

# The diversity of secondary metabolites in Indonesian soybean genotypes

ERİYANTO YUSNAWAN

Indonesian Legumes and Tuber Crops Research Institute. Jl. Raya Kendalpayak Km 8, Po Box 66, Malang, East Java, Indonesia. Tel.: +62-341-801468, Fax.: +62-341-801496. email: eyusnawan@litbang.pertanian.go.id

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**Abstract.** Yusnawan E. 2016. *The diversity of secondary metabolites in Indonesian soybean genotypes. Biodiversitas 17: 704-710.* Soybean secondary metabolites protect the crop from pathogen infection. These secondary metabolites especially phenolic compounds also have functional properties. This study aimed to determine total phenolic and flavonoid contents as well as antioxidant activity of Indonesian soybean genotypes. A total of 63 soybean genotypes with four different seed coat colors (green yellow, light yellow, yellow, and black) and different seed sizes (small, medium and large) was used in this study. Total phenolic content was measured using Folin-Ciocalteu's reagent and antioxidant activity was determined with 2,2-diphenyl-1-picrylhydrazyl. All six genotypes with black seed coat color had higher total phenolic and flavonoid contents as well as antioxidant activity than those in other colors' genotypes. Total phenolic contents of those six black soybeans ranged from 7.19 to 14.72 mg gallic acid equivalent per gram and total flavonoid contents varied from 1.91 to 5.30 mg catechin equivalent per gram. Antioxidant activities of these genotypes ranged from 10.99 to 20.38  $\mu$ mol trolox equivalent per gram. An estimation of the total phenolic contents as well as antioxidant activities particularly in black soybeans was valuable and important for seeking soybeans with high antioxidant properties.

**Keywords:** Antioxidant, flavonoid, phenolic, soybean genotype

**Abbreviations:** AA = antioxidant activity, DPPH = 2,2-diphenyl-1-picrylhydrazyl, GAE = gallic acid equivalent, CE = catechin equivalent, TE = Trolox equivalent.

## INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the legume crops which is rich in phytochemical compounds such as flavonoids, phenolic acids, saponins, and triterpenoids (Lee et al. 2009, 2011; Lee and Cho 2012; Zilic et al. 2013). These plant secondary metabolites protect soybean crops from pathogen infections, such as *Macrophomina phaseolina*, *Phytophthora sojae*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Phakopsora pachyrhizi* (Lygin et al. 2009; 2013; Bellaloui et al. 2012). Phenylpropanoids are known to be responsible during plant-pathogen interactions and have toxic properties or inhibitory to pathogens when either constitutively formed (phytoanticipins) or induced (phytoalexins) (Lygin et al. 2013).

Apart from inhibiting the plant pathogens in chemical defense mechanisms, the secondary metabolites in soybean especially soluble phenolic compounds of isoflavones and anthocyanins have functional and nutritional properties. In most cases, the availability of soluble phenolic compounds is dominant, therefore, contributing higher antioxidant properties than insoluble forms (John and Shahidi 2010; Cho et al. 2013). Flavonoids in soybean particularly isoflavones possess health benefits such as antioxidant, anticancer, antiosteoporosis, antibacterial, and anti-mutagenic (Messina 1999; Gallagher 2001; Kumar et al. 2010; Dixit et al. 2012). As antioxidant, soybean seed extract, genistein and daidzein show scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Malencic et al. 2007; Riedl et al. 2007). Anthocyanins as a dominant compound in black seed coat of soybean also contribute

significant functional properties including antioxidant properties, anticancer, anti-inflammatory as well as cardiovascular disease prevention (He and Giusti 2010; Zhang et al. 2011; Lee and Cho 2012).

Concentration and composition of phytochemicals in legumes vary significantly based on the seed coat colors (Lee et al. 2010; Segev et al. 2010; Zilic et al. 2013). Several seed coat colors of green, brown, black, and yellow are found in soybeans with different hylum color and seed size (Messina 1999; PVT 2007). The role of phenolics in the seed coat is to provide resistance against fungal pathogens and pest insects, to regulate impermeability to water, and to maintain cell integrity (Moise et al. 2005; Bellaloui et al. 2012).

