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Original scientific paper

BLACK AND YELLOW SOYBEAN: CONTRIBUTION OF SEED QUALITY TO OXIDATIVE STRESS RESPONSE DURING PLANT DEVELOPMENT

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The purpose of this paper was to compare quality of seeds of two black and yellow soybean and the response of plants developed from these seeds to oxidative stress during vegetation and reproduction period. Content of carbohydrates: cellulose, starch, total and reduced sugars, as well as oil and protein content varied among all varieties, irrespective the colour. Bearing in mind all differences in seed quality, as well as response to oxidative stresses during development, black cultivars could be proposed as an excellent source of phenolic compounds (flavan-3-ols, antocyanins, genistein, glycitein, quercetin, laricitrin and isorhamnetin derivatives). Due to extreme fluctuation in precipitation amounts in the last years, information on the better performance of soybean varieties in oxidative stress conditions is of great importance to organic and conventional production of this cultivar.

Keywords: antioxidants, black and yellow soybean; HPLC-MS; lipid peroxidation; polyphenols; seed quality

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INTRODUCTION

During the last years, organic production is considered as beneficial to the environment in comparison to conventional. The fundamental difference between the organic and conventional system of production is related to soil fertility, which can affect the nutritional composition of the plant, including secondary metabolites, as well as the yield components (BALISTEIRO *et al*., 2013). Among numerous qualitative characteristics of soybean seed, seed coat or testa colour may be one of the most important factors to consider for soybean seed quality. It has been reported that the pigmentation on soybean seed coat affects the levels of isoflavones, and the composition and levels of fatty acids (SLAVIN *et al*., 2009; LEE *et al*., 2010; CHO *et al*., 2013). According to BALISTEIRO *et al*. (2013), organic soybeans had a higher protein content and lower trypsin inhibitory activity, isoflavones and phenolic compounds than soybean cultivated by the conventional system and the ratio of derivatives of genistein, daidzein and glycitein was affected by cropping system. The same authors concluded that these results seem contrary to what might be expected, since both protease inhibitors and phenolic compounds are associated with plant defense mechanisms, indicating that well-functioning and stable organic systems reduce the abiotic and biotic stress caused initially by the transition.

In general, soybean cultivars have yellow, black, green, brown or mottled seed coats. Most soybean cultivars have a yellow-coloured seed coat, while dark coloured soybeans are also becoming popular worldwide due to their potentially high functional characteristics (DIXIT *et al*., 2011). Dark-coloured soybean seeds accumulate anthocyanins or other phenolics that contribute to their high stress resistance (WU *et al*., 2013; ZHANG *et al*., 2013). Multiple layers of hard seed coat structures and the presence of chemicals in a continuous layer of cells in the seed coat of black soybeans form a barrier against the permeation of water and oxygen (ZHOU *et al*., 2010). The different seed coat cell layers have three main physiological functions: (1) production, transport, and download of metabolites for zygote development, including metabolite inter-conversions, transport of photosynthetic assimilates and photosynthesis, (2) synthesis and deposition of defense-related compounds, both phytoalexins and structural components, and (3) establishment of physical dormancy and mechanical protection (SMÝKAL *et al*., 2014).

In the dry, quiescent state, seeds are protected by their seed coat and are exceptionally tolerant of stress factors (such as temperature extremes that are lethal to adult plants), contrary to highly vulnerable seeds at other developmental stages (during seed development on the mother plant, e.g. drought, or during germination, e.g. pathogen attack) (KRANNER *et al*., 2010). The same authors reported that the ability of seeds to resist various environmental conditions is based on generally applicable protection and repair mechanisms (antioxidant systems), which would further enable seed germination.

Due to various stress tolerance that coincide with developmental switches, the purpose of this paper was to compare the response of plants developed from differently coloured soybean seeds to stress during vegetation and reproduction period.

MATERIALS AND METHODS

Plant material and experimental conditions

Plant material tested in this paper was seeds and leaves of two black (‘NS Blackstar’ and ‘NS Pantera’) and two yellow (‘Fortuna’ and ‘Galina’) soybean cultivars [*Glycine max* (L.) Merr.]. Black varieties are the first black soybean cultivars from the Balkan region. ‘NS Blackstar’ and ‘NS Pantera’ belong to early varieties from 00 maturity group, while ‘Fortuna’

and 'Galina' belong to early varieties from 00 and 0 maturity groups, respectively. Experiments were conducted under conditions of organic experimental field of Institute of Field and Vegetable Crops (Novi Sad, Serbia) which is a certified producer. Trials were conducted in Bački Petrovac (45°420′N 19°35′E) in 2015 and 2016. Before the trials were set up, soil samples were taken and chemical analyses were carried out. The soil was chernozem, moderately supplied with phosphorus and potassium across both years (Table 1). Sowing was performed in the last week of April in 2015 and 2016. The experimental design was a randomized block with five replications. Plot size was 6 m^2. Plant spacing between rows was 0.5 m and within row 4 cm, resulting in population density of 450000 plants per ha. Soil management, pest and disease control was carried out according to standard procedures for organic production. Weather conditions at the experimental field are given in Figure 1. Data (mean minimal and maximal and mean monthly temperatures and monthly precipitation sums) from the local weather station located 500 m away from the trials were used to obtain weather data for the growing seasons studied.

