Seed Isoflavone Content of Blacksoybean Genotypes Growing at Different Altitude

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Abstract

Isoflavones are secondary metabolites contained in black soybean which has role as chemical defense against biotic and chemoatractant in associated with *Rhizobium* bacteria. Isoflavone production in soybean plants is influenced by genotype and environment such as temperature related to altitude of place. The purpose of this study was to study the effect of altitude on the composition and content of isoflavone aglycone group which include daidzein, glycitein and genistein in black soybean genotypes and to identify the genotypes showed stable and high content of isoflavone at different altitude. Four black soybean genotypes grown in planting location with altitude of 868 m and 250 m above sea level using a randomized block design. Daidzein, glycitein and genistein (aglycone form) were extracted from seed in R8 phase (dry seed) and quantified by HPLC. The results showed that the difference in altitude of the place caused different composition and level of daidzein, glycitein and genistein. Altitude of 868 m above sea level with an average temperature of 25,3^{o} C can increase the production levels of daidzein, glycitein and genistein of black soy bean . At altitude of 868 m above sea level (asl), genistein (43.7%) was higher than daidzein (41.2%) and glycitein (15.1%). At an altitude of 250 m asl, daidzein (43.9%) higher than genistein (41.2%) and glycitein (14.9%). UP106 was genotype that showed stable level of daidzein at altitude 868 m above sea level and 250 m above sea level.

Keywords : altitude, blacksoybean, genotype, HPLC, isoflavone

INTRODUCTION

Isoflavones are secondary metabolite found in soybean plants and function in defense systems of biotic stress and in the interaction process of soybean plants with microbes for the formation of root nodules (Long, 1989; Stafford, 1997). In soybean plants, isoflavones are found in various organs, from roots to seeds (Dhaubadel et al., 2003; Chen et al., 2011). At the root, isoflavones act as chemoattractants against Rhizobium bacteria for the nitrogen fixation process (Subramanian et al., 2004). In the leaves, isoflavones act as active defenses against biotic stress (Dhaubadel et al., 2003; Lee et al., 2009).

In seed, isoflavones are the result of the synthesis process in the seed tissue itself and the translocation of tissues that synthesize isoflavones (Dhaubadel et al., 2003). This has been evidenced by the expression of genes controlling the characters of isoflavones on seeds, seed

coat, peas and other tissues in soybean plants with concentrations vary widely (Dhaubadel et al., 2003; Ralston et al., 2005) . The concentration of isoflavones in seeds increases linearly during the process of seed development (Berger et al., 2008). The previous research showed that the content of isoflavones in soybean seeds vary widely. Total isoflavone content has been reported to range from 0.276 mg.g-1 to 3,309 mg.g-1 (Wang et al., 2000; Hoeck et al., 2000). Other studies reported isoflavone levels ranging from 0.25 mg.g-1 - 0.95 mg.g-1 (Lee et al., 2003; Lee et al., 2004; Sequin et al., 2005).

Various variations of isoflavone content were found in a number of studies due to differences in soybean genotypes used, planting site conditions and growing season. This indicates that the concentration of isoflavones is determined by the genetic composition of soybeans and the environment, in which environmental factors and the interaction of genotype environment play a bigger role in causing variation (Lee et al., 2003). According to the control of environmental factors and the interaction of genotype-environment factors to variations in isoflavone content, studies involving a number of genotypes in different environments need to be continuously performed.

The altitude of planting location related to temperature. It's significant in Indonesian agroclimate and affect the growth of soybean included isoflavone content. This study aimed to study the effect of altitude on the composition and content of isoflavone aglycone group which include daidzein, glycitein and genistein in soy genotypes to identify black and black soybean genotypes showed higher levels of daidzein, glycitein and genistein stabilized at a different altitude.

RESEARCH METHOD

Plant Material

Four genotypes of black soybean that showed different isoflavone content of the results of Sumardi *et al.* (2017) were used in this study. The genotypes of UP106 and UP115 are genotypes that show high levels of isoflavones. Genotype UP122 and UP157 are genotypes that show low isoflavone levels. Characteristics of black soybean genotypes are shown in Table 1. The four genotypes were planted at two planting sites at 250 m altitude (February to May 2014), and at an altitude of 868 m asl (April to July 2015).