Overall, soluble phenolic compounds function as initial plant defense against pathogen infection as well as contribute to health benefits (Messina 1999; Lee et al. 2011; Ng et al. 2011; Lygin et al. 2013). This study therefore, focused on the soluble phenolics in soybean seeds. Several studies have investigated soluble phenolics and antioxidant properties of soybean genotypes with varying seed coat colors (Kumar et al. 2010; Zhang et al. 2011; Malencic et al. 2012; Cho et al. 2013; Zilic et al. 2013), however, only few employing different seed sizes. A study on quantifying several secondary metabolites including total phenolic, total flavonoid as well as antioxidant activity in Indonesian soybean germplasm have not been conducted yet. Nine different seed coat colors are described in Indonesian soybean genotypes (PVT 2007). However, only four different seed coat colors were studied since genotypes with those seed coat colors were

commonly cultivated. This study aimed to determine and to compare total phenolic and flavonoid contents as well as antioxidant activity of 63 Indonesian soybean genotypes with different seed coat colors and seed sizes.

## MATERIALS AND METHODS

### Plant materials

Soybean seeds (*G. max*) as many as 63 genotypes with four different seed coat colors (green yellow, light yellow, yellow, and black) and seed sizes (small, medium and large) were obtained from germplasm collection of Indonesian Legumes and Tuber Crops Research Institute (ILETRI). As many as 50 g of each genotype was taken from the collection (moisture content < 9%). Each soybean genotype characteristic is presented in Table 1.

### Chemicals

Gallic acid, catechin, trolox, Folin-Ciocalteu, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma Aldrich (St. Louis, MO, USA). Aluminum chloride, sodium nitrite, sodium hydroxide, sodium carbonate, acetone, ethanol were pro analysis grade from Merck (Darmstadt, Germany).

### Sample preparation and extraction

Intact dried soybean (50 g) were ground using a sample mill (Cyclotec sample mill, Sweden) into fine particles (< 80 mesh) of soybean flour. The flour was kept in sealed plastic bags and stored at -20 °C before used. Moisture content was determined before secondary metabolite analysis to estimate dry weight of the sample.

Extraction was conducted according to Xu and Chang (2007, 2008a) with slight modification. Briefly, the soybean flour was dissolved in 50% acetone (1:10 v v<sup>-1</sup>) for estimating total flavonoid and phenolic contents and in 70% ethanol (1:10 v v<sup>-1</sup>) for studying antioxidant activity. All extraction was conducted in triplicate. Samples were placed on an orbital shaker at 200 rpm (Stuart Scientific, UK) for 2 h at room temperature. After incubation for 18 h in the dark room, the samples were centrifuged (Beckman Allegra 21R, US) at 3000 rpm for 10 min. The same procedure was repeated and the supernatants were combined (total volume ± 10 mL) and stored in amber vials at 4°C.

### Determination of total phenolic content

Folin-Ciocalteu assay according to Singleton et al. (1999); Xu and Chang (2007) was used for estimating total phenolic content in the soybean flour extract. A certain volume of soybean extract was added to distilled water (1:60 v v<sup>-1</sup>), 250 µL of Folin-Ciocalteu's reagent, and 750 µL of sodium carbonate. The mixture was vortexed and incubated for 8 min at room temperature before 950 µL of distilled water was added. The final mixture was incubated for 2 h in the dark at room temperature. The absorbance values were read using a spectrophotometer (Genesys 10s, US) at 765 nm. A linear concentration of gallic acid at 12.5 to 800 µg mL<sup>-1</sup> was used as a standard (r = 0.999). The

