

Performance of agronomical and seed biochemical traits of soybean genotypes in relation to their tolerance and preference by *Nezara viridula*

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Abstract. Kuswanto H, Ginting E, Utomo JS, Yusnawan E. 2019. Performance of agronomical and seed biochemical traits of soybean genotypes in relation to their tolerance to and preference by *Nezara viridula*. *Biodiversitas* 20: 356-363. Qualitative and quantitative variations of biochemical compounds in plant organs is one of the causes leading to infestation by a certain pest which prefers to feed on particular genotypes than other genotypes. Objectives of this study is to find out the relationship of selected agronomical features and seed biochemical contents of soybean genotypes with their tolerance to *Nezara viridula* and also the preference of *N. viridula* to these genotypes. A total of 50 soybean germplasm were grown at Muneng research station during the dry season of 2016. The variability of the tested germplasm was reflected in the diversity of agronomic characters and seed biochemical contents. There was a significant positive correlation among the studied seed biochemical parameters, namely protein, methionine and cysteine contents. The protein and cysteine contents did not relate to *N. viridula* preference, but it was related to soybean tolerance against *N. viridula*. It was indicated by the insignificant positive correlation of protein and cysteine contents with the number of unfilled pods and the negative correlation of protein and cysteine contents with the ratio of number of unfilled pods to the total number of pods. Methionine content increased *N. viridula* preference, but it had no effect on soybean tolerance. Five genotypes, namely MLGG 0164, MLGG 0195, MLGG 0103, MLGG 0896 and MLGG 0196 were selected based on high seed biochemical contents which can be used as gene sources in soybean breeding programs. Among these five genotypes, MLGG 0195 had high protein, methionine and cysteine contents, but it was not tolerant to *N. viridula*. Hence, this genotype should be cultivated by controlling *N. viridula* optimally. MLGG 0196 had the highest methionine content, relatively high cysteine content, and *N. viridula* tolerance. This genotype can be used to improve seed biochemical content and plant tolerance against *N. viridula*.

Keywords: cysteine, methionine, *Nezara viridula*, pest preference, protein, soybean, host tolerance

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) has a strategic position in food security as a source of protein and functional, high quality food ingredients for human needs. Soybean is an important source of vegetable food material with 35-45% protein content and it plays an important role in various aspects of the economy in Indonesia. Major share of soybean produced in Indonesia is used as food while the rest is used as animal feed and seed. Indonesia's soybean consumption is 7.13 kg per capita per year (Ministry of Agriculture 2015). The highest consumption is in the preparation of traditional tofu and tempeh which is 0.144 kg and 0.134 kg per capita per week, respectively. This is even higher than the consumption of beef which is 0.008 kg per capita per week (BPS 2017). The consumption of tofu and tempeh is higher than beef because of the lower price of tofu and tempeh than beef. Therefore, soybean is a valuable protein source for Indonesian people allowing replacement of beef protein.

Besides protein, soybean also contains many amino acids. Two of those amino acids are methionine and cysteine. However, the availability of methionine and cysteine is very limited (Gupta et al. 2010). These amino

acids are necessary because of their linkage to many diseases such as Alzheimer, cardiovascular and diabetes (Ball et al. 2016). The functions of cysteine and methionine are similar—they serve as cellular antioxidants and protein's structure stabilizer (Kim et al. 2014). The human immune system can be stimulated by natural diet ingredients involving amino acids (Wu 2013). Addition of cysteine and methionine in a natural diet can improve human health and avoid certain diseases.

Differences in plant resistance to certain insects are often due to the chemical factors present in those plants, both qualitatively and quantitatively. Biochemical factors in plants, especially toxic alkaloids (Ge et al. 2015), can inhibit the physiological processes of the insects. The lack of one or more of the substances required by the insects, such as protein (Moreno et al. 2011) and amino acids (Karley et al. 2002), also inhibits insect metabolism and influence insect life. Proteins are needed for growth and development of insects. The quality of proteins depends on the amino acids and the requirement of protein and amino acids varies from insect to insect. However, the presence of some proteins may also have negative impact on the insects (Minney et al. 1990; Ryan 1990; Hartweck et al. 1997).

