JURNAL ILMIAH AGROUST VOL 5 NO 2, MARET 2021:166-177

GENETIC VARIATION AND HERITABILITY OF ELEVEN TEA CLONES ON CHARACTERISTIC OF RESISTANCE AGAINST *EMPOASCA* SPP.

Driska Arnanto*
Fakultas Pertanian, Universitas Sarjanawiyata Tamansiswa Yogyakarta
*Email Korespondensi: driska.arnanto@ustjogja.ac.id

ABSTRACT

Genetic variation and heritability of resistance to *Empoasca* spp. was studied in ten clones PGL series and TRI 2025. The research conducted in tea research station in Pagilaran, Batang, Central Java and in the laboratory of Food Chemistry and Nutrition Faculty of Agricultural Technology, LPPT UGM and Genetic Laboratory Faculty of Agricultural, Gadjah Mada University. This research used randomly complete block design of ten clones PGL and TRI 2025 with four blocks as replications. The damage intensity and morphological anatomy data each clone PGL series and TRI 2025 were observed. Analysis of biochemical (tannins content and peroxidase enzyme acitivity) using the spectrophotometric method. Data were analyzed using ANOVA and heritability. The result shows that has high estimated heritability value in damage intensity, hair leaf density, enzym peroxide activity, tannin content, stomata density, and epidermis thickness (abaxial and adaxial). Cluster analysis based on morphological anatomy divide into four group and resistant clone against *Empoasca* spp. consisted of four category, based on principal component analysis.

Key words: heritability, resistant to empoasca, tea clone

INTRODUCTION

There are several groups of important pest that attack tea plants. Setiawan et al (1999) and Setyamidjaja (2000) said the pest attacked the leaves are the major and most dangerous. *Empoasca* spp. attack by piercing and sucking fluid from shoot to young leaf and old leaf of tea. Dharmadi (1999) in 1998 the existence of *Empoasca* spp. has been found for the first time and never be reported before. Sari (2015) identified *Empoasca* spp. based on morphological and molecular approach in Pagilaran tea estate, concluded the species of leaf hoppers was *Empoasca vitis*.

Empoasca spp. attacks and spread were quickly expanding. Dharmadi (1999) said within 45 days, they were disrupting the growth of shoots and decrease production by 50-80%. In a month their attack increase from 85 hectares to 539 hectares. After that *Empoasca* spp. becomes a major pest in the tea plantation

(Setiawan et al., 1999). To avoid leaf hopper attacked could use antagonistic organism (Boll and Herrmann, 2004; Straub et al., 2013), synthetic and bio-insecticides (Tong and Feng, 2016), intercropping (Zhang et al., 2016) and resistant genotype.

Clone resistant assembly against *Empoasca* spp. is one of effort to increase yield in tea plant. The availability of genetic variation in resistance of *Empoasca* spp. is necessary for the development of *Empoasca* spp. tolerance genotypes (Helyanto et al, 2000). Selection of character that resistant against *Empoasca* spp. more easier if it has high variation in population (Sudarmadji, 2007; Bahar and Zein, 1993). Estimating heritability value is quantitatif statement of rule of genotype factor than environment factor to give phenotype variation (Allard, 1960). High heritability value more effective in selection than low (Susiana, 2006). There are two kind of heritability, broad-sense and narrow-sense. Broad-sense heritability considered total genotype variant with phenotype variant, while the effect of aditive variant against phenotype variant more specific in narrow-sense heritability (Nasir, 1999).

The experiment aimed to 1) obtain the morphological anatomy characters and biochemical compounds (tannin content and peroxidase enzym activity) that affect the resistance to *Empoasca* spp., 2) estimate genetic variation and broad-sense heritability of ten PGL series and TRI 2025 against *Empoasca* spp., 3) obtain resistant clone against *Empoasca* spp.

MATERIAL AND METHODS

This experiment consisted of field research and laboratory research from August until December 2016. Field research conducted at Sanderan III in Pagilaran Tea Plantation, Unit Pagilaran Production Batang, Center Java. The PGL 1, PGL 3, PGL 4, PGL 6, PGL 7, PGL 9, PGL 10, PGL 11, PGL 12 and PGL 15 series and TRI 2025 clones were observed. Samples taken using Randomized Complete Block Design (RCBD) with four blocks and each experimental units consisted of ten plants.

