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Variability of pod trichome and agronomic characters of several soybean genotypes

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Abstract. *Adie MM, Krisnawati A. 2017. Variability of pod trichome and agronomic characters of several soybean genotypes. Biodiversitas 18: 416-421.* Trichome potentially act as a plant defense against soybean pod feeders, hence, the identification of trichome variability on soybean pod becomes increasingly important. A total of 150 soybean genotypes was identified for their pod trichome length and density. The experiment was conducted from February to June 2014. All soybean genotypes were planted in Muneng Research Station, Probolinggo (Indonesia). Identification of trichome length and density was performed in Laboratory of Biology, Malang State University. Soybean pods were taken from the third nodes at R7 stage. Soybean yield, pod trichome length and density were grouped using cluster analysis. The days to maturity varied from 72 to 83 days (average 77 days), 100 seed weight ranged from 12.50 to 23.50 g (average 16.50 g), seed yield ranged from 1.51 to 4.28 t/ha (average 2.90 t/ha), pod trichome length varied from 56.9 to 176.5 µm (average 112.8 µm), and trichome density ranged from 12 to 59 per 4 mm^2 (average 27.64 per 4 mm^2). Cluster analysis on 150 genotypes based on yield, trichome length, and trichome density classified soybean genotypes into five major groups. The first group consisted of 61 genotypes, and was characterized by sparse trichome. Cluster II consisted of genotypes with short trichome, whereas cluster III was characterized by medium trichome length and trichome density. Cluster IV and V, each consisted of six genotypes, and was characterized by dense and longer trichome, respectively. G511H/Arg//Arg///Arg///Arg-19-7 was the most densely pubescent genotype, while G511H/Anjasmoro//Anjasmoro-5-6 was the genotype with the longest trichome. These genotypes could be potentially be used as gene sources for further improvement of trichome length or trichome density in soybean varietal production.

Keywords: Density, *Glycine max*, length, trichome

INTRODUCTION

Trichome (plant hairs) is single cells that develop on the surface of the epidermis (Marks 1997; Werker 2000). Various trichomes can be found on leaves, petioles, stems and pods of soybean plants. Trichome characteristics are varied between plants and among plant genotypes in the forms of shape, size, density, length, and hair orientation. The basic terminology that is used to describe the presence of trichome is the glabrous (hairs are completely lacking) and pubescent (having hairs) types. Five different types of trichome were found in pigeon pea and its wild relatives (Romeis et al. 1999). Johnson et al. (1988) found three main forms of trichome on tobacco plant, i.e. glandular, simple and hydathodes. Meanwhile, trichome on soybean was reported to be glabrous, curly, dense, sparse, irregular, slightly dense and normal (Broersma et al. 1972).

Trichome is an important morphological character of soybean plant, which contributes to the resistance to abiotic and biotic stresses. The existence of trichome was expected to play role in increasing soybean resistance to drought stress through suppressing the loss of water (Clawson et al. 1985; Stolf et al. 2009) and salinity (Dolatabadian et al. 2011). The progress of the research shows that the trichome is an important morphological character within soybean resistance against insect pests. A research by Norris and Kogan (1980) revealed that the trichome acts as an important defense against 32 insect pests. Furthermore, 14 insects exhibited antagonistic relationship with the presence of trichome thus increasing the degree of sensitivity. However, other five insects did not show a consistent relationship between trichome with a degree of resistance to the insect pests. The role of mechanism of trichome on *Grapholitha glicinivorella* (Nishijima, 1960) and *Planthypena scabra* (Pedigo 1971) led to spur the process of oviposition (egg laying). As a form of defense against soybean pests, trichome is very important trait to the Indonesian agricultural condition, where pest is a major threat within soybean cultivation.

So far, research on the role of trichome as one form of soybean resistance against pests has been more focusing on leaf-eating pests (Khan et al. 1986; Hulburt et al. 2004; Nobuyuki et al. 2005; Komatsu et al. 2010; Oki et al. 2012; Tong et al. 2013). This is based on the fact that economic losses due to leaf-eating pest infestations in the world soybean production centers were estimated to be higher than that due to the pod-feeders. By contrast, Indonesian soybean yield loss caused by pod-feeders was greater than the yield loss due to the leaf-eating pests.