**Table 1.** Seed coat color, 100 seed weight, and seed size of 63 Indonesian soybean genotypes (ILETRI 2004, 2015)

Genotype ID	Seed coat color	Hylum color	Weight/100 seeds (g)	Seed size category <sup>a</sup>
MLGG 0029	Yellow	Dark brown	13.0	Medium
MLGG 0030	Yellow	Light brown	12.5	Medium
MLGG 0031	Yellow	Dark brown	8.0	Small
MLGG 0032	Yellow	Brown	10.5	Medium
MLGG 0033	Yellow	Brown	10.5	Medium
MLGG 0096	Yellow	Dark brown	10.0	Medium
MLGG 0099	Yellow	Dark brown	10.0	Medium
MLGG 0100	Black	Black	7.5	Small
MLGG 0101	Yellow	Dark brown	8.0	Small
MLGG 0102	Black	Black	8.0	Small
MLGG 0111	Light yellow	Light brown	12.0	Medium
MLGG 0464	Green yellow	Dark brown	7.0	Small
MLGG 0533	Yellow	Brown	13.0	Medium
MLGG 0534	Green yellow	Dark brown	7.5	Small
MLGG 0669	Yellow	Brown	13.5	Medium
MLGG 0681	Green yellow	Dark brown	7.0	Small
MLGG 07xx	Light yellow	Light brown	13.0	Medium
MLGG 0747	Light yellow	Brown	8.3	Small
MLGG 0795	Yellow	Brown	11.0	Medium
MLGG 0796	Yellow	Brown	10.0	Medium
MLGG 0801	Yellow	Black	10.0	Medium
MLGG 0805	Light yellow	Brown	15.0	Large
MLGG 1053	Green yellow	Brown	10.0	Medium
MLGG 1054	Yellow	Dark brown	9.6	Small
MLGG 1058	Light yellow	Dark brown	8.5	Small
MLGG 1059	Yellow	Brown	8.0	Small
MLGG 1061	Black	White	11.5	Medium
MLGG 1062	Yellow	Dark brown	10.0	Medium
MLGG 1063	Yellow	Brown	12.0	Medium
MLGG 1064	Yellow	Light brown	12.0	Medium
MLGG 1065	Yellow	Black	12.0	Medium
MLGG 1066	Yellow	Brown	12.5	Medium
MLGG 1067	Yellow	Brown	10.0	Medium
MLGG 1068	Yellow	Brown	10.5	Medium
MLGG 1069	Yellow	Light brown	12.0	Medium
MLGG 1070	Yellow	Brown	10.6	Medium
MLGG 1071	Yellow	White	16.0	Large
MLGG 1073	Yellow	Yellow	17.0	Large
MLGG 1075	Yellow	Brown	10.7	Medium
MLGG 1076	Yellow	Brown	10.4	Medium
MLGG 1077	Yellow	Dark brown	11.0	Medium
MLGG 1078	Yellow	Brown	11.5	Medium
MLGG 1078	Yellow	Brown	12.5	Medium
MLGG 1080	Yellow	Light brown	16.8	Large
MLGG 1081	Yellow	Light brown	15.1	Large
MLGG 1082	Yellow	Light brown	10.5	Medium
MLGG 1083	Green yellow	Light brown	9.1	Small
MLGG 1085	Yellow	Light brown	16.0	Large
MLGG 1086	Yellow	Brown	11.2	Medium
MLGG 1087	Light yellow	Dark brown	18.5	Large
MLGG 1088	Green yellow	Dark brown	9.5	Small
MLGG 1089	Green yellow	Dark brown	10.5	Medium
MLGG 1091	Green yellow	Brown	15.8	Large
MLGG 1092	Yellow	Light brown	17.8	Large
MLGG 1093	Yellow	Black	23.2	Large
MLGG 1094	Green yellow	Brown	6.8	Small
MLGG 1095	Green yellow	Brown	8.3	Small
MLGG 1096	Light yellow	Brown	11.9	Medium
MLGG 1097	Black	White	14.8	Large
MLGG 1098	Black	Brown	13.5	Medium
MLGG 1099	Yellow	Brown	18.0	Large
MLGG 1100	Black	Light brown	9.5	Small
MLGG 1103	Yellow	Dark brown	10.7	Medium

Note: <sup>a</sup> seeds size category was grouped according to PVT (2007)

total phenolic content was expressed as gallic acid equivalents per gram of sample (mg GAE g<sup>-1</sup> sample) based on dry basis.

#### Determination of total flavonoid content

Total flavonoid content was estimated according to Heimler et al. (2005); Xu and Chang (2007). To 2500 µL of distilled water, aliquot of 500 µL of soybean extract was added in a glass tube and thoroughly mixed using a vortex. Then, 150 µL of 5% NaNO<sub>2</sub> solution was added and incubated for 6 min at room temperature. Another 5 min incubation was conducted after adding 300 µL of aluminum chloride into the mixed solution. A solution of 1 M sodium hydroxide as many as 1000 µL was added into the mixture. The final volume was brought to 5000 µL with distilled water and mixed thoroughly. The absorbance values against blank were read at 510 nm using a spectrophotometer. The results were expressed as catechin equivalents per gram of sample (mg CE g<sup>-1</sup> sample) based on dry basis using the calibration curve of the catechin. A linear range of 12.5 to 400 µg mL<sup>-1</sup> was used as a calibration curve ( $r = 0.999$ ).

