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Anthocyanin Content, Isoflavone Composition, Antioxidant Activities, and Agronomic Performance of Black Soybean Lines

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ABSTRACT

Modern soybean cultivars should have good agronomic performance in addition to high bioactive properties. This study evaluated anthocyanin content, isoflavone composition, antioxidant activity, and agronomic traits of sixteen black soybean genotypes grown in Mojokerto, East Java, Indonesia. Seed samples were analysed for total anthocyanin content (TAC), antioxidant activity, and isoflavone composition, including daidzein, glycitein, and genistein, in both the seed coat and the cotyledon. Significant variation was observed among genotypes for all biochemical and agronomic traits. TAC was higher in the seed coat than in the seed cotyledon. Genotypes with black seed coats exhibited high TAC in the seed coat, whereas green cotyledon genotypes showed higher TAC in the cotyledon. Isoflavone concentrations ranged from 9.42–27.94 mg/kg for daidzein, 25.18–67.14 mg/kg for glycitein, and 12.93–34.52 mg/kg for genistein. Antioxidant activity in the cotyledon was generally low, while the seed coat exhibited antioxidant activity ranging from low to strong, with three genotypes classified as having strong antioxidant potential. The number of nodes was identified as a useful selection criterion for high-yielding genotypes. The Genotype-by-Trait (GT) biplot showed that the genotype G2 (JPG 15/Anj-884) had the highest values for all isoflavone compositions. Genotypes G6 (Khl/Anj-890), G7 (Khl/Anj-891), and G8 (Khl/Anj-893) performed best in terms of seed yield and antioxidant activity. Genotypes G9 (Khl/Anj-894), G11 (Khl/Anj-896), and G15 (Mallika) had large seed size and high TAC in both seed and cotyledon. These genotypes have potential for further improvement, either as promising lines or parental lines in developing new black soybean cultivars.

Keywords: Bioactive properties, GT biplot, high yield, seed coat, seed cotyledon, seed yield

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INTRODUCTION

Black soybean is a promising industrial crop with high nutritional and functional value, such as anthocyanin, isoflavone, and antioxidants (Mitharwal et al., 2024). These functional properties are important for supporting human health. Anthocyanins, which are secondary metabolites and water-soluble pigments found in many fruits, vegetables, and cereal grains, are responsible for the red, purple, or blue colour (Singh et al., 2024). Isoflavones are compounds found in soybeans that act as antioxidants and prevent free radical damage. Similarly, isoflavones from the flavonoid group found in soybean seeds have been shown to benefit people with diabetes mellitus (Kim et al., 2021).

The functional properties of black soybean differ among genotypes, due to variations in genetic, environmental factors, and crop management practices (Choi et al., 2020). A study by Dajanta et al. (2013) found that black soybeans exhibited greater antioxidant properties, total phenolics, and total flavonoids than yellow soybeans. Choi et al. (2020) investigated the relationship between the functional properties and the seed size of 24 black soybeans and found that the large-seeded seeds had higher total anthocyanin and total isoflavone contents than the small seeds, while the small seeds had higher total phenolic content. In another study, it was found that the isoflavone content in the green seed coat and yellow seed coat colours was 2.11 mg/g and 5.24 mg/g, respectively (Bursaca et al. 2017). According to Dhungana et al. (2021), anthocyanins were only found in black soybeans. In contrast to immature soybean seeds, Carrão-Panizzi et al. (2019) found that matured soybean seeds (R8 development stage) have higher levels of isoflavones, anthocyanins, protein, oleic fatty acid, β-glucoside, and aglucone isoflavones. However, it was shown that both anthocyanins and malonyl isoflavones were high in the R7 stage (physiological maturity),

Those research findings offer a potential chance to improve and enhance the functional properties of the soybean genotype. However, the economic value of soybeans, on the other hand, is characterised not only by their nutritional content, but also by commercially valuable agronomic qualities such as seed yield and agronomic traits that maintain high yields (Karges et al., 2022; Ramlal et al., 2023). Several studies on soybeans have shown that seed yield is significantly influenced by yield components, such as the number of pods per plant, the number of seeds per pod, and seed size (Kezar et al., 2023; Tayade et al., 2023; Wang et al., 2025). These traits are frequently used as reliable selection criteria in soybean breeding research and have been shown as significant predictors of seed production (Karyawati & Puspitaningrum, 2021; Mustofa et al., 2021). Furthermore, it has been shown that seed size and seed weight have a significant contribution to yield stability and market value in soybean, especially across different environmental conditions (Egli, 2013). Collectively, these findings emphasise that the integration of bioactive compound evaluation with agronomic performance is essential for the development of superior soybean cultivars.