Trichome on soybean pod could potentially be used as a form of morphological resistance against soybean pod-feeding pests. Suharsono (2009) reported that soybean lines with denser trichome were able to reduce the number of eggs and larvae of pod borer insect as well as the damage intensity it causes. Lam and Pedigo (2001), in their study on the effect of trichome density on soybean pod feeding

by adult bean leaf beetles, found that densely pubescent soybean has the potential to resist the bean leaf beetle feeding on pods. Similar result was also reported in stinkbug where trichome was also able to reduce the non-preference feeding. Various studies (Maulidah 2006; Suharsono and Sulityowati 2012) showed a negative correlation between the density and length of trichome with the damage intensity of pods and seeds caused by pod sucking pest. Genetic studies indicated that trichome characteristic was a simply inherited qualitative trait (Khan et al. 1986), and the number of trichome in a certain area was relatively fixed since the beginning of the growth of leaves. Furthermore, a single gene control of trichome production was also reported by Singh et al. (1971).

The role of trichome as morphological defense lies on its ability to break the interaction between the host and the insects through the intrusion on the perch process, and prevents the insect movement and feeding (Al-Ayedh 1997). Identification of diversity on trichome density and length of soybean pod is important and could potentially as a source of resistance genes within soybean improvement against pod-feeder pests.

The aims of this research were to identify and classify the diversity of trichome length and density on pods of 150 soybean genotypes.

MATERIALS AND METHODS

Research materials consisted of 150 soybean genotypes. The field research was conducted in Muneng Research Station, Probolinggo (East Java, Indonesia), from February to June 2014. The experiment was arranged in a randomized block design, consisted of 150 soybean genotypes as treatment, each was two replicates. Each genotype was planted in a 1.2 m × 4.0 m plot size, 40 cm × 15 cm planting distance, two plants per hill. Fertilizer of 250 kg ha^{-1} Ponska, 100 kg ha^{-1} SP36 and 1 t ha^{-1} organic fertilizer were applied before sowing. Land preparation was zero-tillage due to the land used was previously cultivated with wetland rice crop. Before planting, the planting plots were irrigated to maintain optimum soil moisture. Weed control was performed at two and four weeks after planting. Observations were carried out on days to maturity (when 95% of the leaves have turned yellow), 100 seed weight (by weighting randomly selected 100 seeds from the seed lot, in gram), and seed yield (by weighting total seed yield per plot and was then converted to t/ha).

At R7 stage (Fehr and Caviness 1977), one pod of each genotype in the third nodes was taken for measurements of trichome. The density and length of trichome were measured on adaxial and abaxial surface of the pod, respectively. Trichome density measurement was carried out on the area of 4 mm^2. Observations of trichome density and length were performed using a binocular CX31 microscope in Laboratory of Biology, State University of Malang. The grouping of the genotypes based on seed yield, trichome length and trichome density were conducted by using cluster analysis. Cluster analysis was performed on the Euclidean distance matrix utilizing average linkage method. These analyses were done using MINITAB software version 16 (Minitab 1998).

The selection of trichome length and density was based on general average value and standard deviation (SD) of each parameter. The selection criteria of density and length of pod were as follows:

$X > (\bar{x} + 1SD)$ = dense or long trichome
$X = (\bar{x} + 1SD)$ = moderate trichome
$X < (\bar{x} + 1SD)$ = sparse or short trichome
With,
X = the genotype value
$\bar{x}$ = average value
SD = standard deviation

RESULTS AND DISCUSSION

Agronomic characters

One of the main obstacles faced in improvement of soybean productivity in tropical area of Indonesia is the pest attacks. The availability of high yielding and wide spectrum pest resistant soybean varieties is important to increase the efficiency and productivity of soybean. Results of the present study revealed that observed agronomic characters were varied among the 150 soybean genotypes including days to maturity, 100 seed weight, and seed yield (Table 1) with CV value ranged from 1.03 % to 13.31%.

Days to maturity of the 150 soybean genotypes were varied from 72 to 83 days (average 77 days), 100 seed weight ranged from 12.50 to 23.50 g (average 16.50 g), and seed yield ranged from 1.51 to 4.28 t/ha (average 2.90 t/ha). Nowadays, the soybean users in Indonesia are mostly prefer high yielding varieties with large seed size (> 14 g/100 seeds) and early maturing days (<80 days).