Nezara viridula (Linnaeus, 1758), commonly known as the southern green stink bug, is one of the insect pests feeding on plant organs, especially immature soybean pods. Many studies regarding the preference of *N. viridula* to plant characteristics have been reported. However, studies on preference of *N. viridula* to the biochemical contents of soybean seeds are very limited. Therefore, we have studied the protein, methionine and cysteine contents of soybean seeds in relation to *N. viridula* preference and soybean tolerance.

MATERIALS AND METHODS

Study sites

The field studies with soybean plants were conducted at Muneng Research Station which is located in Muneng Kidul Village, Sumberasih Sub-district, Probolinggo District, East Java Province, Indonesia. Its geographical co-ordinate are 7°48'06.9" S and 113°09'37.2" E, and the altitude is 54 m above sea level. Protein content of seeds was studied at Food laboratory of Indonesian Legume and Tuber Crops Research Institute (Malang Regency, East Java Province, Indonesia; 8°2'50.063" S and 112°37'24.092" E), and methionine and cysteine contents were analysed at Saraswanti Laboratory (Bogor City, West Java Province, Indonesia; 6°33'23.649" S and 106°46'12.579" E).

Plant materials

Fifty types of soybean germplasm were used as planting materials which includes Indonesian landraces and introduced accessions. The Indonesian landraces were originated from various islands of Indonesia, while the introduced accessions were from other countries.

Experimental Design and planting

The planting experiment was conducted as randomized complete block design with three replications. Planting was done in 40 cm x 15 cm planting space. Each genotype was planted in two rows of 3 m length. Fertilization was provided in the form of 50 kg ha⁻¹ of Urea, 75 kg ha⁻¹ of SP36, and 75 kg ha⁻¹ of KCl. To prevent seedling fly pest, seeds were treated with 12.5 g carbosulfan for every 1 kg seed. Weeding was performed at an interval of every three weeks (3rd, 6th and 9th weeks), after planting.

Pest infestation

Infestation of soybean plants with *N. viridula* was carried out naturally by growing infested soybean plants for 40 days around the planting plots. At the time of pod filling, the infestation plants were harvested so that *N. viridula* moved to the test plants and infested them.

Agronomical characters, pest preference and plant tolerance observation

Nezara viridula preference and plant tolerance was analysed by observing certain agronomical characters of soybean such as total number of pods, number of unfilled pods, weight of 100 seeds and seed weight per plant.

Preference of *N. viridula* is indicated mainly by number of unfilled pods, based on the assumption that soybean genotypes having more number of unfilled pods are more favorable genotypes for *N. viridula*. Plant tolerance to *N. viridula* was measured by the ratio of the number of unfilled pods to total number of pods. A higher ratio is indicative of lower tolerance and vice versa.

Protein analysis

The protein content of the fifty genotypes studied was measured using the Mikro Kjeldhal method (AOAC 2005). The seed sample was digested with concentrated H₂SO₄ prior to distillation, the distillate is treated with boric acid and subsequently titrated against a HCl. A blank (without sample) was also prepared and subjected to the entire procedure. The titrate volume was used to calculate the total Nitrogen and protein content. The calculation was done by multiplying the % N with a conversion factor of 5.75.

Methionine and cysteine analysis

Methionine and cysteine content of 20 genotypes which represent low, medium and high protein genotypes were estimated by using AccQ Tag UPLC method, according to Rohman and Gandjar (2007) and Waters System Guide (2012).

RESULTS AND DISCUSSION

Performance of some agronomical and chemical characters

The effect of *N. viridula* infestation on studied agronomical characters of 50 soybean germplasms is shown in Fig. 1. Number of filled pods per plant ranged from 5.8-82.0, with a mean of 28.5 pods. This was lower than the number of unfilled pods per plant for which the mean was 43.7 pods per plant. Although the mean value for the number of filled pods was lower than that for number of unfilled pods, the standard deviation for number of filled pods was higher. The high standard deviation indicated a broad variability. The broad variability may be due to the fact that the plant materials used in this study originated from various regions of Indonesia and also from abroad.