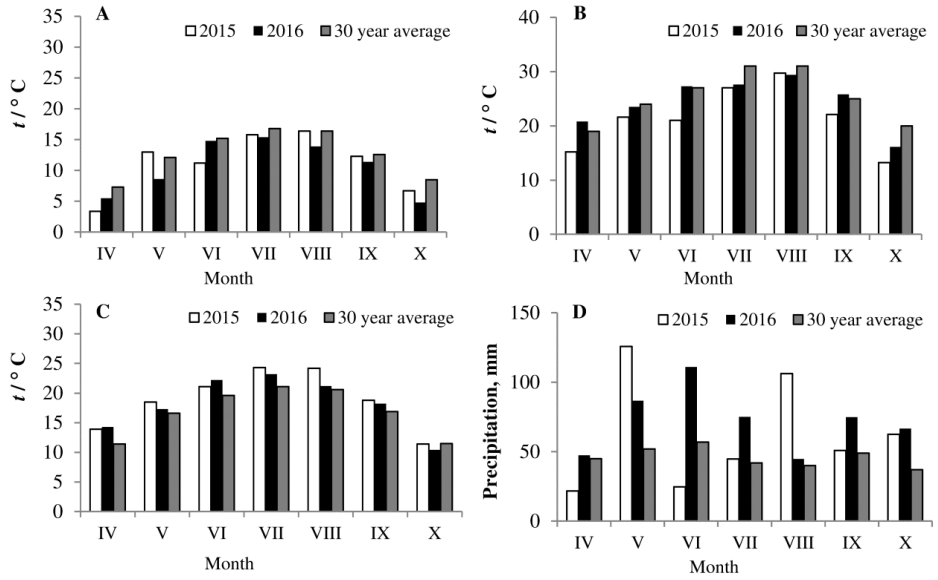

Figure 1. Weather conditions in soybean growing season during 2015, 2016 and 30 years average: minimal monthly air temperatures (A), maximal monthly air temperatures (B), mean monthly air temperatures (C) monthly precipitation sums (D).

Plant material could be divided into two groups: 1) soybean seeds collected in 2015 and 2) leaves developed from these in 2016, sampled in two phases of development, the first period (I) was at the stage of the full bloom or R2, the second (II) was at the seed beginning stage or R5. Leaves (N=30 per repetition) were sampled by hand for biochemical analyses, but final harvest for the yield assessment was performed by sickle. Border plants were not harvested.

Table 1. Soil properties at experimental field in Bački Petrovac, Institute of Field and Vegetable Crops, Serbia (Organic production field).

Year	***pH***		***Content (%)***			***Physiologically active (mg 100 g^{-1} of soil)***	
	in H_2O	***in KCl***	***Total N***	***$CaCO_3$***	***Humus***	***Al-P_2O_5***	***Al-K_2O***
2015	8.05	7.22	0.199	1.61	2.68	26.27	30.35
2016	8.19	7.20	0.189	2.10	2.54	14.80	22.70

Seed quality analysis

A part of the seed of soybean plants collected in 2015 were analysed prior to sowing. Whole seeds were milled in Ika M20 universal mill, under cooling conditions. Soybean seeds (dried to approx. moisture content 11%) were used for the analyses of ash and starch according to Yugoslav Pharmacopoeia (PH JUG, 2000). Total oil content was determined by the extraction of oil from ground seed (8h, 70 °C), performed in a Soxhlet extractor using 5 g of seed and 200 mL petroleum-ether followed by solvent removal under vacuum at 60 °C (SRPS ISO 659:2011). Total protein content (N×6.25) was determined according to the Kjeldahl procedure. Cellulose content was determined by modified Scharrer method (SRPS ISO 6541:1996). Total sugars and reducing sugars were determined according to Luff-Schoorl method (AACC 80-60.01). All results are expressed as % of absolutely dry seed weight (% DW).

Extraction of phenolic compounds and determination of individual phenolic compounds using HPLC-DAD-MS analysis

For extraction of phenolic compounds, after double defatting with n-hexane, 0.5 g of milled seed was mixed with 3 mL of methanol containing 1% (w/v) 2.6-di-tert-butyl-4-methylphenol (BHT) in a cooled ultrasonic bath for 1 hour. BHT was added to the samples to prevent oxidation. After extraction, the extracts were centrifuged for 10 min at 33600x*g*. Each supernatant was filtered through the Chromafil AO-20/25 polyamide filter produced by Macherey-Nagel (Düren, Germany) and transferred to a vial prior to injection into the HPLC (high performance liquid chromatography) system. Phenolic compounds were analysed on a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector. The spectra were collected between 200-600 nm (isoflavones at 254 nm, cinnamic acid derivatives and flavanols at 280 nm, flavonols and flavones at 350 nm and anthocyanins at 530 nm). The column was a Gemini C_{18} (150×4.6 mm 3 μm; Phenomenex, Torrance, USA) operated at 25 °C. The elution solvents were aqueous 0.1% formic acid in twice distilled water (A) and 0.1% formic acid in acetonitrile (B). Samples were eluted according to the linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions (WANG *et al*., 2002). The injection amount was 20 μL and flow rate was 0.6 mL min^{-1}. All phenolic compounds presented in our results were identified by an HPLC-Finnigan MS detector and an LCQ Deca XP MAX (Thermo Finigan, San Jose, CA) instrument with electrospray interface (ESI) operating in negative ion mode. The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 110 to 1500. Column and chromatographic conditions were

identical to those used for the HPLC-DAD analyses. The injection volume was 10 μL and the flow rate maintained at 0.6 mL min^{-1}. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, respectively; the source voltage was 3 kV and normalized collision energy was between 20-35%. Spectral data were elaborated using the Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing retention times and their spectra, as well as by adding the standard solution to the sample and by fragmentation. Concentrations of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg g^{-1} DW. For compounds lacking standards, quantification was carried out using similar compounds as standards.