Several studies on soybeans reported a significant relationship between isoflavones and agronomic traits (Zhang et al., 2014; Kim et al., 2023; Kuswantoro et al., 2023). However, a limited study was found on the relationship between antioxidant activity, anthocyanin, and isoflavone content with agronomic performance. Selecting several soybean genotypes with beneficial functional properties combined with a diversity of agronomic traits, especially seed yield, can help to increase the economic value of soybeans. This study aimed to determine the anthocyanin content, isoflavone composition, and antioxidant activities of black soybean genotypes resulting from crosses of several parental lines, and to examine their relationship with the performance of agronomic traits.

MATERIALS AND METHODS

Plant Genotypes and Study Location

Sixteen black soybean genotypes, consisting of 14 lines derived from crosses and selection among several parental lines and two commercial check cultivars (Mallika and Detam 4), were used in this study (Table 1). Several genotypes listed in Table 1 exhibit brown or blackish-brown seed coats as a result of segregation from crosses between black- and yellow-seeded parents. All genotypes were planted in the experimental field in Mojokerto, East Java, Indonesia, from August to November 2025. The research location has the soil type of Vertisol, C3 climate type based on the Oldeman system, and elevation of 54 a.s.l. The anthocyanin, isoflavone, and antioxidant measurements were performed in the Laboratory of the School of Life Sciences and Technology, Bandung Institute of Technology.

Table 1
The list of soybean genotypes, pedigree, and seed colour

No.	Genotype	Code	Pedigree		Colour	
			Female	Male	Seed Coat	Seed Cotyledon
1	JPG 15/Anj-883	G1	JPG 15	Anjasmoro	Black	Green
2	JPG 15/Anj-884	G2	JPG 15	Anjasmoro	Black	Green
3	JPG 15/Anj-885	G3	JPG 15	Anjasmoro	Black	Green
4	JPG 15/Anj-886	G4	JPG 15	Anjasmoro	Black	Green
5	JPG 15/Anj-887	G5	JPG 15	Anjasmoro	Blackish brown	Green
6	Khl/Anj-890	G6	Khl	Anjasmoro	Blackish brown	Yellow
7	Khl/Anj-891	G7	Khl	Anjasmoro	Blackish brown	Yellow
8	Khl/Anj-893	G8	Khl	Anjasmoro	Black	Yellow
9	Khl/Anj-894	G9	Khl	Anjasmoro	Black	Green
10	Khl/Anj-895	G10	Khl	Anjasmoro	Black	Green
11	Khl/Anj-896	G11	Khl	Anjasmoro	Brown	Green
12	Khl/Anj-898	G12	Khl	Anjasmoro	Brown	Green
13	Khl/Anj-899	G13	Khl	Anjasmoro	Brown	Green
14	Khl/Anj-900	G14	Khl	Anjasmoro	Black	Green
15	Mallika	G15	Local variety		Black	Yellow
16	Detam 4	G16	W9837	G100H	Black	Yellow

Experimental Design and Field Study

The field experiment employed a randomised block design using 16 black soybeans, each of which was replicated four times. The study was conducted in a lowland after rice planting and without any soil tillage. Each genotype was sown in a plot of 2 m × 4.5 m with a planting distance of 40 cm between rows and 15 cm between hills, using two plants per hill. Fertilisers were applied at the time of sowing, consisting of 250 kg/ha Phonska and 100 kg/ha SP-36. Pest, disease, and weed control were managed through regular monitoring. The observations were on the yield and yield components (days to flowering, days to maturity, plant height, number of branches, nodes, filled pods, empty pods, and 100-seed weight).

Determination of Total Anthocyanin Content (TAC)

The seed coat and cotyledon of 16 black soybean genotypes were examined for total anthocyanin content (TAC). Before anthocyanin extraction, the seed coats and cotyledons were manually removed and freeze-dried. After being frozen using liquid nitrogen, 30 grams of each sample were ground with pestles and mortars. The extraction of the TAC followed Giusti and Wrostald (2001). The amount of anthocyanin was quantified by comparing the absorbance to the standard curves at 700 nm and 512 nm at pH 1 and pH 4.5, respectively.

Determination of Antioxidant Activity

The seed coat and cotyledon of black soybeans were examined for antioxidant activity. The DPPH method (Jadid et al. 2017) was used to determine the antioxidant activity. Methanol was used to prepare the DPPH solution, which was subsequently added to extracts at several concentrations (5, 15, 30, 45, and 60 ppm). The absorbance changes were recorded at 517 nm. The standard was ascorbic acid. The following formula was used to calculate the percentage of DPPH inhibition (Pi) as shown in Equation 1:

$$P_i = \frac{(A_b - A_s)}{A_b} \times 100 \qquad [1]$$

Ab is the absorbance of the control, *As* is the absorbance of the compound/standard. The antioxidant ability of the sample was expressed as IC_{50}. The antioxidant activity based on the IC_{50} (ppm) was classified according to Molyneux (2004): < 50 ppm indicated very strong antioxidant activity, 50-100 ppm showed strong antioxidant activity, 100-150 ppm denoted intermediate antioxidant activity, and > 150 ppm indicated weak antioxidant activity.