Observation of damage intensity was done every week in September 2016. Scoring of shoots damage against *Empoasca* spp. (p+1,p+2 and p+3, p=peco) are:

0 = No symptoms.

1 = Symptoms of damage less than 25%

2 = Symptoms of damage between 26-50%

3 = Symptoms of damage between 50-70

4 = Symptoms of damage of 75% or more or leaf were dry

The damage intensity using the following formula:

$$IA = \frac{\Sigma(v\ x\ n)}{Z\ x\ N}\ x\ 100\%$$

Where:

IA = Intensity of damage

v = Score value for each damage category (0, 1, 2, 3, and 4)

n = Number of samples (leaf or peco leaf to p+3) symptomatic included in score v.

Z =The highest score of damage categories.

N = Total number of samples.(Pachrudin, 2006)

Laboratory research consisted of morphological, anatomical characteristics and leaves chemical. The samples used were shoot leaf (p+1, p+2, and p+3; p =peco).

1. Density of leaf hairs.

Valuation based on the spread and density of hairs, due to quantitative calculations is very difficult to do, so it will conducted qualitative :

- Value = 1, were leaf hairs that only in bone leave and hairs density is not prevalent
- Value = 2, were leaf hairs that only in bone leave and hairs density is prevalent
- Value = 3, were leaf hairs that extends to the edges of leaves, with hairs density is not prevalent,
- Value = 4, were leaf hairs that extends to the edges of leaves, with hairs density is prevalent
- Value = 5, were leaf hairs that extends to the edge of leave and leaf stalks, with hairs density is not prevalent,.

- Value = 6, were leaf hairs that extends to the edge of leave and leaf stalks, with hairs density is prevalent,.

Observations using binocular microscope with a magnification of 100 times, each shoot leaf tea was observed (p+1, p+2, dan p+3) and repeated four times (Pachrudin, 2006).

2. Stomata density of tea PGL and TRI 2025.

Sample of fresh and young tea leaves was used (p+1, p+2, dan p+3). Each lower leaf surface samples (lower epidermis) is cleaned using soft tissue to remove impurities. After that leaves smeared with clear nail polish and wait 5-10 minutes until dry. After dry, sample were given masking tape that attaches the leaf mold than leaf mold pasted on the preparations glass. After that, sample were observed on binoculer microscope with magnification 100 times. Determining wide field of view in microscope using a calibrated micrometer optilab objective and adjusted to a magnification used for observation (Rantau et al, 2014).

3. Tea leaf epidermal thickness of PGL and TRI 2025.

Making leaf anatomy preparation using paraffin metode (single coloring). Stages done are fixation, washing and dehydration, infiltration, cloaking, slicing and gluing, coloring, closures, labelling and observations under binocular microscope (Olympus CX 21) with magnification of 400 times (Lab. Struktur Perkembangan Tumbuhan, Fakultas Biologi UGM).

Chemical compound analysis. The analysis are :

1. Peroxidase enzyme analysis.

The analysis based on metode that used by Harni et al (2012) and Zhen (2002). Leaf sample were weighed of five grams and then demolished with mortar in 0.01 M phosphate buffer, pH 6 ratio 1:4. Leaf extract centrifuged at 5000 rpm for 30 min at 4^0C, then filtered using Whatman paper. Supernatant obtained was used as an enzyme preparation.

Peroxidase activity was determine using two tube. First tube contained 5 mL of enzyme extraction and 5 mL of pyrogallol 1%. Second tube contained 5 mL of enzyme extraction, 5 mL of pyrogallol 1% and 5 mL of H_2O_2 1%. The

absorbance was measured at 420 nm wavelength and observed changes in value until constant. Activity determining using this calculation :

$$V = A \times c$$

V, activity of enzyme; A, absorbance difference before and after addition of H_2O_2; c, enzym dosage (mL) / wet weight of sample.

