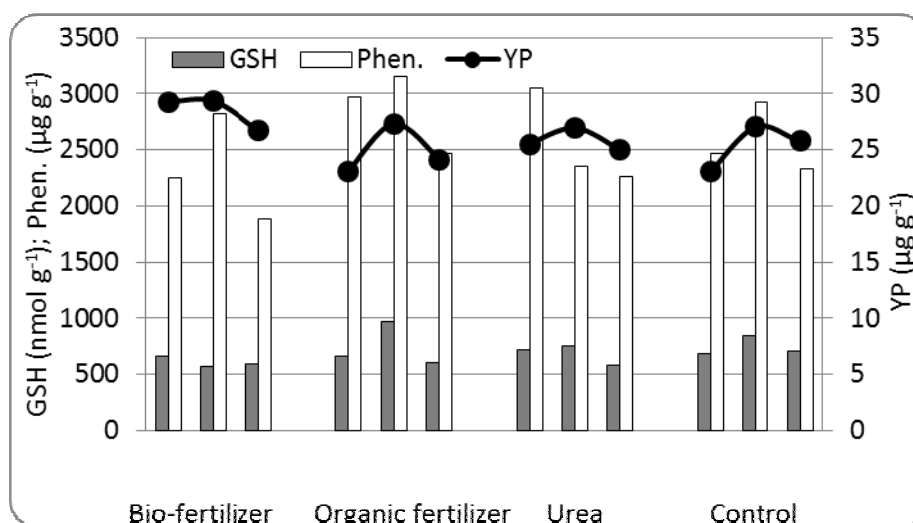


Figure 2. The effect of different cropping systems (SC – single crop, AR – alternating rows, AS – alternating strips) and fertilizer types on variation in glutathione (GSH), soluble phenolics (Phen.) and yellow pigment (YP) in soybean grain (average for 2011–2012).

Irrespective of the present variations in the content of examined antioxidants, relations with the yield parameter, such as seed weight indicated that increased seed weight was followed by the higher GSH and lower YP contents in maize and soybean grain (Figures 3 and 4).

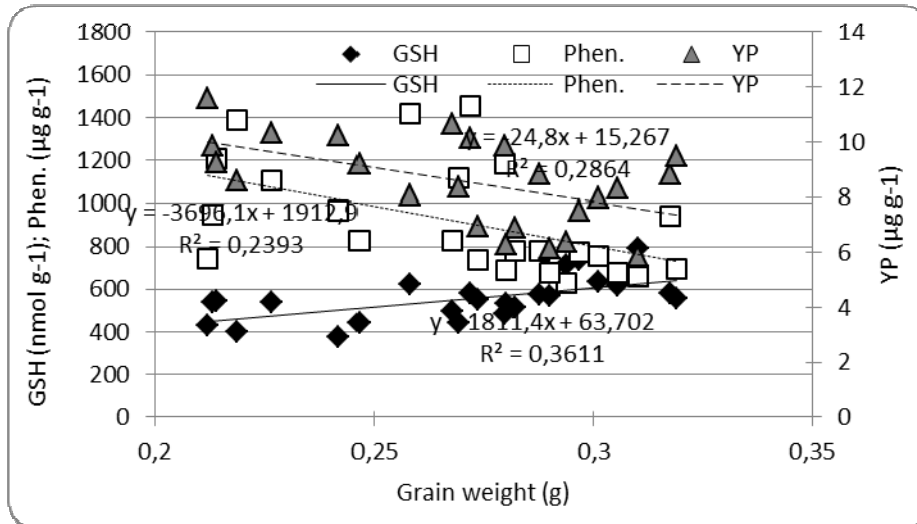


Figure 3. Interdependence between grain weight and glutathione (GSH), phenolics (Phen.) and yellow pigment (YP) content in maize grain.