Leaves analyses

After sampling during 2016, fresh leaves were immediately dipped in liquid nitrogen (fresh plant material), while another part was dried in a shaded and well-ventilated place (dry plant material). All extraction procedures were performed in triplicate. Quantification of investigated biochemical parameters was performed using Perkin Elmer Lambda 25 UV/Visible spectrophotometer.

One g of fresh leaves was ground with cooled mortar and pestle and then homogenized with: 5 mL 20% trichloroacetic acid for lipid peroxidation intensity and 5 mL 0.05 M sodium borate buffer ($Na_2B_4O_7\times 10H_2O$, pH 8.8) for phenylalanine ammonia-lyase activity. After centrifugation at 15000x*g* for 10 min at 4 °C aliquots of the supernatant were used in further analyses. For total contents of phenolic compounds and DPPH-scavenging test, 5 mL methanol ($MeOH/H_2O$, 4/1, v/v) containing 0.1% formic acid, were added to 1 g of powdered plant material and kept in a cooled ultrasonic bath for 1 h. After extraction, leaves extracts were centrifuged for 10 min at 4350x*g*. Each supernatant was rapidly filtered and kept refrigerated until assayed.

Fresh leaves analysis

Lipid peroxidation (LP) was measured as malondialdehyde (MDA) equivalents production, at 532 and 600 nm (A_{600} is a nonspecific absorbance and subtracted from A_{532}) with the thiobarbituric acid (TBA). Concentration of MDA was calculated using Beer-Lambert's equation (extinction coefficient of MDA is 155 mM^{-1} cm^{-1}).The total amount of TBARS (TBA-reactive substances) is given as nmol malondialdehyde (MDA) equivalents g^{-1} fresh weight (FW) (JAMBUNATHAN, 2010).

Phenylalanine ammonialyase (PAL; EC 4.3.1.5) activity in fresh leaves was performed according to GERASIMOVA *et al.* (2005) and expressed in U g^{-1} FW. The reaction mixture contained 1 mL extract, 1 mL 0.1 M borate buffer (pH 8.8), and 1 mL 60 mM mL^{-1}*L*-phenylalanine. Samples were incubated at 37 °C for 1 h. In control samples, the extract was replaced by borate buffer (1 mL). The reaction was stopped by adding 0.5 mL 1M trichloroacetic acid. Absorbance was measured at 290 nm by the formation of *trans*-cinnamic acid.

Dry leaves analysis

Total phenolic content in dried leaves was determined by Folin-Ciocalteu method (MIKULIČ-PETKOVŠEK *et al*., 2012) that is based on the fact that phenolics are reducing agents. Tubes with 8.4 mL H_2O, 0.5 mL 33% Folin-Ciocalteu phenol reagent and 0.1 mL seed extract (except in blank) were vortexed and after 3-6 min 1 mL of 20% Na_2CO_3 were added. Absorbance

at λ=765 nm was recorded after 60 min of incubation at room temperature. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg g^{-1} dry weight (DW).

To determine total flavonoids content (PĘKAL and PYRZYNSKA, 2014), 2.5 mL of methanolic extract, water (2 mL) and $AlCl_3$ reagent (0.1 g crystalline aluminium chloride and 0.4 g crystalline sodium acetate were dissolved in 100 mL of extracting solvent; 5 mL) were added and absorbances were recorded at 430 nm against a blank (without $AlCl_3$ reagent). The amount of flavonoids was calculated from rutin calibration curve and expressed as mg rutin g^{-1} DW. Proanthocyanidins were determined by a butanol-HCl assay (MAKKAR, 2003). To aliquots of prepared extracts (0.5 mL), butanol-HCl reagent (95:5 butanol-HCl, 3.0 mL) was added and 2% ferric reagent (2% ferric ammonium sulfate in 2.0 mol HCl, 0.1 mL). After that, test tubes were vortexed and placed in a boiling water-bath for 60 min. After cooling, absorbances were recorded at 550 nm against a blank containing solvent (0.5 mL) instead of the extract. Proanthocyanidins were expressed as mg leucoanthocyanidin g^{-1} DW.

Monomeric anthocyanins contents were estimated using the differential method (LEE *et al*., 2005). After dilution of MeOH extracts with two buffer solutions at pH 1 and 4.5, the absorbance of each dilution was measured at 510 and 700 nm against a distilled water control. Total monomeric anthocyanin content was calculated as mg cyanidin-3-*O*-glucoside equivalentsg^{-1}DW.

Antioxidant test

Total potential antioxidant activity of investigated seeds and dry leaves extracts was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals (PANDA, 2012). Reaction medium contained 3 mL aliquot of working solution (24 mg DPPH in 100 mL methanol diluted to obtain an absorbance of 0.980 at 517 nm) was mixed with 100 µL of the sample fractions at varying concentrations (25-250 µg mL^{-1}). The change of optical density at 517 nm of DPPH radicals is monitored after incubation in the dark for 15 min at room temperature. DPPH radical scavenging capacity was given as % of neutralized radicals.

