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Potential of local black soybean as a source of the isoflavones daidzein and genistein

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Abstract

Daidzein and genistein are isoflavone compounds produced by soybean plants and have an important role in medical therapy. For the soybean crop itself, daidzein and genistein contribute as a defence mechanism against pathogen attack, and also as chemoattractants for Rhizobium bacteria. The development of black soybean varieties that have high levels of daidzein and genistein is required for functional food and industrial raw materials. The objective of this study was to identify the content of daidzein and genistein in local black soybean genotypes. Thirty-four black soybean genotypes were planted in a randomised block design. Daidzein and genistein content were measured in black soybean seeds using high-performance liquid chromatography. In the 34 black soybean genotypes, daidzein contents (0.01–0.21 mg g^{-1}) were more variable than genistein (0.02–0.03 mg g^{-1}). Compared to genistein, daidzein content was higher in 31 genotypes (0.03–0.21 mg g^{-1}) and the other in three genotypes (0.01–0.02 mg g^{-1}). The UP106 genotype showed the highest content of daidzein (2.06 mg g^{-1}), while UP115 showed the highest genistein content (0.29 mg g^{-1}). UP104 had the highest daidzein content per plant because it had a higher seed weight per plant. The UP106, UP104 and UP115 genotypes have potential to be developed as sources of daidzein and genistein.

Introduction

In Indonesia, the use of black soybean is limited to making soy sauce, while in other countries, black soybeans are used as food and industrial raw materials. Traditionally, in China, India, Japan and Korea, black soybean has been widely consumed as a medicine for hundreds of years for detoxification and anti-inflammation and to improve the quality of red blood cells (Xu and Chang, 2008) because it contains a variety of substances that contribute positively to human health, including isoflavones (Xu and Chang, 2008).

Isoflavones are secondary metabolites mainly found in Leguminosae, especially soybean. There are four forms of isoflavones: the aglycone or free forms (daidzein, genistein and glycitein), glucosides (genistin, daidzin, glycitin), malonylglucosides (6-O-malonylgenistin,6-O-malonyldaidzin and 6-O-malonylglycitin) and acetylglucosides (6-O-acetylgenistin, 6-O-acetyldaidzin,6-O-acetylglycitin) (Kudou *et al*., 1991). Griffith and Collison, 2001). The aglycone form (daidzein and genistein) is the most abundant isoflavone in soybean (Wang and Murphy, 1994; Frank *et al*., 1995; Dhaubadel, 2011). Genistein is a phytoestrogen and has anticancer properties (Barnes *et al*., 1990), and is an inhibitor of protein tyrosine kinase activity (Akiyama *et al*., 1987) and DNA topoisomerase (Okura *et al*., 1988).

In soybean plants, daidzein and genistein act as a phytoalexin, i.e. the compounds produced by plants in response to pathogen attack (Dakora and Phillips, 1996). Daidzein is a potential inducer of the genes controlling nodulation in *Bradirhyzobium japonicum* (Khan and Bauer, 1988) and acts as a chemoattractant for *B. japonicum* (Kosslak *et al*., 1987). Daidzein affects *Phythophthora sojae* by changing the morphology of fungi (Rivera-Vargas

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et al., 1993). Genistein has a strong influence as a fungicide against *P. sojae*, which causes root rot fungi in soybean.

The occurrence of isoflavone in soybean plants starts at the beginning of the seed formation phase (R5) and continues until the seed maturation phase (R7) (Kim and Chung, 2007; Kudou *et al*., 1991). Isoflavone levels increase linearly during seed development and achieve the highest levels in mature seeds (R8) (Berger *et al*., 2008;Dhaubadel *et al*., 2011). Isoflavone contents are influenced by genotype (Wang and Murphy, 2004), environment (Lee *et al*., 2003) and the interaction of genotype with the environment (Hoeck *et al*., 2000; MacDonald *et al.*, 2005).

The potential of black soybean as a source of isoflavone, as well as the importance of daidzein and genistein, have led to the development of the black soybean varieties that have high levels of daidzein and genistein (Morrison *et al*., 2008). One way to achieve that goal is by exploration and identification of local genotypes (Jun, 2014). Local varieties constitute a potential source of genes for the development of commercial soybean varieties (Li *et al.*, 2008). However, research on the development of black soybean as a source of isoflavones is limited, especially in Indonesia (Jeng *et al*., 2013). The limitation of the number of soybean germplasm is one of the causes, and there have been no black soybean varieties with high isoflavone content released by the Indonesian Ministry of Agriculture. This study aims to determine the daidzein and genistein contents in local black soybean from Indonesia using the HPLC method.