Determination of Isoflavone Composition

Isoflavone content consisting of daidzein, genistein, and glycitein was determined using the methods of Vyn et al. (2002). The concentrations of daidzein, genistein, and glycitein were quantified by comparing sample peak areas with known standards based on calibration curves derived from the standard chromatograms.

Data Analysis

Agronomic traits were analysed using analysis of variance (ANOVA). The relationships between agronomic traits, yield, and bioactive properties were assessed using Pearson correlation in RStudio version 1.3.959 (RStudio Team, 2020). Genotypic performance based on selected agronomic traits and bioactive properties was displayed using a "which-won-where" graph of the genotype-by-trait (GT) biplot (Yan & Rajcan, 2002) using RStudio.

RESULTS AND DISCUSSION

Variation in the Seed Coat and Cotyledon Colour

Soybeans have a variety of seed coat colours, including green, brown, black, and yellow, as well as various cotyledon colours (Gao et al., 2021; Lim et al., 2021). Soybeans with black seed coats can be divided into two classes based on the colour of their cotyledons, which can be green or yellow (Jo et al., 2021). In this study, the 16 black soybeans consisted of 10 genotypes with black seed coat colours, three genotypes had blackish brown colours, and the rest (three genotypes) had brown seed coat colours. The cotyledon consisted of the green cotyledon (11 genotypes) and the yellow cotyledon (five genotypes) (Figure 1). The formation of a black seed coat is mainly attributed to the accumulation of anthocyanins and is genetically regulated by the *i* allele. The most common anthocyanins in black-seeded soybean types are delphinidin 3-O-glucoside, petunidin 3-O-glucoside, and cyanidin 3-O-glucoside (Choung et al., 2001).

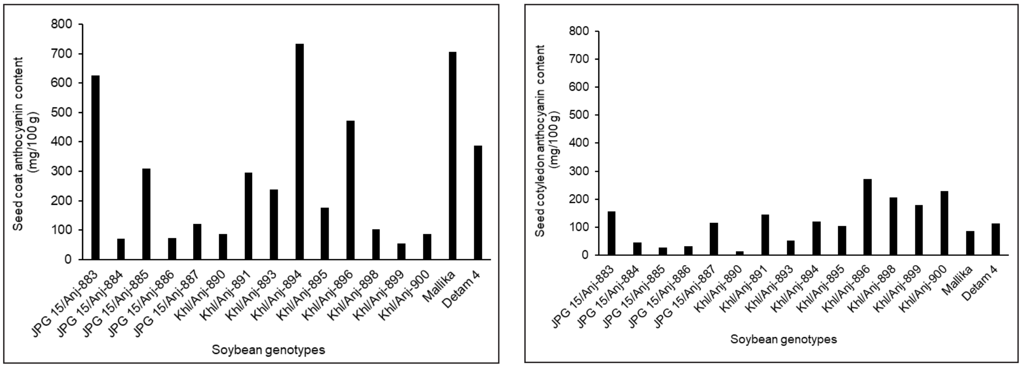
The variation in the seed coat colour of black soybean could be due to gene action. Gene *R* is responsible for seed hilum colour variation, while gene T is responsible for brown pubescent colour. The allelic combination *RT* displays a black seed coat colour, while the allelic combination *rT* displays a brown seed coat colour (Bernard & Weiss, 1973). In Indonesia, information on the genetic variation of the seed coat and cotyledon colours of black soybean is still limited. Meanwhile, in South Korea, soybeans with black seed coats and green cotyledons exhibit narrow genetic variation (Jo et al., 2021).

Figure 1. Variation in seed coat and cotyledon colours of 16 black soybean genotypes (A = seed colour; B = cotyledon colour; C = seed coat colour)

Anthocyanin Content

Black soybeans have the potential to provide nutritional and health benefits for humans (Bhartiya et al., 2020) due to the noticeable variations in compounds compared to yellow soybeans (Anjum et al., 2022; Li et al., 2024), such as anthocyanin. In this study, the TAC was measured in both the seed coat and the cotyledon. The average TAC of the black soybean genotypes was higher in the seed coat (283.69 mg/100 g) than in the cotyledon (118.25 mg/100 g). Previous studies also found a high amount of anthocyanin in the seed coat (Choi et al., 2020; Zhang et al., 2011). It has been estimated that over 99% of the total anthocyanin in black soybeans is found in the epidermal palisade layer of the seed coat, which is primarily responsible for the black colouration (Zhang et al., 2011).