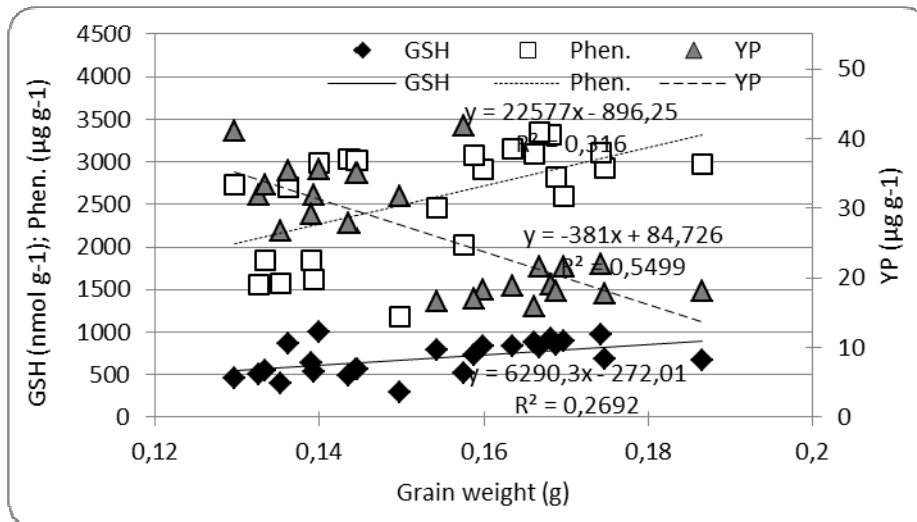


Figure 4. Interdependence between grain weight and glutathione (GSH), phenolics (Phen.) and yellow pigment (YP) content in soybean grain.

Nevertheless, higher grain weight was followed by the decreased phenolic content in maize grain and increased phenolic content in soybean grain. All regression coefficients were significant. Similarly to results obtained for maize grain, Yafang et al. (2011) and Shen (2009) also found higher phenolic level and antioxidant activity of small rice grains. Moreover, grain weight depends on many different factors, so Konopka et al. (2012) found slightly higher content of free phenolics and total carotenoids in wheat grain with smaller kernel weight, produced with organic fertilizers, in comparison to the conventionally produced, with the application of NPK fertilizer.

### Conclusion

Based on the results obtained, it could be concluded that black soybean is richer in antioxidants than red maize. Season had the highest influence on the level of GSH, phenolics and yellow pigment in maize and soybean level, while the cropping system and fertilization regime influenced GSH and phenolics. The antioxidant level in grains with greater weight corresponded with the increased GSH level for maize, as well as increased GSH and phenolic level for soybean, while smaller grains were characterised by the increased YP content. Generally, the antioxidant level was increased mainly by alternating strips and/or alternating rows in maize grain and by alternating rows in soybean grain. The bio-fertilizer had the highest impact on an increase in GSH in maize grain and yellow pigment in soybean grain, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain.

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## SADRŽAJ NEKIH ANTIOKSIDANATA U ZRNU KUKURUZA I SOJE IZ ZDRUŽENOG USEVA

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### R e z i m e

Združeni usev, kao kombinacija različitih useva, koji se gaje u isto vreme i na istom polju, omogućava interakciju njihovih korenova, poboljšava rast i tolerantnost na stres, poboljšavajući tako nutritivni kvalitet proizvedenog zrna. Cilj istraživanja je bio da se ispita efekat različitih sistema gajenja: združeni usev kombinujući naizmenične redove i naizmenične trake kukuruza i soje, kao i pojedinačne useve, zajedno sa različitim režimima đubrenja (konvencionalni, upotreba organskog đubriva, bio-đubriva i kontrola) na sadržaj antioksidanta (glutaciona [GSH], fenola i žutog pigmenta [YP]) u zrnu crvenog kukuruza i crne soje. Zrno crne soje je bogatije antioksidantima od crvenog kukuruza. Sezona je pokazala najveći uticaj na sadržaj GSH, fenola i YP kod kukuruza i soje, dok su sistem gajenja i đubrenje uticali na promene u sadržaju GSH i fenola. Sadržaj antioksidanata u zrnima sa većom masom je odgovarao povećanom nivou GSH kod kukuruza, kao i povećanju nivoa GSH i fenola kod soje, dok su zrna manje mase imala veći sadržaj YP. Uopšteno, sadržaj antioksidanata je uglavnom bio povećan u zrnu kukuruza pri gajenju u naizmeničnim trakama, a u zrnu soje pri gajenju u naizmeničnim redovima. Bio-đubrivo je pokazalo najveći uticaj na povećanje sadržaja GSH u zrnu kukuruza i YP u zrnu soje, dok je organsko đubrivo ispoljilo uticaj na nakupljanje GSH i fenola u zrnu soje.

**Ključne reči:** sadržaj antioksidanata, združeni usev, crveni kukuruz, crna soja.

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