Materials and Methods

Thirty-four black soybean genotypes were planted at an experimental station of the Faculty Agriculture of Padjadjaran University, Jatinangor, and Sumedang District of West Java. The location is about 754 m above sea level with temperatures range between 23°-27°C, and relative humidity range between 60-80%. Planting and maintenance procedure followed the procedures of soybean seed productions. A randomised block design was applied with two replicates. Daidzein and genistein contents were measured from the seed at the R8 growth phase following the methods of Vyn *et al.* (2002). A total of 100 mg of black soybean seed was ground to pass through 35 mesh screens. The fine powder of each seed sample (0.30 g) was mixed with 2 mL of concentrated HCl and 10 mL ethanol (96%), and was then boiled for 2 hours. After being cooled to room temperature and filtered through a 0.45-µm PTFE filter, samples were vortexed and 1.5 mL of each aliquot was centrifuged at 10,000 rpm for 10 minutes.

Analysis of isoflavone content was carried out using HPLC method with a UV detector at 254 nm wavelength and an ODS/C18 (octadecyl silanes) column. The HPLC analysis used methanol and double-distilled water (80:20) as the mobile phase with a flow rate of 1 mL per minute and 10 µL injection volume. Daidzein and genistein concentration of the samples were quantified by comparison with an external daidzein and genistein standard (Sigma Aldrich, USA) using five concentrations: 0.5, 1.0, 1.5, 2.0 and 2.5 µg g^{-1} (dissolved in a mixture of methanol/water 4:1, v/v). Daidzein and genistein were identified by their retention times of the peak. Daidzein and genistein concentrations of samples were calculated by comparing peak areas of samples with those of the standards using a calibration curve based on the chromatogram of the standard. The concentrations of daidzein and genistein of 34 black soybean genotypes were analysed using the K-mean cluster analysis (SPSS version 19) to obtain three levels of isoflavone contents (high, medium and low).

Results and Discussion

Calibration process

Daidzein and genistein standard compound were successfully separated and identified using HPLC. The peaks of the chromatogram of five concentrations of the standard solution appeared at the same retention times with symmetrical shapes. The retention times were 4.8 minutes for daidzein and 7.8 minutes for genistein. The equation of the calibration curve for daidzein was $y = 2.4879x - 1.422$ with R values of 0.9988 and for genistein was $y = 2.5867x - 2.455$ with R values of 0.9977. These results indicate that the calibration curve obtained was fit for calculating daidzein and genistein concentrations of the samples (International Conference on Harmonization, 1994).

Daidzein and genistein content

The content of daidzein was higher than genistein in 31 genotypes (Figure 1), consistent with those previously reported by Malencic *et al.* (2012) (daidzein : genistein; 65.2 : 71.2%) and Cvejic *et al* (2011) (daidzein : genistein; 50.8 : 39.5%). In three genotypes (UP103, UP115 and UP122), the genistein content was higher than the daidzein, consistent with those previously reported by Wang *et al*. (2000) (genistein : daidzein; 56.2 : 43.8%) and Lee and Cho (2012) (genistein : daidzein; 0.518 : 0.416 mg g^{-1}). However, Cesco *et al*. (2011) showed daidzein is a

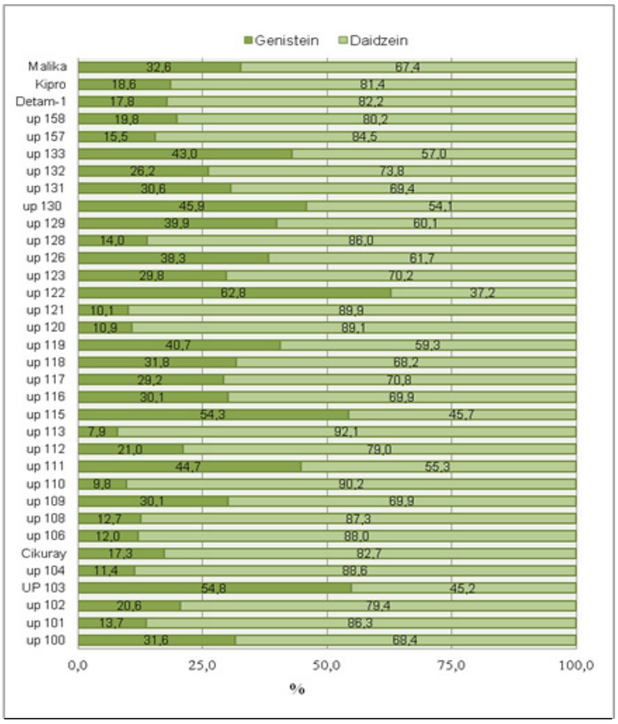

Figure 1. Genistein and daidzein content of 34 local black soybean genotypes.