The number of total pods in this study ranged from 42-114 pods per plant and the mean value was 72 pods (Fig. 2). Usually, in plants under normal conditions without pest infestation, the number of unfilled pods was very low and could be ignored. Similar results were reported in studies related to the effect of environment stresses, such as waterlogging and acidic dryland, which produced maximum of 3 unfilled pods with the mean value of 1.47 pods (Kuswanto 2015) and 6 pods with the mean of 3 pods (Kuswanto 2016), respectively. The number of total pods illustrated the maximum potential for the number of filled pods. This potential may be lower than the true potential number of total pods because some pods may have fallen due to the *N. viridula* infestation. In this study, number of fallen pods was not noted. In the normal condition, Malek et al. (2014) obtained an average of 48.13

total pods per plant which was much lower than the value obtained during the present experiment.

The ratio of number of unfilled pods per plant to the number of total pods is an indication of the plant tolerance against *N. viridula*. Infested plants producing a large number of total pods are having a higher tolerance than those plants producing comparatively less number of total pods. Plant tolerance differs from the pest preference where the number of unfilled pods indicates that a genotype is preferred by the pest. In soybean germplasms viz., MLGG 0564, MLGG 0879, MLGG 0196, and MLGG 0892, the ratio obtained was 0.22, 0.31, 0.37 and 0.38, respectively. These four soybean genotypes had the highest tolerance value than others.

The weight of 100 seeds in this study was also very diverse, which ranged from 8.02 to 20.11 g. Majority of seed sample weights were in the range of 14-16 g/100 seeds (Fig. 3). Some genotypes showed seed size greater than 16 g/100 seeds. These genotypes could be used as a source of genes for improvement in seed size. Kuswanto (2017) also reported a wide genetic diversity in soybean

seed size. *N. viridula* feeds on developing seeds causing yield reduction. Some protein kinases are involved in tolerance response when a developing seed is fed by the pest (Giacometti 2016).

Seed yield ranged from 0.66-11.45 g/plant with a mean of 4.32 g per plant (Fig. 3). The highest number of genotypes are in the class of 3 g plant⁻¹ seed yield, followed by the class of 5 g plant⁻¹. Based on the distribution of the seed yield, it may be opined that the tested germplasms have a broad variability with respect to this character also, as reported earlier by Kuswanto (2017). Naik et al. (2016) also reported a high coefficient of genetic diversity regarding seed yield per plant. In the present study, two genotypes were found to be producing seed yield of about 11 g plant⁻¹, namely MLGG 0564 (11.45 g) and MLGG 0196 (10.95 g). These two are prospective genotypes which may be further used for improving soybean seed yield. The seed yields of these two genotypes are higher than the highest seed yield in normal conditions, as reported by Islam et al. (2016).

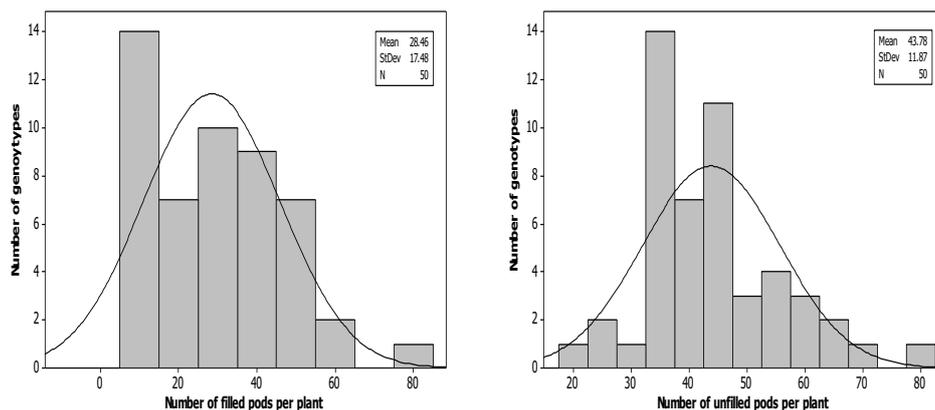


Figure 1. Number of filled pods (left) and unfilled pods (right) per plant of fifty soybean genotypes