Statistical analysis

Values of the biochemical parameters were expressed as means ± standard error of determinations made in triplicates and tested by ANOVA followed by comparisons of means by the Duncan's test ($P<0.05$). All statistical analyses were performed using STATISTICA for Windows version 13 (Dell Software).

RESULTS AND DISCUSSION

The aim of this paper was to determine quality of seeds of two black and yellow soybean grown in 2015 and to compare the change in antioxidants accumulated in leaves of plants developed from these seeds in 2016, with a view to explain in which way they cope with oxidative stress during development.

According to some authors that tested oxidative stress parameters in light- and dark-coloured soybeans (lipid peroxidation, genetic damage and integrity loss of cell membranes in the age-associated physiological processes that occur during storage), and how it further affects development of the seed and plants after germination, the light-coloured seeds deteriorate more easily, whereas the dark-coloured soybean seeds maintain higher seed viability (KRANNER *et al.*,

2010; LIU *et al*., 2017). LIU *et al.* (2017) reported that hardness of the seed, physical structure and the chemical constituents, such as: multiple hard layers and phenolics in the dark-coloured seed coat, effectively prevent external injuries (oxidization and insect damage) and possibly prevent inactivation of dark soybean seeds.

Nutrients and bioactive compounds present in soybean seeds vary greatly with the cultivar, environmental conditions and geographical sowing region (BALISTEIRO *et al*., 2013). Our results showed that black cultivars had more carbohydrates: starch, total and reduced sugars, while yellow cultivars had more protein content (Table 2). KIM *et al*. (2005) explained that relationship between protein and lipid positively correlated to testa colour. Our results showed that black varieties had higher oil content (Table 2).

Table 2. Chemical composition of soybean seed varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina') (% DW), mean ± standard error (SE). Values marked with the same letter, do not differ significantly at P<0.05 (Duncan's test).

variety		*total oil*	*ash*	*starch*	*total sugars*	*reduced sugars*	*cellulose*	*protein*
black	**B**	19.4±1.1^{b}	5.4±0.9^{a}	9.3±0.7^{a}	9.0±0.1^{b}	0.2±0.0^{a}	5.3±0.9^{b}	41.5±2.8^{b}
	P	22.2±2.3^{a}	4.7±0.7^{c}	8.1±0.2^{b}	10.4±0.1^{a}	0.2±0.0^{a}	4.8±0.7^{d}	41.0±5.9^{b}
yellow	**F**	18.7±0.9^{b}	5.2±0.5^{b}	7.0±0.1^{c}	7.9±0.4^{c}	0.0±0.0^{c}	5.1±0.8^{c}	44.6±7.8^{a}
	G	21.1±1.6^{a}	4.8±0.5^{c}	6.9±0.4^{c}	8.9±0.3^{b}	0.1±0.0^{b}	5.9±1.5^{a}	39.4±4.1^{c}

WILCOX and SHIBLES (2001) observed that the increase in the level of carbohydrates was accompanied with the decrease in protein level. In addition, they demonstrated that the inverse relationship of protein with total carbohydrates was strongly associated with sucrose level. The composition of fatty acids and contents of isoflavones have been demonstrated to be in strong connection with testa colour (CHO *et al*., 2013; LEE *et al*., 2010; SLAVIN *et al*., 2009).

According to our results, there is quite difference in isoflavones contents among individual cultivars, as well as between black and yellow groups. Genistin was accumulated only in black cultivars, glycitin in 'NS Pantera', and daidzin in 'Fortuna' and 'Galina' (Table 3 and 4). Acetylgenistin was detected in all tested cultivars, but dominantly in 'NS Blackstar' (3 to 15-fold higher). Acetyldaidzin was detected in yellow cultivars, along with acetylglicitin, however the latter was accumulated in 'NS Pantera' in greater amount (approx. 3-fold) (Table 4). Similar to other authors (XU and CHANG, 2008a, 2008b), subtotal genistein derivatives content was the highest, followed with daidzein and glycitein derivatives. Cluster analysis of metabolic responses to various seed coats colours revealed that daidzein and genistein were tightly associated with each other but less with glycitein, irrespective of seed coat colour, since glycitein content is the least among total isoflavones (LEE *et al*. 2017). Our results showed that black varieties had more genistein derivatives, yellow had more daidzein derivatives, and as it was already mentioned 'NS Pantera' accumulated glycitein derivatives.

The innermost seed coat cell layer, the endothelium, is a metabolically active cell layer and is the main site of synthesis of proanthocyanidins (PAs). The PAs, oligomers of flavan-3-ol units, have received particular attention due to their abundance in seed coats (DIXON *et al*., 2005; ZHAO *et al*., 2010). PAs are also known as the chemical basis for tannins, polymeric flavonoids that comprise part of the broad and diverse group of phenolic compounds that plants produce as secondary metabolites (WINKEL-SHIRLEY, 2001).

Contrary to yellow, black cultivars had flavan-3-ols and antocyanins in their seeds (Table 3 and 4). 'NS Blackstar' accumulated higher content of all detected flavan-3-ols, almost double the amount determined in 'NS Pantera' (Table 4).