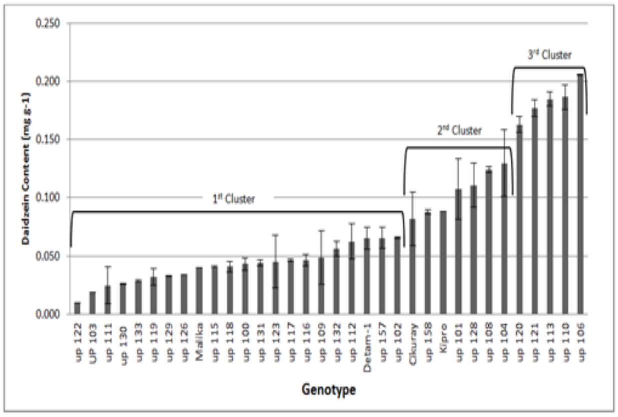

Figure 2. Three clusters of daidzein content from 34 black soybean genotypes. Malika, Kipro and Detam-1 are commercial varieties of black soybean.

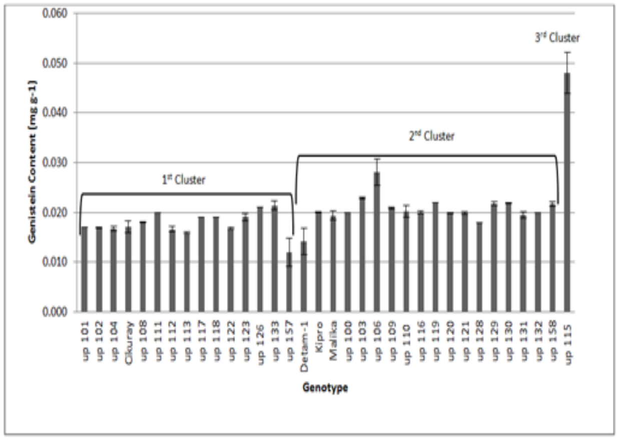

Figure 3. Genistein content of three clusters from 34 of black soybean genotypes.

ubiquitous compound identified in all cultivars, while genistein is strongly cultivar specific.

Although the genistein content was lower than daidzein in the majority of soybean genotypes, genistein has an important role in human health and the soybean crop itself. As a phytoestrogen, genistein can prevent and suppress cancer (Messina *et al*., 1994; Pavese *et al*., 2010). A few studies have clearly shown that genistein, together with other isoflavones released from soybean roots, can affect soil microbial growth and diversity, particularly fungi populations (Werner, 2001; Colpas *et al*., 2003).

Daidzein was more variable than genistein and the range of values was wider. Daidzein content ranged between 0.010-0.206 mg g^{-1} and genistein ranged between 0.012-0.048 mg g^{-1}. In this study, daidzein and genistein content were lower than the results of Malencic *et al.* (2012), but had a wider range of daidzein concentration. This suggests that the character of the daidzein content of the black soybean population has a greater variety than that of genistein.

The cluster analysis of the daidzein content (Figure 2) showed that there are five genotypes that have a high daidzein content, while seven genotypes have a medium and 25 genotypes have a low daidzein content. The clustering results of the genistein content (Figure 3) showed that there was one genotype that had a high genistein content, while 18 genotypes had a medium and 15 genotypes a low genistein content.

The daidzein contents (average: 0.08 mg g^{-1}) and genistein contents (average 0.02 mg g^{-1}) of black soybean in this study are lower than the results of Malencic *et al.* (2012) with two genotypes of black soybean grown in Novi Sad, Serbia, and those of Correa *et al.* (2010) with five genotypes of black soybean grown in central Korea. Malencic *et al.* (2012) reported that the average daidzein content was 2.35 mg g^{-1} and that of genistein was 0.83 mg g^{-1}, while Correa *et al.* (2010) found an average daidzein content of 0.25 mg g^{-1} and an average genistein content of 0.18 mg g^{-1} seed. The daidzein and genistein contents of this study were different with both previous studies due to differences in the genetic material used and the location of research. The proportion of daidzein and genistein are genetically and environmentally determined (Lee *et al*., 2010). It has been reported in previous studies that genotype significantly influences the content and composition of isoflavones in soybean seeds and the potential for isoflavone production is largely under genetic control (Hoeck *et al*., 2000; Primomo *et al*., 2005). According to Seguin *et al*. (2007), environmental factors (air temperature, soil moisture