The TAC range in the seed coat was 54.98-734.51 mg/100 g (Figure 2a) while it was 13.47-272.40 mg/100 g in the cotyledon (Figure 2b). The highest anthocyanin content in the seed coat was Khl/Anj-894 (734.51 mg/100 g), higher than that of the check cultivar of Mallika (707.29 mg/100 g). Both genotypes had black seed coat colour. The highest anthocyanin content in seed cotyledons was Khl/Anj-896 (272.40 mg/100 g) and Khl/Anj-900 (229.02 mg/100 g)—both had green cotyledon colour. Soybeans with black seed coats and green cotyledons have been commonly utilised in medicinal treatments in Japan, Korea, and China (Jo et al., 2021). A study on Korean black soybean revealed that the range of TAC was from 19.8 to 1,420.4 mg/100 g (Lee et al., 2016). Another study conducted by Choi et al. (2020) linked anthocyanin content with soybean seed size and reported that the TAC varied in small seeds (280.16-1085.89 mg/100 g), medium seeds (189.461-1191.028 mg/100 g), and large seeds (457.508-2633.454 mg/100 g). The available information on the variation and anthocyanin content among soybean genotypes provides an excellent opportunity to develop soybean varieties with high anthocyanin content.



(a) (b)

Figure 2. Anthocyanin content of 16 black soybean genotypes in the (a) seed coat and (b) seed cotyledon

Isoflavone Composition

The isoflavone composition, which consisted of daidzein, glycitein, and genistein, varied among black soybean genotypes. The contents of daidzein, glycitein, and genistein ranged from 9.42 to 27.94 mg/kg, 26.80 to 67.14 mg/kg, and 12.93 to 34.52 mg/kg, respectively. Among 16 genotypes, the average glycitein was higher than daidzein and genistein. Several studies found a higher variation in daidzein than in genistein (Anjum et al., 2022; Sumardi et al., 2017). Furthermore, another study found a higher genistein content (de Oliveira et al. 2024). These results suggest that the variation in the isoflavone content was influenced by environment (E), genotype (G), and the GE interaction (Choi et al., 2020).

The check cultivars of Mallika and Detam 4 had the highest daidzein (27.94 and 25.39 mg/kg, respectively). The Detam 4 had the highest glycitein (67.14 mg/kg), followed by Khl/Anj-896 (49.30 mg/kg). The highest genistein was found in JPG 15/Anj-883 and Mallika n (34.52 and 33.66 mg/kg, respectively). These findings show that Mallika has excellent daidzein and genistein contents, while Detam 4 has excellent daidzein and glycitein contents. The JPG 15/Anj-883 showed high glycitein and genistein (Figure 3). The biological activities of genistein and daidzein are widely recognised. However, genistein was reported to have about ten times higher biological activity than daidzein and glycitein (Kocar et al., 2019). Numerous significant effects of genistein have been reported, including anti-cancer activity (Yu et al., 2020), suppression of axillary osmidrosis (Saito et al., 2020), and potential nematocidal activities against soybean cyst nematodes (Ma et al., 2022). Several studies have also shown that genistein and other isoflavones derived from soybean roots affect the diversity of microbial development, notably fungus populations (Colpas et al., 2003; Guo et al., 2011).

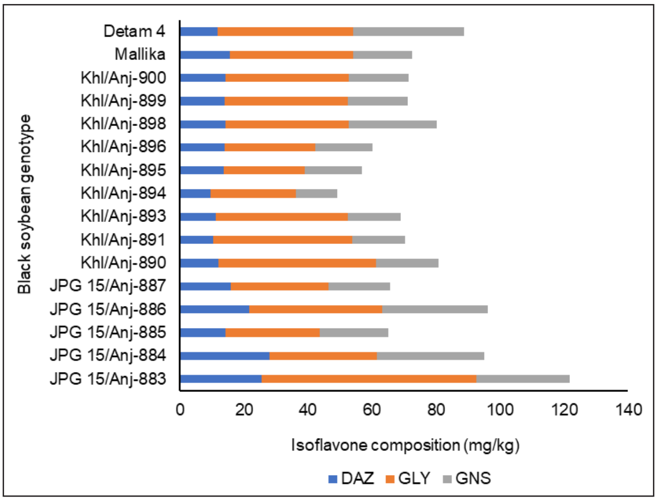


Figure 3. Isoflavone composition (daidzein, glycitein, and genistein) of 16 black soybean genotypes