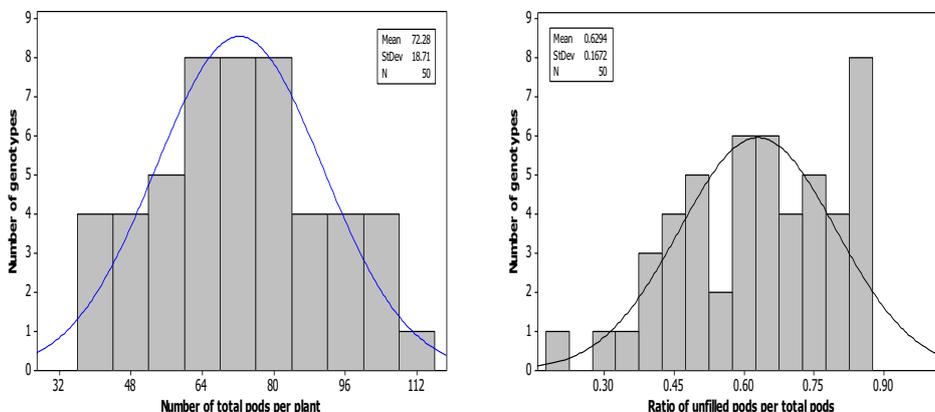


Figure 2. Number of total pods (left) and ratio of unfilled pods (right) to total pods of fifty soybean genotypes

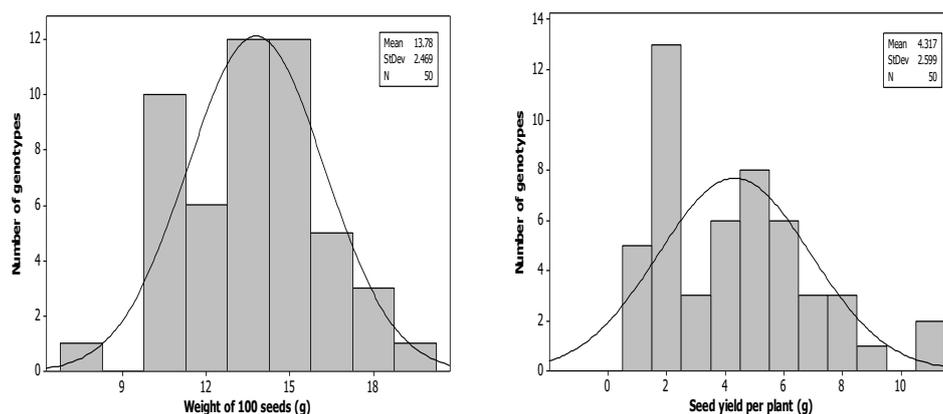


Figure 3. Weight of 100 seeds (*left*) and seed (*right*) yield per plants of fifty soybean genotypes

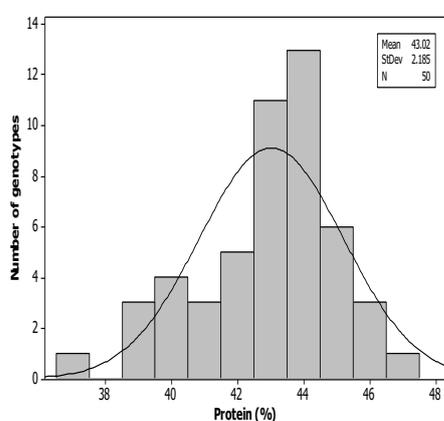


Figure 4. Seed protein content of fifty soybean genotypes

The seed protein content of 50 tested genotypes ranged from 36.82% to 47.23%. The mean of the protein content for this population was 43.02%. In general, the protein contents were in a class of 33% and 34% (Fig. 4). Hwang et al. (2014) reported that a soybean genotype may contain 35-50% of protein. A protein content of more than 45% is considered as high. In this study, there were several genotypes with a high protein content, such as MLGG 0164, MLGG 0195, MLGG 0169 and MLGG 0091 with protein contents of 47.23, 46.44, 46.32 and 46.00% respectively. Seed protein content is a quantitative trait controlled by many genes, and the interaction with the environment effects seed protein content (Hwang et al 2014). The proteomic adjustments during seed filling and increasing selective accumulation to maintain normal protein content due to the compensation for shortage of major proteins (Schmidt et al. 2011) are the indication of genes control existence. Interaction between genes and environment is also reflected in the soil nutrient such as nitrogen and sulfur which are involved in the biosynthesis of protein and sulfur-containing amino acids (Yi et al. 2010).