#### DPPH free radical scavenging activity

DPPH scavenging activity or antioxidant activity of soybean extract was determined according to Xu and Chang (2008a, b). Aliquot of soybean extract was mixed with 0.1 mM ethanolic DPPH solution (1:19 v v<sup>-1</sup>). The solution was vortexed and incubated in the dark room for 30 min at room temperature. The absorbance values of sample ( $A_{\text{sample}}$ ) and blank ( $A_{\text{control}}$ ) were recorded using spectrophotometer at 515 nm. The radical scavenging activity as reflected by the percent DPPH discoloration was calculated as follows: percent discoloration =  $[1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$ .  $A_{\text{control}}$  was recorded after the DPPH solution was reacted with the extraction solvent. The DPPH scavenging activity of each sample was expressed as micromoles of Trolox equivalent per gram of sample (µmol TE g<sup>-1</sup> sample).

## RESULTS AND DISCUSSION

#### Total phenolic content in soybean genotypes

Total phenolic contents of soybean genotypes between four different seed coat color groups were significantly different ( $p < 0.05$ ), showing seed coat color based genotypic variation among them (Table 2). However, no significant difference was observed in three seed size groups (small, medium and large) ( $p > 0.05$ ). Black soybean genotypes showed higher total phenolic contents than those in green yellow, light yellow and yellow soybeans. Total phenolic contents among 63 soybean genotypes varied from 3.49 to 14.72 mg GAE g<sup>-1</sup> (Figure 1). The genotype of MLGG 0030 with yellow seed coat had the lowest ( $3.49 \pm 0.03$  mg GAE g<sup>-1</sup>), whereas MLGG 1098 with black seed coat had the highest phenolic contents ( $14.72 \pm 0.07$  mg GAE g<sup>-1</sup>). Significant genotypic variations ( $p < 0.05$ ) of soybeans within different seed coat

color groups were also investigated by Xu and Chang (2007, 2008c) as well as Kumar et al. (2010), in which black soybean genotypes expressed the highest phenolic contents.

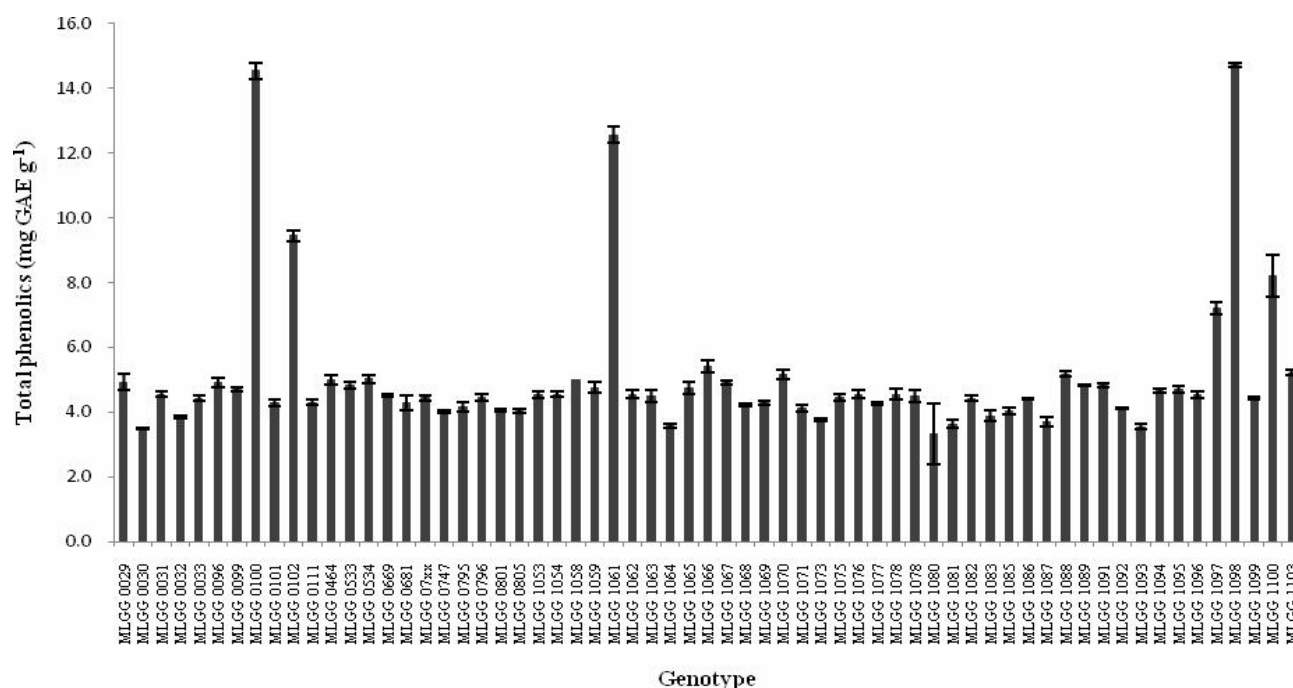
Six genotypes with black seed coat color, i.e. MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097, MLGG 1098, and MLGG 1100 were rich in total phenolic contents (from 7.19 to 14.72 mg GAE g<sup>-1</sup>). Results suggested that seed coat contributed higher phenolic contents than those in embryonic axis and cotyledon (Jeng et al. 2010). Higher phenolic contents in black soybean compared to yellow soybeans were also reported by Xu and Chang (2007, 2008a), where the variability among Indonesian soybeans was 2.05-fold in those six genotypes. However, more variation among genotypes up to 7.27-fold was observed in six Indian black soybeans (Kumar et al. 2010). Genotypic variations of black soybeans were also found in three Taiwan black soybean varieties, where these varieties contained total phenolics ranged from 4.38 to 7.49 mg GAE g<sup>-1</sup> (Jeng et al. 2010). The two varieties containing  $7.05 \pm 0.50$  mg GAE g<sup>-1</sup> and  $7.49 \pm 0.39$  mg GAE g<sup>-1</sup> of total phenolics (Jeng et al. 2010) were similar to MLGG 1097 ( $7.19 \pm 0.19$  mg GAE g<sup>-1</sup>) and MLGG 1100 ( $8.22 \pm 0.64$  mg GAE g<sup>-1</sup>) determined in this study. Different extraction and quantification methods may attribute to different phenolic measured, apart from different soybean genotypes used in the study as reported by Zilic et al. (2013).