Of all detected antocyanins, cyanidin-3-rhamnoside was detected only in 'NS Blackstar' and pelargonidin-3-rutinoside in 'NS Pantera'. 'NS Blackstar' synthetized cyanidin and peonidin derivatives in higher content (2-3 fold) than 'NS Pantera' which was highlighted with pelargonidin derivatives in seed (Table 4). As it was confirmed earlier[26], cyanidin-glucoside was the major anthocyanin in black soybeans.

Syringic acid hexosides were mostly accumulated in black cultivars. Syringoyl-galactoside was dominantly synthetized in 'NS Blackstar', and syringoyl-glucoside in 'NS Pantera' and in lower amounts in tested yellow cultivars (Table 4). 'NS Pantera' had the highest content of eridictyol-hexoside. Eridictyol-rhamnoside was only detected in yellow cultivars, contrary to 'NS Blackstar', which had no flavanones in the seed.

Only black cultivars had flavonols: quercetin, laricitrin and isorhamnetin derivatives (Table 4). Except quercetin-glucoside which was equally presented, 'Blackstar' had higher amounts of all other flavonols in comparison to 'NS Pantera'. The same was with phloretin hexoside which was only detected in black cultivars, as well.

As previously determined by different authors (XU and CHANG, 2008a, 2008b; SHAHIDI and AMBIGAIPALAN, 2015; ŽILIĆ *et al.,* 2013), seed extracts showed high antioxidant capacities (over 80% neutralized radicals) and it was clear that black cultivars had higher antioxidant activity than yellow ones, due to higher total polyphenols content (Figure 4).

Lipid peroxidation represents a valuable marker of membrane degradation and it is measured as content of malondialdehyde (MDA). Comparing MDA content in leaves of investigated cultivars and our previous findings (MALENČIĆ *et al*. 2010), it is clear that plants did not suffer from intensive peroxidation of membrane lipids (Figure 2A). 'Galina' and 'NS Blackstar' leaves had higher MDA contents during the flowering and seed filling period, respectively, which points to important periods of oxidative stress in these plants. 'NS Pantera' and 'Fortuna' leaves had similar MDA content during investigated periods (Figure 2A).

During flowering period, all tested cultivars had similar PAL activity in leaves (Figure 2B), however during seed filling period activity of the enzyme intensified (13-16%). PAL is the entry-point enzyme into the phenylpropanoid pathway responsible for the synthesis of plant phenylpropanoids or phenolics (BIAŁA and JASIŃSKI, 2018). Higher PAL activity in seed filling period points to higher demand for synthesis of phenolic compounds.

Higher total polyphenols content in leaves of 'NS Blackstar' was due to higher total flavonoids content (Figure 3A), but leaves of 'NS Pantera' plants showed higher accumulation of proantocyanidins and antocyanins (Figure 3C and 3D).

Having higher total polyphenols content in leaves, 'NS Blackstar', 'Galina' and 'Fortuna' had higher antioxidant capacities than 'NS Pantera' (Figure 4). Nevertheless, all cultivars showed very high antioxidant capacity (over 70% neutralized radicals).

Table 3. Detected polyphenols in seeds of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina').

in variety	**$[M]^-$ *(m/z)***	**MS^2 $[M-H]^-$ *(m/z)***	***tentative identification***
B, P	577	289, 335	*procyanidin dimer*
B, P	865	739, 695, 575, 289	*procyanidin trimer*
B, P	1153	1027, 1135, 863, 701, 407	*procyanidin tetramer 1*
B, P	1153	1135, 863, 739, 425	*procyanidin tetramer 2*
B, P	335	289	*epicatechin*
P, F&G	449	287	*eriodictyol-hexoside*
F&G	433	287	*eriodiyctiol-rhamnoside*
B, P	507	345	*syringoyl-3-galactoside*
B, F&G	507	345	*syringoyl-3-glucoside*
B,P	431	269	*genistin*
B, P, F&G	473	431/269	*acetylgenistin*
F&G	415	253	*daidzin*
F&G	457	415/253	*acetyldaidzin*
P	445	268	*glycitin*
P, F&G	487	445/268	*acetyl glycitin*
B, P	463	301	*quercetin-3-galactoside*
B, P	463	301	*quercetin-3-glucoside*
B, P	609	301	*quercetin-3-rutinoside*
B	493	331	*laricitrin-3-galactoside*
B	493	331	*laricitrin-3-glucoside*
B, P	477	315	*isorhamnetin-hexoside 1*
B, P	477	315	*isorhamnetin-hexoside 2*
B, P	435	273	*phloretin hexoside*
in variety	**$[M]^+$ *(m/z)***	**MS^2 $[M-H]^-$ *(m/z)***	***tentative identification***
B, P	449	287	*cyanidin-3-galactoside*
B, P	449	287	*cyanidin-3-glucoside*
B, P	433	271	*pelargonidin-3-glucoside*
B, P	463	301	*peonidin-3-glucoside*
B	433	287	*cyanidin-3-rhamnoside*
P	579	433, 271	*pelargonidin-3-rutinoside*

Table 4. Individual polyphenols content in seeds of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina') (mg g^{-1} DW), mean ± standard error (SE). Values marked with the same letter, do not differ significantly at P<0.05 (Duncan's test), nd- not detected.