2. Tannin analysis (group of phenolic).

The analysis using colorimetry metode. This methode estimates the tannin based on measurements of blue coloration which is formed from reduction of fosfomolibdat acid by tannin like compound in alkaline solution. (Ranganna, 1977). Stages of analysis process includes :

- Standard curve preparation**.**

  Tannin acid standard solution 1 mL put in a 100 mL volumetric flask and then added 7.5 mL of distilled water. Inserted 0.5 mL reagent Folin-denin and 1 mL solution of sodium carbonate into volumetric flask and added water until it reaches the limit. Mixed well and measure coloring after 30 minutes at 760 nm with experimental blank customized to the absorption rate of zero.

- Sample preparation.

  A total of 5 grams are inserted 500 mL volumetric flask and the diluted with distilled water until reaches limit. Then pipette 10 mL into 100 mL of volumetric flask, add 5 mL reagenFolin-denin and 10 mL sodium carbonate diluted with distilled water to the limit. Mixed well and formed the color, wait 30 minutes and then analyzed by spectrophotometry at 760 nm.

- Determination**.**

  Of standard solution of tannin 0.1 mg (1 mL) can be determined mg tannin acid from standard curve. The treated samples can be compared with the tube Nassler to acid standars tannins treated in the same experiment.

- Calculation**.**

$$\text{TA (\%)} = \frac{\text{mg TA} \times \text{dilution} \times 100}{\text{V} \times \text{Wt from taken samples from coloring} \times 1000}$$

Information :

TA = Tannin Acid

V = mL of taken samples

Data were analyzed using ANOVA on morphology, anatomy, damage intensity, analysis of tannins content and enzyme peroxide activity. Component of variant were carried out through SAS program on character that observed. Estimating heritability value using this formula :

$$h = \frac{\sigma^2 g}{\sigma^2 p}$$

Classification of heritability based on MC Whirter (Sudarmadji, 2007) are high (H≥0.5), middle (0.20≥H>0.5) and low (H<0.2). Grouping morphological anatomy based on scoring data of ten PGL clone and TRI 2025 description plant using cluster analysis. Determined of resistant clone using PCA analysis.

RESULT AND DISCUSSION

The yield of tea plantation is depending on the quantity and quality of leaves fresh weight. Many factors affect the quantity and quality of tea leaves, include unfavorable environment such as abiotic and biotic stress. Selection of tea clone adapted to a broad environment or suitable for a specific location is required for maximizing the potency of tea production (Astika, 1978). Murti et al. (2014) show the response of tea clones was diverse on the fifteen selected clones of PGL series in different environments (location and years) and drought condition (Krisyando et al., 2012).

Table 1. Analysis of variant of Damage Intensity, tannin content, density of leaf hair and stomata density, peroxidase enzyme activity sixteen clones in Pagilaran tea plantation

Character	Mean	Mean square	F value
Damage intensity	13.844	111.842	4.4*
Hair leaf density	3.115	2.068	42.09**
Peroxidase enzyme activity	0.249	0.007	87350.5**

Tannin content	2.044	0.282	6.42*
Stomata density	295.725	8954.575	2.59*
Abaxial	15.721	25.669	30.23**
Adaxial	24.349	9.547	9.39**

** = highly different significant, * = different significant

The appearance of tea character is controlled by gene and environment. The genotype-environment interaction get much concern because makes it difficult to assess the advantages of plants through genetic analysis and reduces efficiency in crop improvement through breeding. To overcome this, required adaptability testing on a variety of differen test sites (Cooper dan Byth, 1999 *cit.* Sriyadi, 2009). Astika (1978) said, it is necessary to do the selection of superior tea clones suitable for an area.

Tabel 2. Componen of variant and heritability on resistant character against *Empoasca* spp.

Character	CV	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	h
Damage intensity	36.432	28.8017	25.437	37.280667	0.773
Hair leaf density	7.115	0.67267	0.05	0.6893333	0.976
Peroxidase enzyme activity	0.113	0.00233	0.00000008	0.0023333	0.999
Tannin content	10.251	0.07933	0.044	0.094	0.844
Stomata density	19.858	1834.1	3452.268	2984.8583	0.615
Abaxial	5.861	8.273	0.85	8.5563333	0.967
Adaxial	4.141	2.84333	1.017	3.1823333	0.894