Pod trichome characters

Characterization of the 150 soybean genotypes revealed a substantial variability in both trichome density and trichome length. Observation on a pod surface area revealed that trichome density of the evaluated genotypes ranged from 12 to 59 trichome/ 4 mm^2 (average 27.64 trichome/4 mm^2) while the trichome length of the genotypes ranged from 56.9 to 176.4 µm (average 112.82 µm) (Table 2). Observation of the trichome of the 150 soybean genotypes showed that 66 genotypes exhibited a higher trichome density than the average density of the 150 soybean genotypes. Additionally, the trichome lengths of 75 genotypes were longer than that of the average of the total 150 soybean genotypes. These results demonstrate that soybean genotypes with dense trichome or long trichome are likely to be obtained from this germplasm collection.

Correlation analysis revealed the absence of correlation between trichome density and trichome length with a correlation value of $r = 0.057^{ns}$ (Figure 1). This shows that of the 150 characterized genotypes, no relationship was found between the trichome density and length. This implies the low chance to obtain soybean genotypes which posses both dense and long trichome.

Table 1. Analysis of variance of yield and yield component 150 soybean genotypes in 2014

Parameter	Mean Square		CV (%)
	Replication	Genotype	
Days to maturity (days)	2.253333^{ns}	11.266219**	1.03
100 seed weight (g)	0.9633333^{ns}	5.7281655**	9.02
Seed yield (t/ha)	0.70664533*	0.26443688**	13.31

CV = coefficient of variation, * = significant at 5% probability level ($p < 0.05$), ** = significant at 1 % probability level ($p < 0.01$), ns = not significant.

Table 2. Descriptive statistics of yield component and pod trichome of 150 soybean genotypes in 2014

Statistic	Days to maturity (days)	100-seed weight (g)	Yield (t/h)	Trichome density/4 mm^2	Trichome length (µm)
Average	77.00	16.50	2.90	27.64	112.8
Standard deviation	6.13	2.12	0.44	9.69	27.3
Minimal	72.00	12.50	1.51	12.00	56.9
Maximal	83.00	23.50	4.28	59.00	176.4

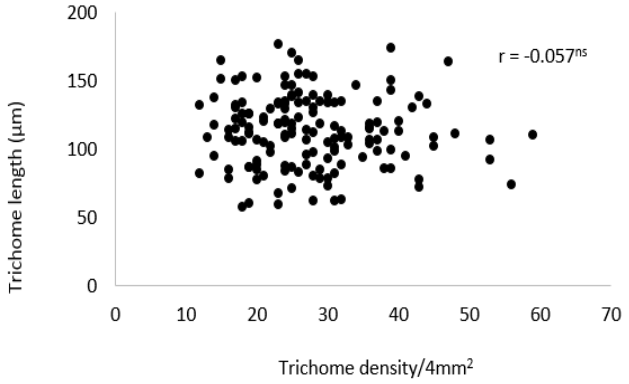

Figure 1. Relationship between trichome length and density of 150 soybean genotypes

Grouping of the soybean genotypes based on pod trichome characters and seed yield

Grouping of 150 soybean genotypes based on the trichome density, trichome length, and seed yield are presented in Table 3. Based on these three observed variables, 150 soybean genotypes were clustered into five groups. Group I consisted of 61 genotypes which was characterized by sparse trichome and high yield potential. Group II with a total of 36 genotypes was characterized by short trichome. Group III consisted of 41 genotypes that was classified as medium for trichome length, trichome density and seed yield characters. Group IV consisted of six genotypes with dense trichome, and group V also consisted of six groups with long trichome.

Grouping of the 150 characterized soybean genotypes based on trichome length and density showed that dense trichome falls in the range of 45-59 trichome/4 mm^2, while the long trichome falls in the range of 163.3 to 176.48 µm of trichome length. Further observation of the genotypes in clusters IV and V associated with some agronomic characters was presented in Table 4. Based on the selection category of the pod trichome density, three genotypes with dense trichome were selected. These genotypes have days to maturity ranged from 75-80 days and the seed size ranged from 14.50-18.5 g/100 seeds. Three soybean genotypes were also selected for their long trichome character. These genotypes have days to maturity ranged from 75-79 days and seed size ranged from 14.50-17.00 g/100 seeds. This means that these six genotypes are classified as early maturing and relatively large seed size soybean genotypes.