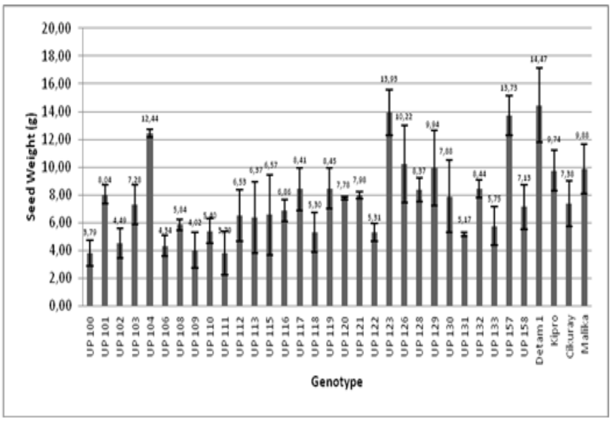

Figure 4. Seed weight per plant of 34 local black soybean genotypes

levels, soil fertility, CO_2 levels, light quality and pest pressure) affect isoflavone concentrations in soybean. Numerous studies have reported that some varieties may have relatively stable isoflavone concentrations across a range of environments (Hoeck *et al*., 2000; Lee *et al*., 2003b; Seguin *et al*., 2004).

Based on the daidzein and genistein contents, the 34 genotypes were grouped into three clusters. The first cluster included genotypes with daidzein at concentrations from 0.01- 0.07 mg g^{-1}, the second cluster 0.08-0.13 mg g^{-1}, and the third cluster 0.16-0.21 mg g^{-1} (Figure 2). Analyses of variance of the three clusters showed a significant difference (Table 2 in supplementary material). The third cluster can be categorised as having a high content of daidzein, while the second cluster had a medium content and the first cluster was low. The genotype of UP106, UP113, UP110, UP120 and UP121 were categorised as being in the third cluster because they have a high content of daidzein per gram of seed. The genotypes of Cikuray, Kipro, UP158, UP101, UP108, UP128 and UP104 have a medium content of daidzein and were categorised into the second cluster. Twenty-two genotypes were categorised as being in the first cluster because they had a low daidzein content.

Genotype UP106 had a high content of daidzein per gram of seed (0.21 mg g^{-1}), which was higher than UP113 (0.19 mg g^{-1}), UP110 (0.18 mg g^{-1}), UP120 (0.18 mg g^{-1}) and UP121 (0.16 mg g^{-1}), but it had lower daidzein content per plant (0.89 mg) than these genotypes. This is because the seed weight per plant of UP113 (6.37 g), UP110 (5.4 g), UP120 (7.78 g) and UP121 (7.98 g) were higher than UP106 (4.33 g) (Figure 4).

The genotype UP104 showed different results to those of UP106. It has a medium content of daidzein per gram of seed (0.13 mg g^{-1}, second cluster), but has higher daidzein content per plant (1.61 mg) than the other genotypes, which was caused by the high seed weight per plant (12.43 g). It is important to consider that the relationship between isoflavone concentrations and agronomic characteristics is important in breeding and selection programs, as the presence of positive correlations indicates that selection for several desirable traits can be carried out concurrently (Lee *et al*., 2010). Seguin *et al.* (2004) reported that there are positive correlations between isoflavone concentrations and seed yield, 100-seed weight and protein.

The clustering result of genistein content showed three clusters (Figure 3). The first cluster showed a genistein content in the range 0.012–0.019 mg g^{-1}, while the second cluster ranged from 0.195-0.028 mg g^{-1}, and the third cluster ranged from 0.029-0.048 mg g^{-1}. Based on analysis of variance, there were no significant differences between the groups (Table 2 in supplementary material) and all 34 genotypes had the same relative content of genistein. Nevertheless, the UP115 genotype had the highest content of genistein (0.048 mg g^{-1}).

The results of the study showed that among 34 local black soybean genotypes, there are genotypes, such as UP115, that have the potential to be developed as a commercial variety as a source of genistein, and UP106 as a source of daidzein. These varieties have potential as a raw material for medicine. In humans, genistein acts as a phytoestrogen and it has potential in cancer prevention and suppression (Messina *et al*., 1994). Eighty per cent of antioxidants in soybean were daidzein and genistein (Arora *et al*., 2000; Hsu *et al*., 2001; Hwang *et al*., 2001). UP106 and UP104 genotypes have the highest potential as a source of daidzein, while the UP115 genotype has potential as a genistein source. This result is supported by Hoeck *et al*. (2000) who showed that isoflavone content seems to be a quantitative trait and thus soybean cultivars containing high isoflavone content can be bred. Therefore, on the basis of the results of this study, the genotypes of UP106, UP104 and UP115 could be recommended as potential cultivars with high isoflavone content.

Conclusion

The contents of daidzein in 34 black soybean genotypes were more variable than those of genistein. The daidzein content of local black soybean was higher in 31 genotypes and lower in three genotypes compared to genistein. The UP106, UP104 and UP115 genotypes have potential to be developed as a source of daidzein and genistein.

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