Relationship between protein content and agronomical characters

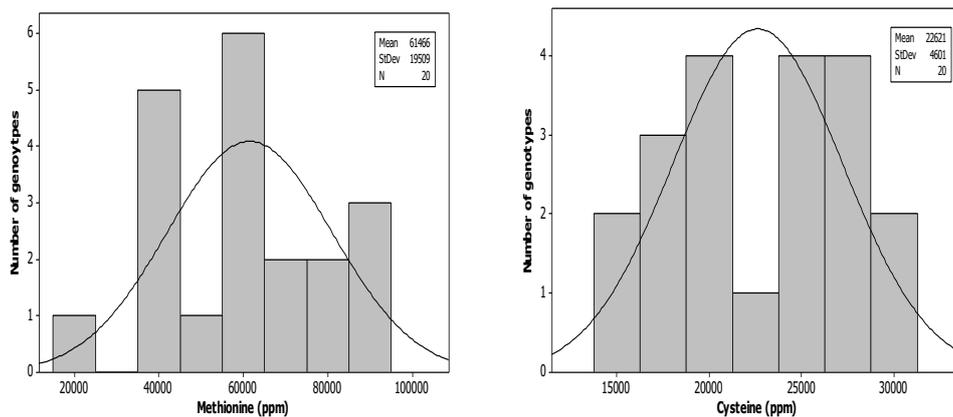
The protein content is closely linked with all observed agronomical characters, except the number of unfilled pods per plant (Table 1). Seed protein content can affect soybean agronomical characters in conjunction with the infestation of *N. viridula*. In this study, the characters that can describe causal relationship between the protein content and infestation of *N. viridula* is the number of unfilled pods and the ratio of unfilled pods to total pods. The number of unfilled pods per plant is indicative of the preference of *N. viridula* to a genotype. More the number of unfilled pods, more is the preference of *N. viridula* for that genotype. In this study, as mentioned earlier, there was no correlation between the number of unfilled pods and the seed protein content. There can be two possible explanations for this. The first is that protein is not a substrate attracting *N. viridula* to feed on soybean seeds. The second is that *N. viridula* does not feed on the seeds completely, but only feed partially on small sized seeds. This study corroborates the second possibility because there is a correlation between the weight of 100 seeds and protein content (Table 1). The resistance of soybean genotypes varies from the least infested, low yield reduction, low levels of damage in seeds and low levels of leaf retention (Souza et al. 2016). There are some gene families associated with nucleotide-binding and receptor-like proteins serving in the plant's biotic resistance (McHale et al. 2012). The preference is caused by metabolic processes in the pest showing a pest having different characteristics compared to other pests.

Soybean plant has tolerance mechanism to limit reduction in seed yield (Souza et al. 2016). The ratio of unfilled pods to total pods describes the tolerance of a genotype to *N. viridula* infestation. Therefore, as the ratio of unfilled pods to total pods increases, the tolerance of a genotype against *N. viridula* decreases. In this study, the protein content correlated negatively with the ratio of unfilled pods to total pods. This means that the higher protein content, the lower is the ratio of unfilled pods to total pods. This is also supported by the significant correlation between the number of filled pods and protein content.