At each planting site, each genotype is planted on a 10 kg (Grumusol Order) soil that is inserted into a polybag measuring 30 cm x 40 cm. Polibag is arranged in a randomized block design consisting of 3 replications. The process of cultivation following soybean seed productions (Adie et.al., 2013

Table 1. The characteristics of UP106, UP115, UP122 and UP157 genotypes

No	Genotype	Population Origin	Characteristic			
			Seed colour	Seed size *)	Days of harvest (DAP)	Yield potency (ton ha^{-1})
1	UP 106	Cikuray (Farmer population)	Black	Small	92	0.98
2	UP 115	Lokal Jatiwangi	Black	Small	82	1,1
3	UP 122	Lokal Kebumen	Black	Medium	93	1.24
4	UP 157		Black	Medium	85	1.11

Note : ** = Based on weight of 100 seed (small : < 10 g, medium : 10 g – 12 g, big :12 g <) (Adie *et.al*., 2013)

Extraction of isoflavones

Isoflavones are extracted from black soybean seeds harvested at the R8 phase (dry seeds). The extraction of isoflavones is done by the method of Vyn et al. (2002). A total of three grams of dried soybean seed (R8 phase) is mashed with a blender. The extraction solvent was prepared by preparing a 2 mL mixture of 18 molar HCL with 10 mL of 96% ethanol in a 100 mL erlenmeyer glass. Finely ground of soybean flour is weighed as much as 0.3 grams and put into the solvent then heated in waterbath shaker at 70^{o} C for 2 hours. The solution is then filtered using Whatman Filter Paper. The filtrate was used to analyze daidzein, glycitein and genistein content.Before injection to the HPLC machine, the extract is rectified using a 45 mm PTFE filter. A total of 25 mL of extract is injected into the HPLC machine.

Determination of HPLC condition analysis

The HPLC system uses ODS / C18 (octadecyl silane) columns, 250 mm in length, 4.6 mm inner diameter, and 10 μm particle size, 254 nm UV detector, mobile phase of methanol: double distilled water (80:20) with flow rate 1 ml / min and injection volume of 10 μl sample. The reliability of the HPLC analysis process is tested by the calibration process to calculate linearity parameters. The linearity parameter is used to show the ability of the analysis method to give a good and proportional response to the concentration of the analyte in the sample. In this case the relationship between the concentration of standard solution of mixture of daidzein, glycitein and genistein to the area under the curve on the chromatogram. The linearity parameter is expressed in r or the correlation coefficient (ICH, 1994). The calibration curve

was made from 5 variations of daidzein, glycitein and genistein standard was 5; 10; 15; 20 and 25 ppm. Based on calibration standard of daidzein, glycitein, and genistein, obtained linear regression equation for daidzein y = 1.32x - 1.76 with correlation coefficient value of 0.9943, glycitein y = 1.25x - 2.06 with correlation coefficient of 0.9940 and for genistein y = 1.79x - 2.17 with correlation coefficient value of 0.9962.

RESULTS

The composition of daidzein, glycitein and genistein in black soybean seeds at different altitudes

Comparison of daidzein, glycitein, and genistein seeds of black soybean showed a difference between 868 m asl (above sea level) and 250 m asl (Figure 1). At altitude of 868 m asl, genistein (43.7%) was higher than daidzein (41.2%) and glycitein (15.1%). At altitude of 250 m asl daidzein (43.9%) higher than genistein (41.2%) and glycitein (14.9%). At altitude of 868 m above sea level and 250 m asl, genistein is lower than daidzein and genistein. At altitude of 868 asl, total isoflavones (5.20 mg g^{-1}) were higher than at 250 m asl (3.31 mg g^{-1}). Levels of daidzein (2.14 mg g^{-1}), glycitein (0.79 mg g^{-1}) and genistein (2.27 mg g^{-1}) at an altitude of 868 m asl were higher than at 250 m altitude (daidzein 1.46 mg g^{-1}; glycitein 0.50 mg g^{-1}, genistein 1.36 mg g^{-1}).

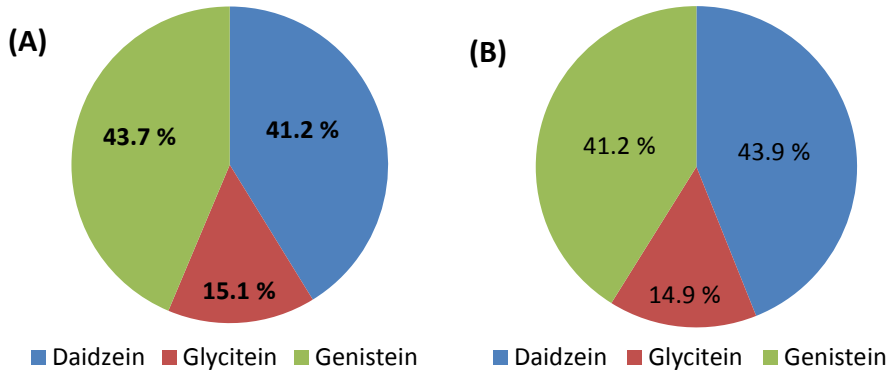

Figure 1. Daidzein, glycitein dan genistein ratio of black soybean seed at 868 m asl(A) and 250 m asl (B)