Of the green yellow, light yellow and yellow seed color genotype groups, no difference of total phenolic contents was observed (Table 2). The ranges of total phenolic contents of these three seed coat classes were 3.89 – 5.17, 3.70 – 5.02 and 3.49 – 5.42 mg GAE g<sup>-1</sup>, respectively. Higher range of total phenolic contents of yellow soybeans was found in Indonesian genotypes compared to that of Indian genotypes, which was 1.06 – 1.54 mg GAE g<sup>-1</sup> (Kumar et al. 2010). Average of total phenolic contents of green yellow soybean genotypes ( $4.70 \pm 0.38$  mg GAE g<sup>-1</sup>) in this study was also higher than total phenolic content of green soybean ( $3.46 \pm 0.09$  mg GAE g<sup>-1</sup>) as observed by Malencic et al. (2012).

**Table 2.** Average values of total phenolic, flavonoid contents, and DPPH scavenging activity of 63 soybeans with different seed coat colors and different seed size

Seed coat color	Total phenolic content (mg GAE g <sup>-1</sup> )	Total flavonoid content (mg CE g <sup>-1</sup> )	Antioxidant activity (µmol TE g <sup>-1</sup> )
Black (n = 6)	11.11 ± 3.27 a	3.47 ± 1.50 a	15.58 ± 4.53 a
Green yellow (n = 10)	4.70 ± 0.38 b	0.34 ± 0.05 b	6.68 ± 0.75 b
Light yellow (n = 7)	4.29 ± 0.43 b	0.36 ± 0.04 b	7.21 ± 1.01 b
Yellow (n = 40)	4.39 ± 0.46 b	0.34 ± 0.06 b	6.78 ± 0.77 b

Note: n = number of genotypes with the same seed coat color. Values followed by the same number in the same column were not significantly different based on the Duncan test ( $p < 0.05$ ).



**Figure 1.** Total phenolic contents of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.

### Total flavonoid content in soybean genotypes

All soybean genotypes with black seed coat accumulated more flavonoid contents than green yellow, light yellow and yellow genotypes and the difference among these groups was significant ( $p < 0.05$ ). However, no difference in total flavonoids among green yellow, light yellow and yellow soybean genotypes was found (Table 2). No difference was also observed in the three seed size groups ( $p > 0.05$ ). The average total flavonoid contents in Indonesian black soybeans was higher than that in black soybeans ( $2.57 \pm 0.03$  mg CE g<sup>-1</sup>) as investigated by Xu and Chang (2007). However, the yellow soybeans in this present study did not exhibit so high total flavonoid value (1.47-fold) as reported by Xu and Chang (2007). This finding clearly showed that genotypic variation within seed coat color group may be responsible for different flavonoid contents. Environmental factors such as geographic condition, light, temperature, planting year, and soil moisture also contributed to the difference of the distribution and concentration of bioactive compounds (Lee et al. 2003; Lee and Cho 2012).