compound	**B**	**P**	**F**	**G**
procyanidin dimer	1.850±0.363[a]	1.347±0.146[a]	nd	nd
procyanidin trimer	0.621±0.106[a]	0.344±0.049[b]	nd	nd
procyanidin tetramer 1	0.041±0.016[a]	0.024±0.004[b]	nd	nd
procyanidin tetramer 2	0.220±0.067[a]	0.111±0.020[b]	nd	nd
epicatechin	0.222±0.038[a]	1.657±0.134[b]	nd	nd
eriodictyol-hexoside	nd	0.072±0.007[a]	0.005±0.001[b]	0.009±0.004[b]
eriodiyctiol-rhamnoside	nd	nd	0.005±0.000[a]	0.003±0.001[b]
syringoyl-3-galactoside	0.028±0.000[a]	0.009±0.003[b]	nd	nd
syringoyl-3-glucoside	0.033±0.002[b]	0.077±0.039[a]	0.001±0.000[c]	0.004±0.000[c]
genistin	0.185±0.006[a]	0.157±0.048[a]	nd	nd
acetylgenistin	0.291±0.004[a]	0.093±0.029[b]	0.022±0.005[c]	0.024±0.007[c]
daidzin	nd	nd	0.001±0.000	nd
acetyldaidzin	nd	nd	0.022±0.005[a]	0.024±0.007[a]
glycitin	nd	0.008±0.001	nd	nd
acetyl glycitin	nd	0.050±0.021[a]	0.016±0.009[b]	0.022±0.009[b]
quercetin-3-galactoside	0.075±0.010[a]	0.076±0.011[a]	nd	nd
quercetin-3-glucoside	0.127±0.021[a]	0.008±0.003[b]	nd	nd
quercetin-3-rutinoside	0.055±0.004[a]	0.033±0.008[b]	nd	nd
laricitin-3-galactoside	0.021±0.001[a]	0.005±0.002[b]	nd	nd
laricitin-3-glucoside	0.058±0.005[a]	0.038±0.007[b]	nd	nd
isorhamnetin-hexoside 1	0.027±0.001[a]	0.006±0.002[b]	nd	nd
isorhamnetin-hexoside 2	0.017±0.000	nd	nd	nd
phloretin hexoside	0.126±0.010[a]	0.048±0.024[b]	nd	nd
cyanidin-3-galactoside	0.255±0.110[a]	0.179±0.081[b]	nd	nd
cyanidin-3-glucoside	2.324±0.463[a]	1.170±0.109[b]	nd	nd
pelargonidin-3-glucoside	0.375±0.165[a]	0.414±0.158[a]	nd	nd
peonidin-3-glucoside	0.777±0.337[a]	0.254±0.113[b]	nd	nd
cyanidin-3-rhamnoside	0.098±0.047	nd	nd	nd
pelargonidin-3-rutinoside	nd	0.084±0.035	nd	nd

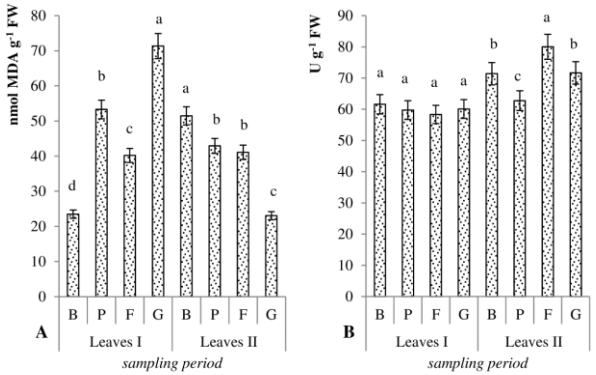

Figure 2. Lipid peroxidation intensity (A) and phenylalanine ammonialyase (A) in leaves of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina'), mean ± standard error (SE). Values marked with the same letter do not differ significantly at P<0.05 (Duncan's test).

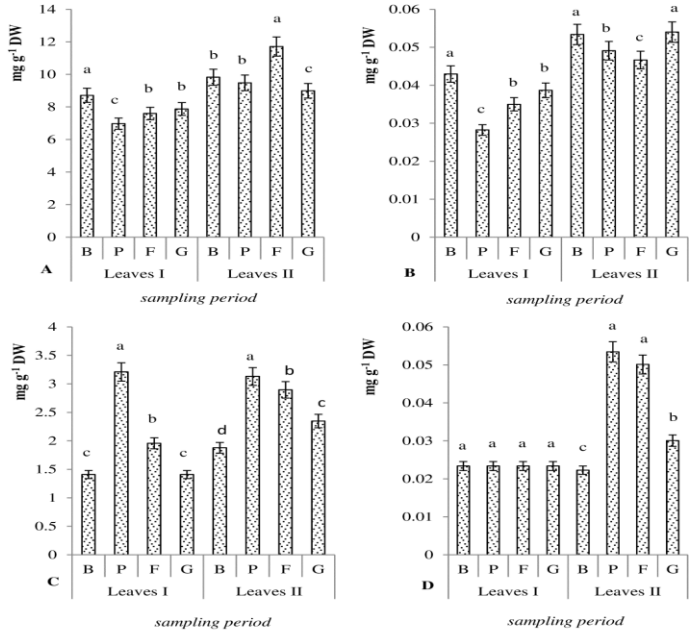

Figure 3. Total polyphenols (A), flavonoids (B), proanthocyanidins (C) and anthocyanins (D) in leaves of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina'), mean ± standard error (SE). Values marked with the same letter do not differ significantly at P<0.05 (Duncan's test).