The four genotypes showed differences in the ratio of daidzein, glycitein and genistein between at an altitude of 868 m asl with 250 m asl (Figure 2). At an altitude of 868 m above sea level, the ratio of daidzein, glycitein and genistein showed a consistent condition in which genistein was higher than daidzein and glycitein in all genotypes. At an altitude of 250 m asl, there is a difference in the ratio of daidzein to genistein between the four genotypes. In

genotype UP106, daidzein (52.4%) was higher than genistein (35.3%). In the genotype UP115, daidzein (39.9%) was lower than that of genistein (42.9%). In the genotypes of UP122 and UP157, genistein (43.9% and 43.3%) were higher than daidzein (40.9% and 40.8%). Glycitein showed lower levels than daidzein and genistein in all genotypes at altitudes of 868 m asl (13.2 - 16.3%) and 250 m asl (12.2 - 17.2%).

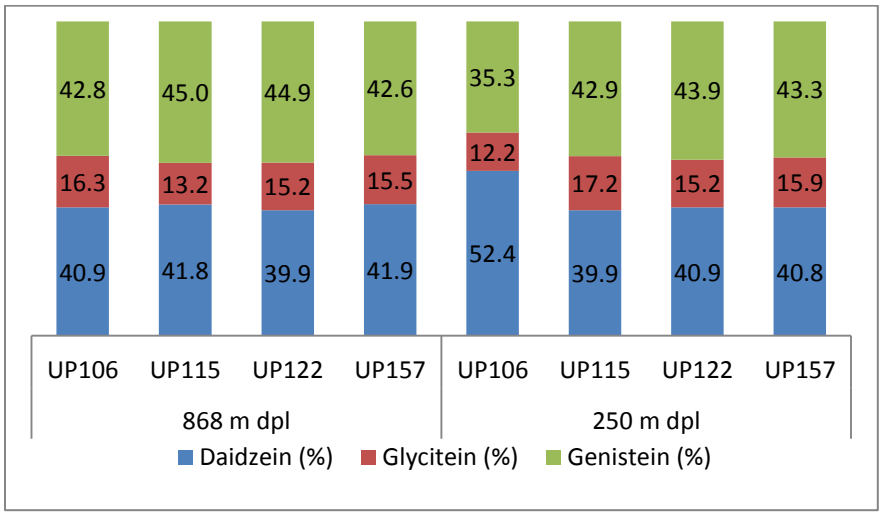

Figure 2. Daidzein, glycitein dan genistein ratio of UP106, UP115, UP122 dan UP157 black soybean genotypes at 868 and 250 m above sea level.

Daidzein, glycitein and genistein content of black soybean genotypes

Daidzein, glycitein and genistein content showed differences between different genotypes at the same altitude and the same genotypes between different altitudes (Figure 3). At an altitude of 868 m above sea level, the genotype of UP157 showed high levels of daidzein (0.67 mg g^{-1}), glycitein (0.25 mg g^{-1}) and genistein (0.68 mg g^{-1}) compared to UP106, UP122 and UP157 genotypes. The genotypes of UP122 showed the lowest levels of daidzein (0.40 mg g^{-1}), glycitein (0.15 mg g^{-1}), and genistein (0.45 mg g^{-1}) compared to UP106, UP115 and UP157 genotypes.