Table 3. Grouping of genotypes based on yield, trichome length and density of 150 soybean genotypes in 2014

Cluster	No. of genotypes	Range of values					Characteristic
		Yield (t/ha)	Days to maturity (day)	100 seed weight (g)	Trichome density/4 mm^2	Trichome length (µm)	
I	61	1.51-4.28	72-83	13.50-23.50	13-14	92.79-125.52	Sparse trichome
II	36	2.25-3.36	73-83	14.00-21.50	14-43	56.94-94.97	Short trichome
III	41	2.16-3.46	73-83	13.50-19.00	12-44	126.88-154.66	Moderate
IV	6	2.63-3.55	75-80	14.50-18.50	45-59	92.20-110.88	Dense trichome
V	6	2.25-3.27	75-79	14.50-17.00	15-47	163.34-176.48	Long trichome

Table 4. Selection of genotypes within cluster IV (dense trichome) and cluster V (long trichome) and their agronomic characters in 2014

Genotype	Days to maturity (days)	Seed weight (g/100 seed)	Yield (t/h)	Trichome density/4 mm^2	Trichome length (µm)
G 511 H/Anj//Anj///Anj////Anjs-2-5	75 f	15.00 cd	2.81 abcd	45	108.06
G 511 H/Anjasmoro//Anjasmoro-2-8	76 ef	17.00 bcd	3.18 ab	47	163.35
G 511 H/Arg//Arg///Arg///Arg-12-15	75 f	18.50 b	3.00 abcd	53 *)	92.21
G 511 H/Arg//Arg///Arg///Arg-19-6	75 ef	16.00 bcd	3.27 ab	26	164.74
G 511 H/Arg//Arg///Arg///Arg-19-7	75 f	14.50 d	3.55 a	59 *)	110.31
L.Jtg/Sbg//Burangrang-10	75 ef	15.50 cd	2.81 abcd	39	174.00 **)
Wilis x Grobogan-11	77 cd	16.00 bcd	2.34 cd	15	164.60
G 511 H/Anj//Anj///Anj////Anjs-6-13	78 bc	17.50 bc	2.63 bcd	48	110.88
G 511 H/Anjasmoro//Anjasmoro-5-6	78 bc	15.50 cd	2.25 d	23	176.48 **)
G 511 H/Anjasmoro//Anjasmoro-2-22	76 de	16.50 bcd	2.72 bcd	45	101.85
G 511 H/Anj//Anj///Anj////Anjs-6-2	80 a	16.00 bcd	3.36 ab	53 *)	106.65
G 511 H/Anj//Anj///Anj////Anjs-5-4	79 ab	14.50 d	3.08 abc	25	170.59 **)
Check varieties: Argomulyo	73 g	15.00 cd	3.08 abc	24	134.56
Grobogan	73 g	23.50 a	3.28 ab	16	113.34

Note: Values within the same column followed by the same letter are not significantly different at the 0.05 level according to LSD test. The selection of trichome length and density was based on general average value and standard deviation of each parameter: *) = selected in cluster IV (dense trichome), **) = selected in cluster V (long trichome)

Discussion

Trichome are potentially be used as a form of plant defense against pests in soybean plant. In Indonesia, soybeans are mostly cultivated in the dry season, and yield losses due to pod-feeding pests are greater than that caused by leaf-eating pests. The soybean pod feeders are grouped into pod sucking insects (*Nezara viridula* L., *Piezodorus hybneri* Gmel., and *Riptortus linearis* F.) and pod borers (*Etiella zinckenella* Tr. and *E. hobsoni* Butler). Until now, soybean cultivation practices at the farmer's level are mostly relying on the use of insecticides as pest control measure. This is due to the fact the pest resistant soybean varieties are not available yet at the moment.