Table 1. Correlation among some agronomical characters and protein contents

	Fpod	Upod	Tpod	Rpod	W100	Yield
Protein	0.422**	0.065	0.434**	-0.399**	-0.404**	0.317*
Fpod		-0.233	0.783**	-0.943**	-0.383**	0.938**
Upod			0.421**	0.461**	-0.111	-0.312*
Tpod				-0.585**	-0.428**	0.676**
Rpod					0.277	-0.928**
W100						-0.124

Fpod = number of filled pods per plant, Upod = number of unfilled pods per plant, Tpod = number of total pods per plant, Rpod = ratio of unfilled pods to total pods per plant, W100 = weight of 100 seeds (g), Yield = seed yield per plant (g), Protein = protein content of seeds (%)

**Figure 5.** Methionine (*left*) and cysteine (*right*) contents of twenty soybean genotypes

Relationship between methionine-cysteine content and agronomical characters

Protein content has a close relationship with the profile of essential amino acids, such as methionine and cysteine contents (Vaughn et al. 2014). The content of methionine and cysteine was measured in 20 genotypes, which are chosen to represent the entire range of protein content (lowest to highest) of all the 50 genotypes included in this study. Methionine content ranged from 23508 to 91128 ppm with a mean of 61466 ppm (Figure 5). Most genotype had methionine content between 40000 to 60000 ppm. Song et al. (2013) reported that methionine content of soybeans varies from 9592 ± 328 to 2616 ± 22 340 mmol g⁻¹ fresh weight seed. Certain biosynthesis pathways contribute to seed methionine content (Amir et al. 2012), leading to a complex metabolism originating from multiple gene expression. Three genotypes studied, i.e. MLGG 0196, MLGG 0164, and MLGG 0195, showed methionine contents up to 90000 ppm. These three genotypes can be used as source genes for the improvement of methionine content of soybean.

The cysteine content varied between 15112 to 30122 ppm, with the mean value of 22621 ppm (Figure 5). Majority of the tested genotypes showed cysteine content around 22500 ppm. Two genotypes, i.e. MLGG 0896 and

MLGG 0195, had cysteine content of as high as 30000 ppm. Cysteine protease is the primary protease in the gut of *N. viridula* (Lomate and Bonning 2016) due to which *N. viridula* prefer cysteine rich soybeans. The soybeans with higher free cysteine also had a high concentration of Bowman-Birk protease inhibitor, a protein with rich in cysteine (Kim et al. 2012). Bowman-Birk protease inhibitor is also a known anticarcinogenic agent (Kennedy 1998).

Methionine content was significantly correlated with the number of unfilled pods. This fact indicates that increase in methionine content also increases the preference of *N. viridula*. Besides being an important plant nutrient required for the biosynthesis of methionine and cysteine, sulfur can be also metabolized to form molecules which are capable of protecting plants from oxidative and environmental stresses (Yi et al. 2010). The total number of pods, which is a combination of both filled and unfilled pods, were significantly correlated with methionine content (Table 2). As the number of filled pods is not significantly correlated, the greater influence on the correlation was due to the number of unfilled pods. In most cases, a high methionine content correlates with abnormal phenotypes causing yield reduction (Galili and Amir 2013). However, there were no evidences for this report in the present study.

Table 4. Agronomical performance of five selected soybean genotypes with high protein, methionine and cysteine contents

Genotype	Fpod	Upod	Tpod	Rpod	W100	Yield	Protein	Methionine	Cysteine
MLGG 0164	36	70	106	0.66	10.69	4.21	47.23	90623.92	24782.27
MLGG 0195	33	55	87	0.63	13.50	5.12	46.44	90341.43	29069.35
MLGG 0103	48	66	114	0.58	10.74	6.45	45.14	84762.43	26948.54
MLGG 0896	45	47	92	0.51	10.17	4.70	44.84	74548.48	30122.02
MLGG 0196	63	37	100	0.37	14.84	10.95	44.73	91127.94	26265.43

Note: Fpod = number of filled pods per plant, Upod = number of unfilled pods per plant, Tpod = number of total pods per plant, Rpod = ratio of unfilled pods to total pods per plant, W100 = weight of 100 seeds (g), Yield = seed yield per plant (g), Protein = protein content (%dw), Methionine = methionine content (ppm), Cysteine = cysteine content (ppm)

Table 2. Correlation of methionine and cysteine contents with agronomical characters