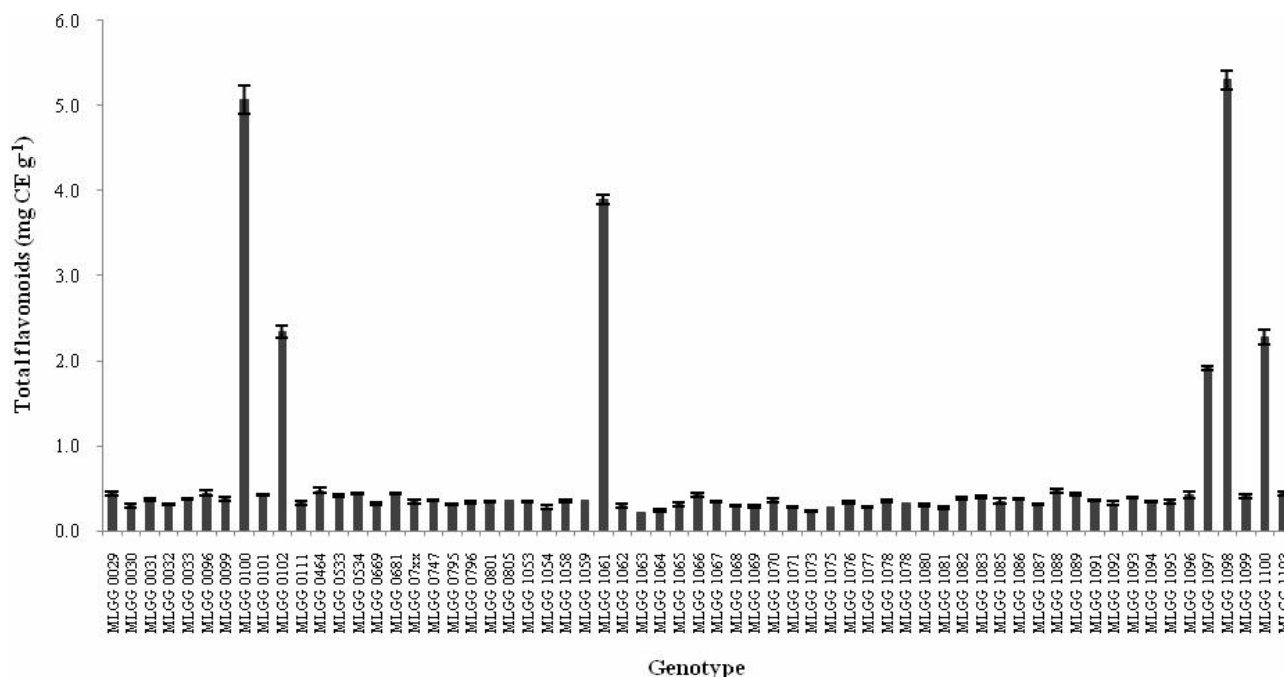
Major groups of flavonoids accumulated in seeds were anthocyanins, proanthocyanidins, and isoflavones (Lepiniec et al. 2006). Anthocyanins and proanthocyanidins were more concentrated in black seed coat of soybeans (Xu and Chang 2008b; Lee et al. 2009; Jeng et al. 2010). However, anthocyanins were not detected in yellow and green seed coats of many soybean genotypes (Cho et al. 2013). In whole green and yellow soybeans, isoflavones are a group of compounds which are responsible for the high total flavonoid contents (Cho et al. 2013; Zilic et al. 2013). Total isoflavones in different seed coat color of

soybeans from the highest to the lowest followed the order of green > yellow > black > brown soybean genotypes (Cho et al. 2013). However, no variation among green yellow and yellow soybean genotypes was observed in the present study, suggesting other compounds than isoflavones may also contribute to the total flavonoid contents. In fact, carotenoids and luteins were reported high in green and yellow soybeans (monma et al. 1994; Kumar et al. 2010).