$\sigma^2 g$ = variant of genetic, $\sigma^2 p$ = variant of phenotypic, $\sigma^2 e$ = variant of environment

h = heritability

Estimated broad-sense heritability shows more than 50% in damage intensity, hair leaf density, enzym peroxide activity, tannin content, stomata density, and epidermis thickness (abaxial and adaxial). These show that all resistant character control by genotype factor than environment (Suharsono et al, 2006; Suprapto and Narimah, 2007; Pinaria et al, 1995). Malik et al (2006) and Sreelathakumary & Rajamony (2004) also said that high heritability show that aditive gene more active. Improvement character in plant breeding can be done with selection on character that has high estimated heritability value and also high variation in genotype and phenotype (Handayani & Hidayat, 2012; Vidya et al, 2002; Gothil et al, 2006;

Selvaraj et al, 2011; Tyagi & Khan, 2011; Mohamed et al, 2012). The clustering analysis based on morphological anatomy of ten PGL clone and TRI 2025 description divided into four group. PGL 1 and PGL 3 have similar with TRI 2025, while PGL 4, PGL 6, PGL 7 and PGL 15 into same group. PGL 10 has similar with all clones and the rest PGL 9, PGL 11 and PGL 12 are similar.

The result of principal component analysis shows in Figure 2. This analysis used data of resistant plant character against *Empoasca* spp. There have four category of resistant. PGL 6, PGL 4 and PGL 9 in the first quadran are resistant, followed by PGL 1 (second quadran) was rather resistant. While susceptible clone against *Empoasca* spp. were TRI 2025, PGL 7, PGL 11 and PGL 15 (third quadran). The most susceptible clone were PGL 3, PGL 10, and PGL 12 (fourth quadran).

CONCLUSION

Estimated broad-sense heritability value shows more than 50% (high) in damage intensity, hair leaf density, enzym peroxide activity, tannin content, stomata density, and epidermis thickness (abaxial and adaxial). Selection process more effective if it has high heritability on resistant character in tea clone. Based on principal component analysis there have four group of resistant. Resistant clone consisted of PGL 4, PGL 6, and PGL 9, rather resistant was PGL 1. While susceptible clone were TRI 2025, PGL 7, PGL 11 and PGL 15. The most susceptible clone consisted of PGL 3, PGL 10, and PGL 12.