Antioxidant Activity

Antioxidants are compounds that support human health by neutralising damaging free radicals in the body. These substances help to minimise oxidative stress, which is linked to a variety of chronic diseases and the ageing process. Previous research has revealed that the extract of black soybean showed strong antioxidant activity, indicating its potential use as an alternative anti-ageing agent due to the presence of phenolic chemicals that work as antioxidants (Asan et al., 2019). In this study, the determination of antioxidant activity was performed in the seed coat and cotyledon. The IC_{50} value was used to measure the antioxidant activity. It is determined as the substrate concentration required that causes a 50% loss of the DPPH activity (Molyneux, 2004). Hence, the lower the IC_{50} value, the higher the antioxidant activity (Rivero-Cruz et al., 2020). Accordingly, the black soybean cotyledon of all genotypes has a weak antioxidant activity, with the IC_{50} value ranging from 4,115.47 - 6,813.84 ppm. In contrast, the antioxidant activity in the seed coat ranged from strong to weak, with a range of 5,595.05 -78.29 ppm. These findings are consistent with previous studies, which reported that antioxidant activity was primarily found in the seed coat (Soedarjo, 2019; Lim et al., 2021).

In this study, black soybean lines (Khl/Anj-894, Khl/Anj-896, and Khl/Anj-898 showed strong antioxidant activity in the seed coat. Meanwhile, eight lines exhibited intermediate antioxidant activity, while the other lines had low antioxidant activity (Table 2). Three lines with strong antioxidant activity had black and brown seed coats. Significant polymerised procyanidin and anthocyanin content in the black seed coat of soybeans may explain the excellent antioxidant effect (Takahata et al., 2001). In line with research, Lee et al. (2020) found that soybean genotype 42 KSBLs exhibited higher levels of phytochemicals and antioxidant activities.

A study by Prvulović et al. (2016) showed variation in the antioxidant activity of five soybean genotypes. In other crops, such as *Vigna unguiculata*, there was also variation in antioxidant potential (Saeed et al., 2020). A high variation in the antioxidant activity of black soybean (10.99-20.38 µmol TE/g) was also reported by Yusnawan et al. (2016).

Table 2
The IC_{50} values and antioxidant activities of 16 black soybean genotypes

No.	Genotype	Cotyledon		Seed coat	
		IC_{50} value	**Category**	**IC_{50} value**	**Category**
1	JPG 15/Anj-883	4,606.23	Weak	116.23	Intermediate
2	JPG 15/Anj-884	4,115.47	Weak	129.85	Intermediate
3	JPG 15/Anj-885	4,876.50	Weak	164.70	Intermediate
4	JPG 15/Anj-886	4,701.73	Weak	114.72	Intermediate
5	JPG 15/Anj-887	5,212.34	Weak	105.26	Intermediate
6	Khl/Anj-890	6,782.94	Weak	4,127.60	Weak
7	Khl/Anj-891	5,115.20	Weak	5,595.05	Weak
8	Khl/Anj-893	5,928.30	Weak	4,381.18	Weak
9	Khl/Anj-894	4,273.08	Weak	78.29	Strong
10	Khl/Anj-895	4,479.00	Weak	104.89	Intermediate
11	Khl/Anj-896	4,989.82	Weak	82.77	Strong
12	Khl/Anj-898	5,312.81	Weak	84.09	Strong
13	Khl/Anj-899	4,365.55	Weak	3,292.59	Weak
14	Khl/Anj-900	4,875.08	Weak	2,892.42	Weak
15	Malika	6,813.84	Weak	130.07	Intermediate
16	Detam 4	4,833.56	Weak	104.89	Intermediate

Performance and Correlation of Agronomic Traits

The agronomic traits showed variation among the black soybean genotypes (Table 3). The performance of the agronomic traits was presented in Figure 4. Based on the performance of the agronomic traits, it was noted that the plants grew normally. The range of seed was 2.54 to 3.89 t/ha, with a range of days to maturity from 81 to 87 days. These indicate that soybean genotypes have a high genetic potential.

Genotype and environment have a significant impact on soybean seed yield and yield components. As new cultivars are developed through breeding, it is important for breeders to investigate the correlations between yield and yield components (Mecha et al., 2017). The study of the effect of agronomic traits on seed yield showed that plant height (r = -0.602*), number of nodes (r = 0.502*), and number of empty pods (r = -0.703*) had a significant correlation with seed yield (Figure 5).