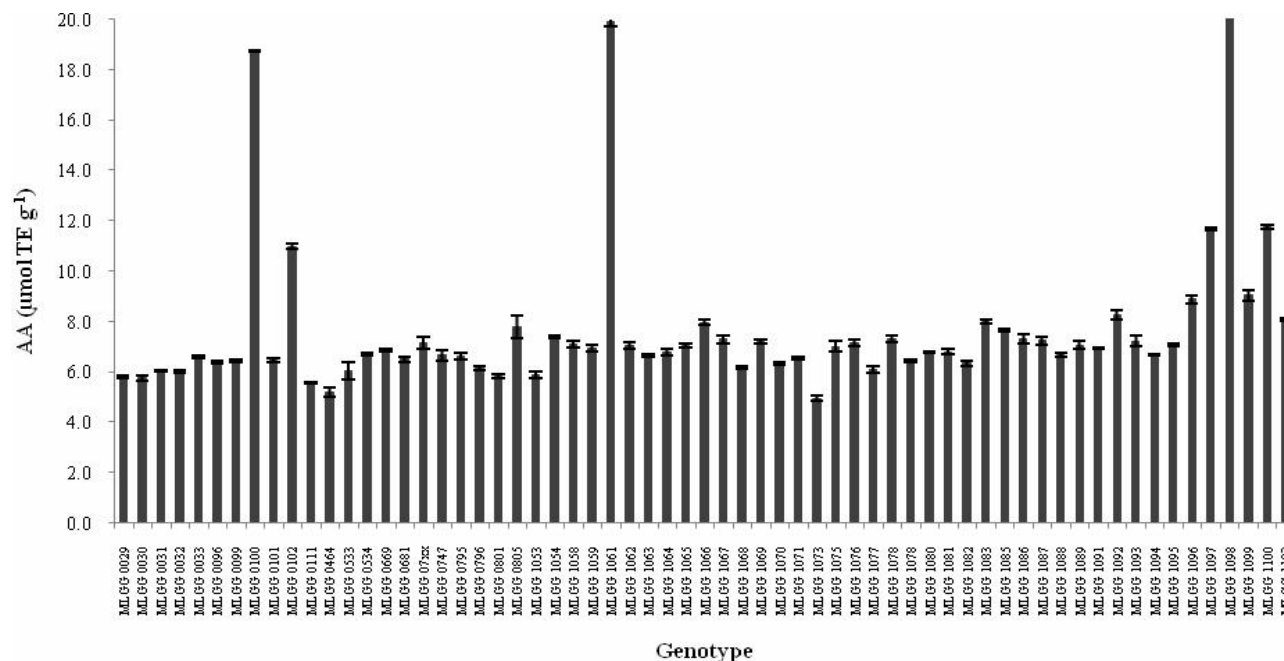
Total flavonoids of 63 soybean genotypes were in the range from 0.22 to 5.30 mg CE g<sup>-1</sup> (Figure 2). As observed in total phenolic contents, black soybean genotypes consistently also contained high flavonoid compared to yellow soybeans. MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097, MLGG 1098, and MLGG 1100 with black seed coat had 1.91 to 5.30 mg CE g<sup>-1</sup> of total flavonoid contents. Unlike black soybeans, higher flavonoid contents in yellow, light yellow and green yellow soybean genotypes did not always contain higher phenolic contents. Total flavonoid contents of the yellow, light yellow, and green yellow seed color were from 0.22 to 0.48 mg CE g<sup>-1</sup>. It is difficult to compare the flavonoid contents in this study to the previous studies since a different flavonoid standard was utilized to estimate total flavonoid equivalent (Malencic et al. 2012). However, a trend of black soybeans contained more flavonoids than those in yellow soybeans were in agreement.

### DPPH scavenging activity in soybean genotypes

Genotypes with black seed coat possessed significantly higher DPPH scavenging activity than yellow soybean groups ( $p < 0.05$ ) (Table 2). However, no significant



**Figure 2.** Total flavonoid contents of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.



**Figure 3.** Antioxidant activity (AA) of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.

difference was found within three seed sizes ( $p > 0.05$ ). The DPPH values of the antioxidant extracts of black soybean genotypes ranged from 10.99 to 20.38  $\mu\text{mol TE g}^{-1}$  for MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097,

MLGG 1098, and MLGG 1100 (Figure 3). The average DPPH value of black soybean genotypes ( $15.58 \pm 4.53 \mu\text{mol TE g}^{-1}$ ) was lower than in another study (Xu and Chang 2007), which was  $17.93 \pm 0.03 \mu\text{mol TE g}^{-1}$ .