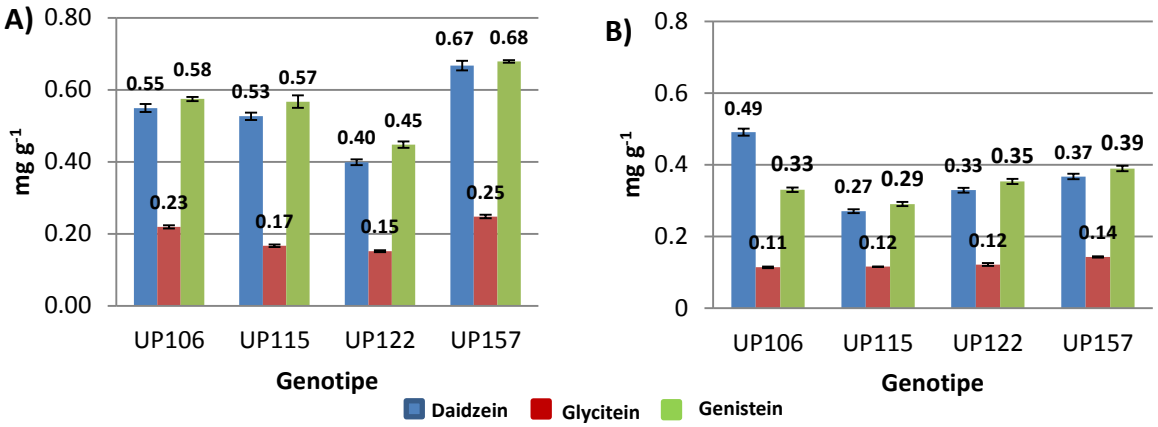

Figure 3. Daidzein, glycitein and genistein content of UP106, UP115, UP122 and UP157 black soybean genotypes at 868 m asl (A) dan 250 m asl (B)

At an altitude of 250 m asl, the UP106 genotype showed the highest daidzein (0.49 mg g^{-1}) and the lowest glycitein (0.11 mg g^{-1}) levels compared to the UP115, UP122 and UP157 genotypes. The genotype UP157 showed the highest levels of glycitein (0.14 mg g^{-1}) and genistein (0.39 mg g-1) versus the UP106, UP115 and UP157 genotypes.

Differences in altitude lead to changes in daidzein, glycitein, and genistein levels in all genotypes presented as differences in levels (Table 2). The genotype of UP122 showed the smallest difference in glycitein (0.03 mg g^{-1}) and genistein (0.09 mg g^{-1}) between altitude of 868 m dpl and 250 m asl. The genotype of UP106 showed the smallest difference in daidzein (0.06 mg g^{-1}) between altitudes of 868 m dpl and 250 m asl compared to the genotypes UP115, UP157 and UP122. The genotypes of UP157 showed the greatest differences in daidzein levels (0.30 mg g^{-1}), glycitein (0.10 mg g^{-1}) and genistein (0.29 mg g^{-1}) between altitudes of 868 m dpl and 250 m asl.

Table 2. Daidzein, glycitein and genistein content of UP106, UP115, UP122 and UP157 black soybean genotypes at 868 m asl dan 250 m asl.

Genotype	868 m asl			250 m asl			Difference*		
	Daid	Gly	Gen	Daid	Gly	Gen	Daid	Gly	Gen
UP106	0.55	0.22	0.57	0.49	0.11	0.33	**0.06**	0.10	0.24
UP115	0.53	0.17	0.57	0.27	0.12	0.29	0.26	**0.05**	0.28
UP122	0.40	0.15	0.45	0.33	0.12	0.35	**0.07**	**0.03**	**0.09**
UP157	0.67	0.25	0.68	0.37	0.14	0.39	0.30	0.10	0.29

Note : * = Daidzein/glycitein/genistein content at 868 m asl less at 250 m asl; Daid = daidzein; Gly = glycitein; Gen = genistein

DISCUSSION

Isoflavones consist of four groups : aglycons, β-glycosides, malonyl glycosides and acetyl glycosides. The glycoside group is the most dominant but inactive form (Day et al., 1998). Aglicon group is the least isoflavone form in soybeans but is the most biologically active form of isoflavones (Tepapcevic et al., 2011). Aglicon group consist of daidzein, glycitein and genistein. Glycitein is the lowest-grade isoflavone on the soybean.

The results of Kim et al. (2005) showed that glycitein can not be detected by the HPLC method on seven yellow soybean genotypes. This is thought to be due to very low levels of glycitein. The levels of glycitein from the results of this study are in line with the results of studies that have been reported. At altitude of 868 m asl and 250 m asl glycitein showed lower than daidzein and genistein in all genotypes. In all genotypes glycitein content at altitudes of 868 m above sea level higher than at 250 m asl. These results indicate that the production of glycitein in soybean is affected altitude (environmental factor).