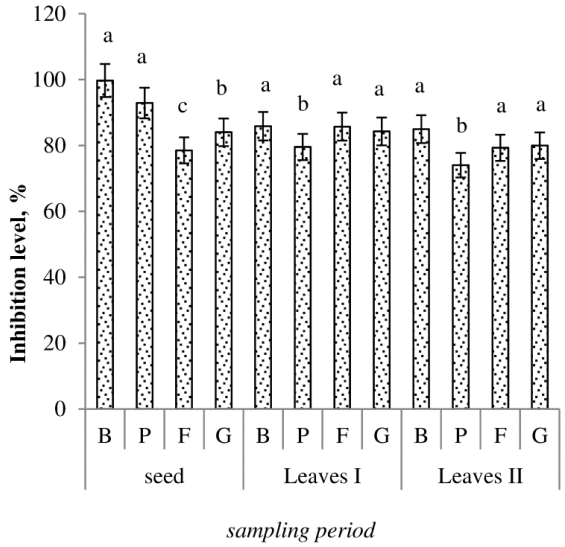

Figure 4. Antioxidant capacity of seeds and leaves of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina'), mean ± standard error (SE). Values marked with the same letter do not differ significantly at P<0.05 (Duncan's test).

When comparing our previous experiments with yellow soybean grown under organic conditions (KIPROVSKI *et al*., 2016), with obtained results of all investigated parameters in leaves, it could be concluded that plants had overall favorable conditions for development. According to environmental conditions report (Figure 1), 2015 and 2016 had similar temperature regime. In both years, plants had enough water supplies, as well as favorable temperature, for growth. However, in the vegetation and R5 periods of both years, plants were exposed to heavy rains which flooded the field. In 2015, as well as in recent years, excessive precipitation in August prolong harvest of early varieties from 00 and 0 maturity groups, which leads to lower yield due to dispersal of seeds. Yield parameters were presented in Table 5.

Table 5. Yield of soybean varieties coloured black (B-'NS Blackstar' and P-'NS Pantera') and yellow (F-'Fortuna' and G-'Galina') in 2015 and 2016 (kg ha^{-1}). Values marked with the same letter, do not differ significantly at P<0.05 (Duncan's test).

variety	***Year***	
	2015	***2016***
B	1629^{d}	3190^{c}
P	3025^{a}	3230^{c}
F	1825^{c}	3550^{b}
G	2975^{b}	3780^{a}

CONCLUSIONS

Bearing in mind all differences in seed quality, as well as response to oxidative stresses during development, black cultivars could be proposed as an excellent source of phenolic compounds, along with nutrients, however, the impact of phenolics in seeds are not crucial for the yield of soybean cultivars. Owing to its biochemical composition, 'NS Pantera' could be proposed as valuable source of phenolics and other nutrients. Due to extreme fluctuation in precipitation amounts in the last years, information on the better performance of soybean varieties in oxidative stress conditions is of great importance to organic and conventional production of this cultivar.

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REFERENCES

AACC, INTERNATIONAL APPROVED METHODS OF ANALYSIS (1978): AACCI Method 80-60.01determination of reducing and nonreducing sugars. 11th Ed. St. Paul, MN.

BALISTEIRO, D.M., C.V., ROMBALDI, M.I., GENOVESE (2013): Protein, isoflavones, trypsin inhibitory and *in vitro* antioxidant capacities: Comparison among conventionally and organically grown soybeans. Food Res. Int., *51*(1): 8-14.

BIAŁA, W., M., JASIŃSKI (2018): The Phenylpropanoid case–it is transport that matters. Front. Plant Sci., *9*.

CHO, K.M., T.J., HA, Y.B., LEE, W.D., SEO, J.Y., KIM, H.W., RYU, S.H., JEONG, Y.M., KANG, J.H., LEE (2013): Soluble phenolics and antioxidant properties of soybean (*Glycine max* L.) cultivars with varying seed coat colours. J. Funct. Foods, *5*(3): 1065-1076.

DIXIT, A.K., V., KUMAR, A., RANI, J.G., MANJAYA, D., BHATNAGAR (2011): Effect of gamma irradiation on lipoxygenases, trypsin inhibitor, raffinose family oligosaccharides and nutritional factors of different seed coat colored soybean (*Glycine max* L.). Radiat. Phys. Chem., *80*(4): 597-603.

DIXON, R.A., D.Y., XIE, S.B., SHARMA (2005): Proanthocyanidins–a final frontier in flavonoid research? New Phytologist, *165*(1): 9-28.

GERASIMOVA, N.G., S.M., PRIDVOROVA, O.L., OZERETSKOVSKAYA (2005): Role of L-phenylalanine ammonia Lyase in the induced resistance and susceptibility of potato plants. Appl. Biochem. Microbiol., *41*(1): 103-105.

JAMBUNATHAN, N. (2010): Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. In: Sunkar R, editor.Plant stress tolerance: methods and protocols. Humana press, Springer, p. 291-297.

KIM, S.L., H.Y., CHI, J.R., SON, N.K., PARK, S.N., RYU (2005): Physicochemical characteristics of soybean seed coat and their relationship to seed lustre. Korean J. Crop Sci., *50*(spc1): 123-131.

KIPROVSKI, B., DJ., MALENČIĆ, S., ĐURIĆ, M., BURSAĆ, J., CVEJIĆ, V., SIKORA (2016): Isoflavone content and antioxidant activity of soybean inoculated with plant-growth promoting rhizobacteria. J. Serb. Chem. Soc., *81*(11):1239-1249.