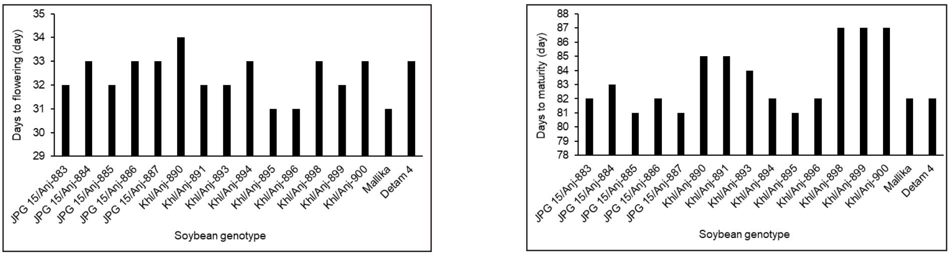
Table 3
Analysis of variance for agronomic traits of 16 black soybean genotypes

Traits	**Symbol**	**Mean Square**	
		Replication	**Genotype**
Days to flowering (days)	DTF	0.5208^{ns}	3.2291**
Days to maturity (days)	DTM	7.8541**	19.0958**
Plant height (cm)	PHG	694.9980**	192.8692**
Number of branches	NOB	0.1297^{ns}	3.3707**
Number of nodes	NON	54.1696^{ns}	118.2683**
Number of filled pods	NFP	388.0618**	322.8526**
Number of empty pods	NEP	2.4340^{ns}	4.6653**
100 seed weight (g)	SWG	0.3536*	4.7384**
Seed yield (t/ha)	YIELD	0.0858**	0.4085**

Note. ns = not significant; * = significant at 5 % probability level ($p < 0.05$); ** = significant at 1 % probability level ($p < 0.01$)

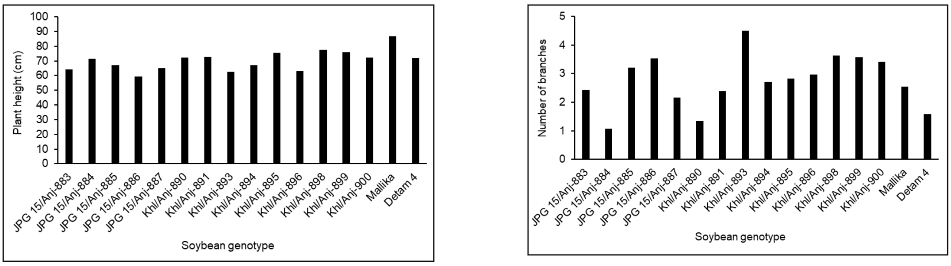
In this study, plant height was negatively correlated with seed yield. A higher plant may not consistently demonstrate a higher yield because tall plants may have a denser canopy, which limits the interception of photosynthetically active radiation in the plant's lower parts (Vu et al., 2019). Higher seed yields have been observed in soybean cultivars with greater efficiency in intercepting photosynthetically active radiation within the vegetative canopy (Müller et al., 2017).

In this study, the number of nodes showed a positive correlation with seed yield. This result is similar to previous studies (Hapsari et al., 2021; Karyawati & Puspitaningrum, 2021). This finding implies that the number of filled pods was influenced by the number of nodes. The number of pods per unit area is frequently correlated with seed yield, suggesting that the number of pods with nodes affects soybean yield (Egli, 2013). Additionally, the number of nodes per plant could contribute to the changes in the number of pods per plant (Egli, 2013; Wei & Molin, 2020). The present study also revealed a significant negative correlation between the number of empty pods and seed yield, indicating that an increase in non-reproductive nodes may reduce seed yield potential (Du et al., 2020; Egli, 2013). Information on the relationship between soybean yield and yield components can be beneficial in the selection of parental lines for producing cultivars with higher genetic yield potential. Based on these findings, the indirect selection for short plants could increase the seed yield in soybean, and the number of nodes can be used as one of the indicators to obtain high-yielding soybean genotypes in the breeding selection stage.