Comparison of daidzein, and genistein content in soybean seeds varied based on the results of reported studies. Sumardi et al. (2017), reported that in 30 local black soybean genotypes, daidzein levels were higher than genistein and in 4 genotypes, genistein levels were higher than daidzein. Correa et al. (2010) reported on black soy daidzein levels ranged from 0.193 mg g-1 - 0.288 mg g-1 and genistein ranged from 0.145 mg g-1 - 0.223 mg g-1 seeds. The same result reported Wang et al. (2014) varieties of Zhongdou (high isoflavones) and Jiunong (low isoflavones), glycitein showed the lowest levels (0.36 μg / 100 g and 0.42 μg / 100 g) and daidzein levels (8.92 μg / 100 g and 4.79 μg / 100 g) Were higher than genistein (4.22 μg / 100 g and 2.81 μg / 100 g). Different results are reported Gutierrez-Gonzalez et al. (2010) in the yellow soybean population of the F7 generation indicating that levels. The results of this study indicate that the ratio of daidzein and genistein levels varies between genotypes and altitude of different places. At an altitude of 868 m above sea level, genistein levels were higher than daidzein in all genotypes. At an altitude of 250 m asl, genistein levels were higher in the UP115, UP122 and UP157 genotypes and lower in the UP106 genotype compared to daidzein. Distribution of individual isoflavones within these values can vary significantly, although it is known that daidzein with its conjugates and genistein with its conjugates are present in nearly equal amounts in soybeans, whereas glycitein and its conjugates are present in lesser amounts (Lee et al., 2004). The variation of soybean isoflavones in content and composition occur as a

consequence of variation in the genotype of the seed, as well as the year and location of seeding.

Daidzein levels show variation between genotype and altitude. Sumardi et al. (2017) has reported that black soybean genotype UP106 planted at altitudes of 754 m asl showed higher daidzein (0.21 mg g-1) than the UP157 (0.07 mg g-1) genotype. This study showed different results where the daidzein genotype UP106 level was lower than the UP157 genotype at altitude of 868 m asl. In the UP106 genotype study showed higher daidzein levels when planted at altitudes 254 m asl.

The same condition occurs in genistein content. Results of Sumardi *et al*. (2017) showed that the black soybean genotype UP115 planted at altitude 754 m above sea level has higher genistein (0.048 mg g^{-1}) than the UP157 genotype. This study shows different results. Genistein genotype UP115 level at altitude 868 m asl and 254 m above sea level lower than genistein genotype UP157. According to the result, Tsukamoto et. al. (1995) suggested that the effects attributed to years and locations in research studies may reflect differences in the temperatures that occur during seed development, as a result of the date of planting. It can be concluded that the diversity of isoflavone contents depend on unknown climatic and environmental factors and genetic variation.

The results of this study indicate that daidzein, genistein and glycitein levels in black soybeans are influenced by genotype, environment and genotype interaction with the environment. The effect of genotypic interaction on daidzein, genistein and glycitein levels can be known when different levels of daidzein, genistein and glycitein are among the different genotypes planted at the same planting site. Similarly, when differences in daidzein, genistein and glycitein levels of the same genotype are planted at different plant sites.

The UP122 genotype showed the lowest but most stable levels of glycitein and genistein compared to other genotypes. This can be seen from the difference of daidzein content (0.07 mg g^{-1}), glycitein (0.03 mg g^{-1}) and genistein (0.09 mg g^{-1}) between at altitude of 868 m asl with a height of 250 m asl showing the smallest value compared to the others. While genotype UP106 (0.06 mg g^{-1}) showed the most stable daidzein levels compared to other genotypes. The genotype UP157 is a genotype that shows unstable levels of daidzein, glycitein and genistein because it shows the greatest difference value among other genotypes. Among the four genotypes tested, the UP106 genotype was a genotype that showed high and stable daidzein

levels. The UP106 genotype can be used as a parent in the development of a hope line that has isoflavontinggi content.

The results of this study indicate that at altitude of 868 m above sea level, daidzei, glycitein and genistein levels are higher than at 250 m asl. This means that the altitude of 868 m asl with an average temperature of 25.3o C, can increase the production of daidzein, glycitein and genistein. This is in accordance with the results of research in Korea reported Ha et al. (2009), that isoflavone content in yellow soybean planted at location with height 600 m asl with mean temperature 12,8-24,3^{o} C higher than that planted at location with height 12 m asl with average temperature 16.0 -27.7^{o} C. According to Tsukamoto (1994) and Lozovaya (2005), low temperatures and high rainfall during seed development can produce high contentof isoflavone.

CONCLUSION

Differences in altitude lead to differences in composition and content of daidzein, glycitein, and genistein of black soybean. The altitude of 868 m above sea level with an average temperature of 25.3^{o} C can increase the production of daidzein, glycitein and genistein of black soybean. The genotype of UP106 is a genotype that showed stable content of daidzein at altitude of 868 m and 250 m asl.

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