However, higher antioxidant activity of black soybeans in comparison to other seed color groups was in agreement with a study conducted by Zhang et al. (2011). In their study, high antioxidant activities were noted with DPPH, oxygen radical absorbance capacity (ORAC) as well as ferric reducing antioxidant power (FRAP) methods (Zhang et al. 2011). This high antioxidant activity in dark seed coat of soybeans could possibly due to high polymerized procyanidin and anthocyanin contents (Takahata et al. 2001).

The green yellow, light yellow and yellow soybean genotypes possessed DPPH scavenging activities from 4.97 to 9.04  $\mu\text{mol TE g}^{-1}$ . The average antioxidant activity of the yellow soybeans was 3.5-fold higher than that DPPH scavenging activity observed in Xu and Chang (2007) study. The reason may be that phenolic compounds such as isoflavones and ferulic acid were dominant in cotyledons and embryo contributing to high antioxidant activity as observed by Zilic et al. (2013).

Another contributing reason could be that the DPPH scavenging activity of the four groups of soybean with different seed coat colors may strongly correlate with isoflavones and anthocyanins as previously described (Cho et al. 2013). However, the scavenging activity against DPPH of isoflavones was less than procyanidins and anthocyanins (Takahata et al. 2001; Jeng et al. 2010).

#### Correlations between total phenolic, flavonoid contents and DPPH scavenging activity

Correlation analyses between total phenolics, total flavonoids and DPPH scavenging activity among all soybean genotypes were also determined (Table 3). Black soybeans exhibited significant ( $p < 0.01$ ) linear correlations between total phenolic content and total flavonoid content ( $r = 0.99$ ), total phenolic content and DPPH scavenging activity ( $r = 0.92$ ), and total flavonoid content and DPPH scavenging activity ( $r = 0.93$ ). These results confirmed the previous findings that the three parameters were significantly correlated (Xu and Chang 2007). Linear correlations among total phenolics, flavonoid contents and DPPH activity of black soybeans were also investigated by Jeng et al. (2010) and Kumar et al. (2010).

Unlike black soybeans, a significant linear correlation ( $p < 0.01$ ) in yellow soybeans was only observed in total phenolic contents and total flavonoid contents ( $r = 0.57$ ). This correlation was not surprising because flavonoids are a large group of phenolic compounds (Jeng et al. 2010). However, this finding was not in agreement with results reported by Xu and Chang (2007). These authors mentioned that linear correlations among the three parameters were not only observed in black soybeans, but also noted in yellow soybeans. Other different antioxidant activity assays such as ORAC and FRAP could possibly be conducted to obtain better understanding of the correlations among the three parameters in this present study.

Flavonoids including anthocyanins, proanthocyanidins and isoflavones found in soybeans are most likely responsible for the antioxidant activities in the tested samples (Lee et al. 2005; McGhie and Walton 2007; Jeng et al. 2010). Significant linear correlations among total

**Table 3.** Correlation coefficient values observed among total phenolic content, total flavonoid content, and antioxidant activity of varying seed coat color soybean genotypes.

Parameters	Correlation coefficient (r) of genotype			
	Black	Green yellow	Light yellow	Yellow
TPC and TFC	0.987**	0.392 <sup>ns</sup>	0.391 <sup>ns</sup>	0.567**
TPC and AA	0.924**	0.481 <sup>ns</sup>	0.106 <sup>ns</sup>	0.246 <sup>ns</sup>
TFC and AA	0.929**	0.285 <sup>ns</sup>	0.750*	0.211 <sup>ns</sup>

Note: \* significance at  $p < 0.05$ , \*\* significance at  $p < 0.01$ , ns = non significance. TPC = total phenolic content, TFC = total flavonoid content, AA = DPPH scavenging activity

phenolics, total flavonoids and DPPH scavenging activity determined in those black soybeans were consistent with other reports in which total phenolics contributed to a high antioxidant activity found in colored legumes (Segev et al. 2010; Zhao et al. 2014). Therefore, it can be assumed that antioxidant activities could be estimated based on the degree of total phenolic contents, especially for the black soybeans (Jeng et al. 2010; Segev et al. 2010).

In conclusion, this study revealed that significant radical scavenging activities in soybean genotypes may be influenced by high phenolic contents. Results presented in this study suggest that estimation of phenolic contents as well as antioxidant activities in soybean genotypes may be useful for seeking soybeans with high antioxidant properties.

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