Trichome orientation varies among soybean genotypes, thus providing the opportunity to perform selection to obtain soybean genotypes with dense and/or long trichome. Development of soybean varieties that are resistant to pod feeder is highly dependent on the availability of sources of gene(s) determining the resistance and the effective and efficient selection methods employed. Various studies showed that antixenosis resistance can be improved by increasing the length and density of pod trichome. In this study, we successfully obtained six soybean genotypes with long trichome (163.3-176.4 µm) and also identified six genotypes with dense trichome (45-59/4 mm^2) but none of these genotypes was found to have both dense and long trichome. There are two possibilities for utilizing these 12 soybean genotypes: (i) the genotypes are used as a source of genes for improvement of soybean resistance to pod-feeding pests through increasing the trichome density or length, or (ii) if one of the 12 genotypes has high yield potential, it can be proposed to be released as new soybean variety.

Trichome is physical parameter, which has the potential to be used as a mechanical barrier against insect pest infestations (Tingey 2001; Haq et al. 2013). Glas et al. (2012) studied the relationship between the plant glandular trichome with the resistance to herbivore insects and stated that the glandular trichome was potentially be used as plant defense against herbivore, especially in the Solanaceae plant groups. Furthermore, glandular trichome can be improved through classical breeding for crop protection purposes. Hulburt et al. (2004) examined the relationship between trichome with a various leaf-eating pests, and reported that the sharp pubescence tip was significantly capable of reducing levels of leaves defoliation by insects. It was then concluded that sharp tip pubescence could potentially be used as a character to increase the soybean resistance against various leaf-eating pests. The density of glandular trichome had negative effect on the decreasing oviposition process, but on the contrary, it had a positive effect in trapping the pest population (Oriani and Vendrami 2010).

Several researchers found that denser and longer trichome could act as a morphological defense. Suharsono and Sulistyowati (2012) found two soybean genotypes with dense trichome (IAC 100 and IAC 596-2) became susceptible to pod sucking bug if the trichome were eliminated. This is reasonable because the trichome act as a barrier to pest stylet for piercing the soybean pod. In the pod borer pests, research by Susanto and Adie (2008) revealed a positive correlation between the trichome density and the number of eggs with damage intensity in both soybean pods and seeds; meanwhile the trichome length had a positive correlation with the damage intensity of seeds and pods. Different results were found in studies conducted by Permana et al. (2012), where number of eggs and oviposition preferences were found to be not correlated with trichome density and trichome length. It is, therefore, interesting to further examine the results of these two contrasting research conclusions. Trichome has different roles in the pod borer, depending on the biological phase of the pest. Moreover, trichome becomes a favorite place for pod borer to perform egg laying. However, the larvae of the pod borer will be hindered if the soybean pod has a dense trichome as well as long trichome.

Until now, soybean varieties relatively resistant to pod-attacking pests have not been released yet, especially for resistance to pod sucking and pod borer pests, respectively. Of the three soybean genotypes classified as having dense trichome, the genotype G511H/Arg//Arg///Arg///Arg-19-7 had seed yield of 3.55 t/ha, days to maturity of 75 days and 100 seed weight of 14.50 g/100 seeds. The seed yield of this genotype was higher than that of the check varieties as well as the average seed yield of the 150 genotypes. Of the three genotypes classified as having long trichome, the genotype G511H/Anjasmoro//Anjasmoro-5-6 had a seed yield of 2.81 t/ha but was lower than the check varieties as well as the average seed yield of the 150 evaluated genotypes. The agronomic characters of these genotypes were early maturing day and large seed size. In Indonesia, the farmer's are mostly prefer soybean variety with high yield, early maturing (<80 days) and large seeded (> 14 g/100 seeds). The availability of soybean varieties with dense trichome or long trichome, coupled with high seed yield, are important to minimize the yield loss due to pod-feeding pests, and this will potentially reduce the use of chemical insecticides.

From the present study results, it can be concluded that the chance to obtain soybean genotypes that have both dense and long trichome is very low. Two selected soybean genotypes, i.e. G511H/Arg//Arg///Arg///Arg-19-7 with a dense trichome and G511H/Anjasmoro//Anjasmoro-5-6 with a long trichome, can be used as genes sources to produce soybean varieties that are resistant to pod-feeder pests through increasing the density or length of trichome. These genotypes had early days to maturity, which suited the tropical climate of Indonesia.

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