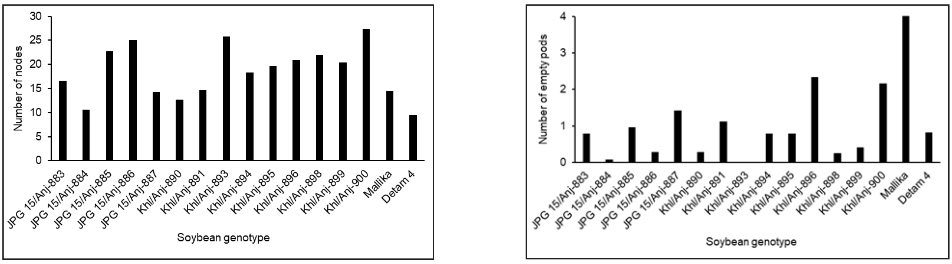
(a) Days to maturity

(b) Days to flowering



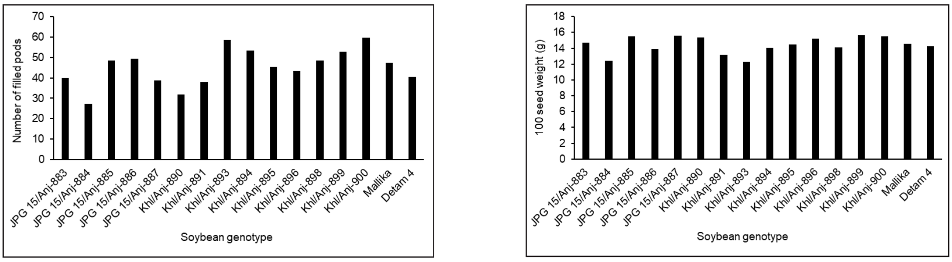
(c) Plant height

(d) Number of branches



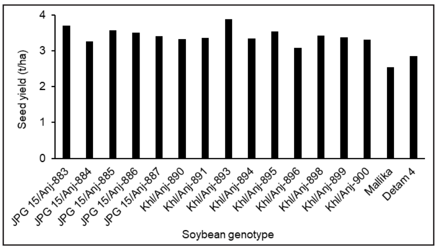
(e) Number of nodes

(f) Number of filled pods



(g) Number of empty pods

(h) 100-seed weight



(i) Seed yield

Figure 4. The agronomic trait performance of 16 black soybean genotypes

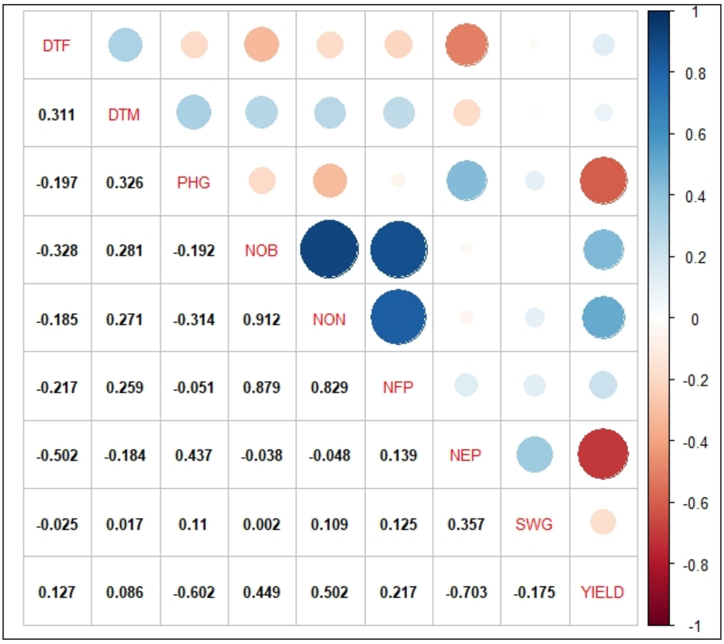


Figure 5. Correlation among agronomic traits of 16 black soybean genotypes. The limit of significance correlation is ≥ ± 0.50; the agronomic trait codes are shown in Table 3

Correlation between Yield and Bioactive Properties

No significant correlation was found between seed yield and the bioactive properties (TAC, antioxidant activities, isoflavone), nor among the bioactive properties, except for daidzein with genistein (Figure 6). A non-significant correlation between seed yield and both daidzein and genistein was also reported by Laurenz et al. (2017). However, a previous study by Vyn et al. (2002) revealed a positive correlation between isoflavone concentrations and seed yield. In the present study, daidzein and genistein were strongly and positively correlated with each other ($r = 0.676^{**}$).

The individual isoflavones (daidzein, glycitein, genistein) also showed no significant correlation with the TAC and antioxidant activity, respectively. This finding is in line with earlier studies, which reported no significant correlation between anthocyanins and isoflavones, or between antioxidant activity and isoflavone content (Choi et al., 2020; Lee et al., 2016). Another study found negative or weak associations between individual isoflavones with particular anthocyanins, indicating a trade-off between their biosynthesis pathways (Wu et al., 2017). However, the lack of significant correlation between the seed yield and the functional properties found in this study indicates the possibility of improving these functional properties without significantly lowering seed yield.

DAZ							
0.334	GLY						
0.676	0.364	GNS					
-0.164	-0.047	-0.201	TACs				
-0.118	0.105	-0.221	0.127	TACsc			
-0.323	0.238	-0.279	-0.257	0.096	ANTs		
-0.422	0.286	-0.427	-0.03	-0.317	0.331	ANTsc	
0.105	0.162	-0.073	-0.17	-0.355	-0.29	0.254	YLD

Figure 6. Correlation between yield and functional properties of 16 black soybean genotypes. The limit of significance correlation is ≥ ± 0.50; DAZ = daidzein; GLY = glycitein; GNS = genistein; TACs = total anthocyanin content in seed; TACsc = total anthocyanin content in seed cotyledon; ANTs = antioxidant activity in seed; ANTsc = antioxidant activity in seed cotyledon; YLD = seed yield

Genotype Evaluation Based on Selected Agronomic Traits and Bioactive Properties

The characteristics of modern soybean varieties are determined by bioactive properties, yield potential, and agronomic performance. Large seed size is one of these characteristics that could be improved to meet the specific demand of society. In addition, large-seeded soybeans were used by the industry as raw material for tempeh production (Krisnawati & Adie, 2015). In this study, the genotype evaluation was based on important agronomic traits (large seed size and high yield) and bioactive properties.