	Fpod	Upod	Tpod	Rpod	W100	Yield
Methionine	0.436	0.444*	0.649**	-0.212	-0.205	0.373
Cysteine	0.663**	0.066	0.601**	-0.605**	-0.386	0.532*

Note: Fpod = number of filled pods per plant, Upod = number of unfilled pods per plant, Tpod = number of total pods per plant, Rpod = ratio of unfilled pods to total pods per plant, W100 = weight of 100 seeds (g), Yield = seed yield per plant (g), Methionine = methionine content (ppm), Cysteine = cysteine content (ppm)

Table 3. Correlation of among protein, methionine and cysteine contents

	Methionine	Cysteine
Protein	0.961**	0.999**
Methionine		0.963**

The cysteine content shows significant positive correlation with the number of filled pods, total number of total pods and seed yield per plant, but it has significant negative correlation with ratio of unfilled pods to total pods. There was no significant correlation between cysteine content and number of unfilled pods. Phytocystatins has the capability to inhibit heterologous enzymes and serve in plant defense. Decreased pest development and reproduction has been reported in the presence of high phytocystatins (Carrillo et al. 2012).

Relationship among protein, methionine and cysteine contents

The protein content was significantly correlated with methionine and cysteine contents. Similarly, the methionine content was significantly correlated to the cysteine content (Table 3). Ramamurthy et al. (2014) has also reported the correlation between methionine and cysteine content. This relationship also has a genetic correlation (Carlson 2011) having a high role in a plant breeding program. However, such a positive genetic correlations are not found between protein content and cysteine content (Carlson 2011). During seed development, biosynthetic pathway of cysteine is active, but the supply of cysteine does not affect methionine or protein synthesis

(Tabe et al. 2010). Song et al. (2013) proposed a positive relationship between methionine content and other amino acids synthesis which causes more seed proteins synthesis.

Performance of five genotypes with high biochemical contents

Five genotypes were selected from the 50 studied genotypes, based on their high biochemical contents namely, protein, methionine and cysteine. Their agronomical features and biochemical contents are presented in Table 4. The highest number of total pods was found in MLGG 0164 and MLGG 0196, which produced 106 and 100 pods respectively. However, these two genotypes had a different ratio of unfilled pods to total pods indicating their different tolerance levels against *N. viridula*. MLGG 0196 was more tolerant than MLGG 0164. Methionine and cysteine contents in these two genotypes were almost similar. This observation is not coinciding with the earlier finding that cysteine content has a negative correlation with plant tolerance. This deviation may be due to the presence of some morphological characters of the plant which are limiting the feeding of seeds of MLGG 0196 by *N. viridula*. In this study, however, the morphological characters were not observed. The MLGG 0195 had high protein, methionine and cysteine content. Similar to MLGG 0164, it also had a high ratio of unfilled pods to total pods describing low tolerance to *N. viridula*. The cysteine content in this genotype was similar to MLGG 0896, also having relatively low tolerance.

In conclusion, performance of the agronomical and chemical characters varied among the tested soybean germplasm. Most of the agronomical characters showed significant correlation with protein content. A significant correlation was also found among protein, methionine and cysteine contents. The protein and cysteine contents did not relate to *N. viridula* preference, but related to soybean tolerance against *N. viridula*. It is indicated by the insignificant correlation between protein and cysteine contents with number of unfilled pods, and also the negative correlation between protein and cysteine contents with the ratio of unfilled pods to total pods. Methionine content increased *N. viridula* preference, but had no effect on soybean tolerance to *N. viridula* infestation. Five genotypes, namely MLGG 0164, MLGG 0195, MLGG 0103, MLGG 0896, and MLGG 0196 were selected based on high seed biochemical contents. These genotypes can be used as gene sources in soybean breeding program. Among these five genotypes, MLGG 0195 had high protein,

methionine and cysteine contents, but it was not tolerant to *N. viridula*. Hence, this genotype should be cultivated by controlling *N. viridula* optimally. MLGG 0196 had the highest methionine content, relatively high cysteine content, and it was also tolerant to *N. viridula*. This genotype can be used in breeding programs to improve seed biochemical contents and soybean plant's tolerance against *N. viridula*.

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