KRANNER, I., F.V., MINIBAYEVA, R.P., BECKETT, C.E., SEAL (2010): What is stress? Concepts, definitions and applications in seed science. New Phytologist., *188*(3): 655-673.

LEE, J., R.W., DURST, R.E., WROLSTAD (2005): Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Int., *88*: 1269-1278.

LEE, J., Y.S., HWANG, S.T., KIM, W.B., YOON, W.Y., HAN, I.K., KANG, M.G., CHOUNG (2017): Seed coat color and seed weight contribute differential responses of targeted metabolites in soybean seeds. Food Chem., *214*: 248-258.

LEE, S.J., P., SEGUIN, J.J., KIM, H.I., MOON, H.M., RO, E.H., KIM, S.H., SEO, E.Y., KANG, J.K., AHN, I.M. CHUNG (2010): Isoflavones in Korean soybeans differing in seed coat and cotyledon color. J. Food Composit. Anal., *23*(2): 160-165.

MAKKAR, H.P.S. (2003): Quantification of tannins in tree and shrub foliage: a laboratory manual. The Netherlands: Springer Kluwer Academic Publishers, p. 49-53.

MALENČIĆ, DJ., B., KIPROVSKI, M., POPOVIĆ, D., PRVULOVIĆ, J., MILADINOVIĆ, V., DJORDJEVIĆ (2010): Changes in antioxidant systems in soybean as affected by *Sclerotinia sclerotiorum* (Lib.) de Bary. Plant Physiol. Biochem., *48*(10): 903-908.

MIKULIČ-PETKOVŠEK, M., A., SLATNAR, F., ŠTAMPAR, R., VEBERIČ (2012): HPLC-MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species. Food Chem., *135*: 2138-2146.

PANDA, S.K. (2012): Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. In: El-Missiry MA, editor. Biochemistry, Genetics and Molecular Biology. 'Antioxidant Enzyme': InTech.

PĘKAL, A., K., PYRZYNSKA (2014): Evaluation of aluminium complexation reaction for flavonoid content assay. Food Anal. Methods, *7*: 1776-782.

SHAHIDI, F., P., AMBIGAIPALAN (2015): Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. J. Funct. Foods, *18*: 820-897.

SLAVIN, M., W., KENWORTHY, L., YU (2009): Antioxidant properties, phytochemical composition, and antiproliferative activity of Maryland-grown soybeans with colored seed coats. J. Agri. Food Chem., *57*(23): 11174-11185.

SMÝKAL, P., V., VERNOUD, M.W., BLAIR, A., SOUKUP, R.D., THOMPSON (2014): The role of the testa during development and in establishment of dormancy of the legume seed. Front. Plant Sci., *5*: 351.

SRPS ISO 6541:1996 Agricultural food products - Determination of crude fibre content - Modified Scharrer method (identical with ISO 6541:1981)

SRPS ISO 659:2011 Oilseed – Determination of oil content (Reference method) (identical with ISO 659:2009)

WILCOX, J.R., R.M., SHIBLES (2001): Interrelationships among seed quality attributes in soybean. Crop Sci., *41*(1): 11-14.

WINKEL-SHIRLEY, B. (2001): Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol., *126*(2): 485-493.

WU, K., S., XIAO, Q., CHEN, Q., WANG, Y., ZHANG, K., LI, Y., YU, L., CHEN (2013): Changes in the activity and transcription of antioxidant enzymes in response to Al stress in black soybeans. Plant Mol. Biol. Report, *31*(1): 141-150.

XU, B., S.K., CHANG (2008a): Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota− Minnesota region. J. Agri. Food Chem., *56*(19): 9102-9113.

XU, B., S.K., CHANG (2008b): Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones. J. Agri. Food Chem., *56*(18): 8365-8373.

YUGOSLAV PHARMACOPOEIA (Ph. Jug. V) (2000): Pharmacopoeia (5th ed.), Adjusted translation of European Pharmacopoeia 1997 (Ph. Eur. III), Kovac M, editor. Belgrade, Serbia: Federal Institute for Health Protection and Improvement.

ZHANG, T., K., KAWABATA, R., KITANO, H., ASHIDA (2013): Preventive effects of black soybean seed coat polyphenols against DNA damage in *Salmonella typhimurium*. Food Sci. Tech. Res., *19*(4): 685-690.

ZHAO, J., Y., PANG, R.A., DIXON (2010): The mysteries of proanthocyanidin transport and polymerization. Plant Physiol., *153*(2): 437-443

ZHOU, S., H., SEKIZAKI, Z., YANG, S., SAWA, J., PAN (2010): Phenolics in the seed coat of wild soybean (*Glycine soja*) and their significance for seed hardness and seed germination. J. Agri. Food Chem., *58*(20): 10972-10978.

ŽILIĆ, S., A., SERPEN, G., AKILLIOĞLU, V., PERIĆ, V., GÖKMEN (2013): Comparisons of phenolic compounds, isoflavones, antioxidant capacity and oxidative enzymes in yellow and black soybeans seed coat and dehulled bean. Eur. Food Res. Technol., *237*(3): 409-418.

CRNA I ŽUTA SOJA: DOPRINOS KVALITETA SEMENA ODGOVORU BILJKE NA OKSIDATIVNI STRES U RAZLIČITIM FAZAMA RAZVOJA

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