A GT biplot with a polygon view was used to help identify genotypes with the highest values for one or more traits (Figure 7). The biplot was divided into six sectors, with ten traits falling into three sectors. The genotype with the greatest value of the traits within each sector was considered to be the vertex genotype (Paramesh et al., 2016). Figure 7 illustrates that the vertex genotypes are G2, G6, G7, G8, G9, G11, and G15. The GT biplot suggests that G2 has the highest or near-highest value for all isoflavones (daidzein, glycitein, genistein). The genotypes G6, G7, and G8 demonstrated superior performance in seed yield and antioxidant activity in the seed and cotyledon. The genotypes G9, G11, and G15 exhibited superior performance in the large seed size and TAC in the seed and seed cotyledon.

The identified soybean genotypes with specific superior traits could be used as elite genetic resources for soybean breeding programs or be further developed to improve black soybean cultivars for industrial purposes. For example, genotypes with high daidzein, glycitein, and genistein contents could be utilised as parents to breed soybean lines with enhanced isoflavone levels and composition, which are desirable by the industry.

Previous research findings have focused on evaluating the content of bioactive properties. However, research that simultaneously assesses both yield-related agronomic traits and functional properties remains limited. By applying the GT biplot approach, the results of this study provide valuable insights for identifying modern black soybean varieties that combine excellent agronomic performance with high health benefits.

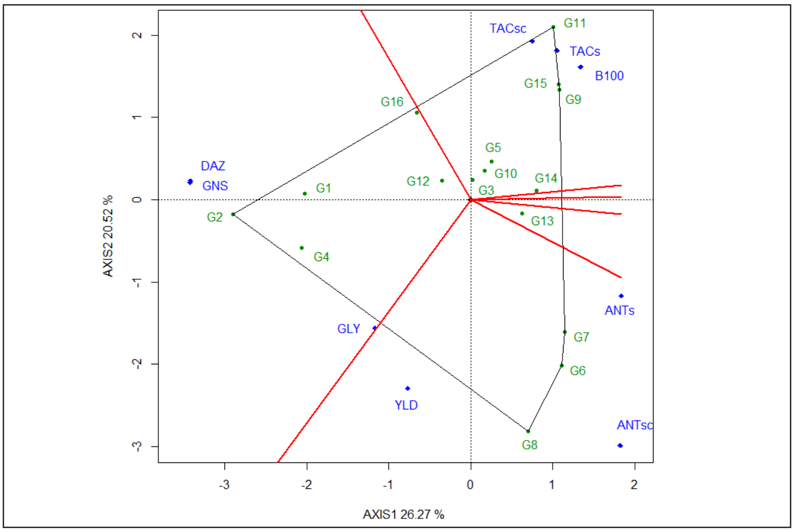


Figure 7. Polygon view of the "which-won-where" graph of genotype-by-trait biplot, illustrating which genotypes exhibit the highest values for specific traits. DAZ = daidzein; GLY = glycitein; GNS = genistein; TACs = total anthocyanin content in seed; TACsc = total anthocyanin content in seed cotyledon; ANTs = antioxidant activity in seed; ANTsc = antioxidant activity in seed cotyledon; B100 = 100-seed weight; YLD = seed yield

CONCLUSION

The anthocyanin content, isoflavone composition, antioxidant activity, and agronomic traits of black soybeans varied among genotypes. The anthocyanin content of black soybeans in the seed coat was higher than in the seed cotyledon. The isoflavone composition showed that the average glycitein content was higher than daidzein and genistein. The antioxidant activity in the seed coat ranged from low to strong, while the seed cotyledon showed a moderate to strong antioxidant activity. The number of nodes can be used as one of the selection indicators to obtain high-yielding soybean genotypes.

The soybean genotype G2 (JPG 15/Anj-884) had the highest values for the traits of all isoflavones (daidzein, glycitein, genistein). Genotypes G6 (Khl/Anj-890), G7 (Khl/Anj-891), and G8 (Khl/Anj-893) exhibited superior performance in seed yield and antioxidant activity in both seed and cotyledon. Genotypes G9 (Khl/Anj-894), G11 (Khl/Anj-896), and G15 (Mallika) showed superior performance in seed size and total anthocyanin content in both the seed and the cotyledon. These genotypes represent promising candidates for further improvement of new black soybean cultivars, either through selection as pure lines or as parental lines in the breeding program.

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