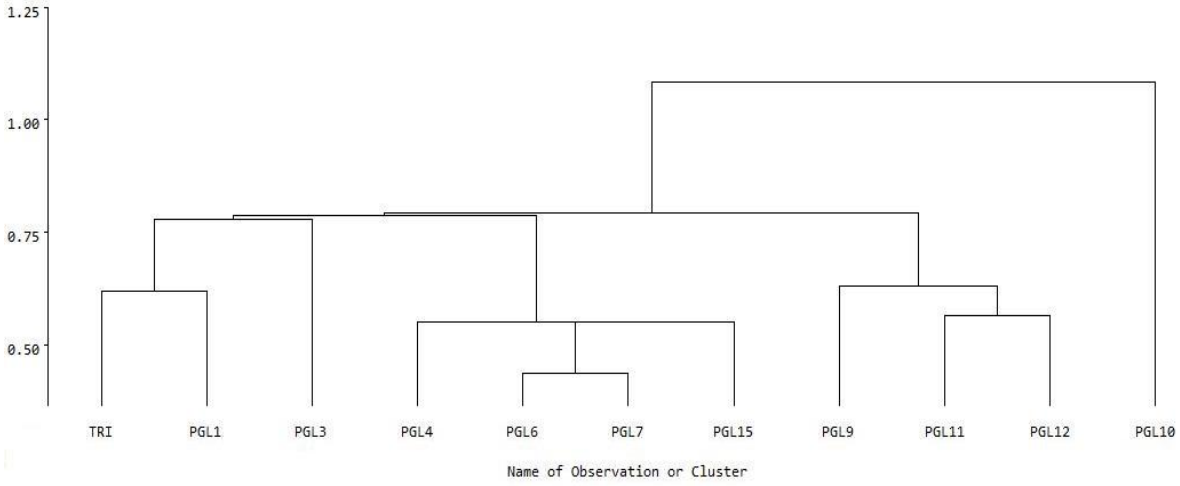

Figure 1. Dendogram of cluster analysis of ten PGL clones and TRI 2025

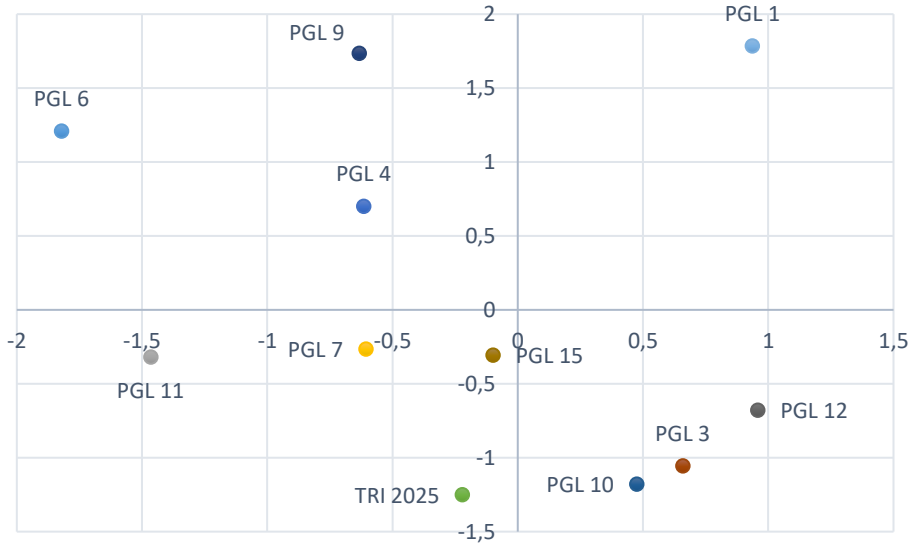

Figure 2. Scatter diagram of principal component analysis of ten PGL clone and TRI 2025

References

Allard RW. 1960. Principles of Plant Breeding. John wiley & Sons, Inc. New York.

Astika W. Dan D. Muchtar. 1978. Anjuran bahan tanaman teh tahun 1978. Warta BPTK. 4 : 297-306.

Bahar, M., dan A. Zein, 1993. Parameter genetik pertumbuhan tanaman, hasil dan komponen hasil jagung. Zuriat 4(1):4-7p

Boll, S. and J. V. Herrmann. 2004. A long-term study on the population dynamics of the grape leafhopper (Empoasca vitis) and antagonistic mymarid species. J Pest Sci. 77: 33–42p

Dharmadi, A. 1999. Empoasca sp. hama baru di Perkebunan Teh. Pusat Penelitian Teh dan Kina. Bandung, Jawa Barat. 5 p

Gothil, V. N., H.M. Pandya, and dDR. Mehta. 2006. Genetic Variability for Seed Yield and Its Component Traits in soybean. Agric. Sci. Digest. Vol. 26. 4-73p

Handyani, T. and I.M. Hidayat. Keragaman Genetik dan Heritabilitas Beberapa Karakter Utama pada Kedelai Sayur dan implikasinya untuk seleksi Perbaikan Produksi. J. Hort. Vol. 22. 327-333p

Harni, R. Supramana, M. S. Sinaga, Giyanto dan Supriadi. 2012. Mekanisme Bakteri Endofit Mengendalikan Nematoda *Pratylenchus brachyurus* Pada Tanaman Nilam. Buletin Penelitian Tanaman rempah dan Obat. Vol. 23 No. 1. 102 - 114. 102.

Helyanto, B., U. Setyo Budi, A. Kartamidjaja, dan D. Sunardi. 2000. Studi parameter genetik hasil serat dan komponennya pada plasma nutfah rosela. Jurnal Pertanian Tropika. 8(1):82-87p

Kallenbach, M., G. Bonaventure, P. A. Gilardoni, A. Wissgott, and I.T. Baldwin. 2012. Empoasca leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. http://www.pnas.org/content/109/24/E1548.full.pdf

Krisyando, P., D. Indradewa, & S. Waluyo. 2012. Potensi Hasil dan Toleransi Kekeringan Seri Klon Teh (Camelia sinensis (L.) Kuntze) PGL di Kebun Poduksi Pagilaran Bagian Andongsili. Vegetalika 1(1): 161-172

Miklas, P.N.,J.D. Kelly, S.E. Beebe and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding.Euphytica (2006) 147: 105–131 DOI: 10.1007/s10681-006-4600-5 C

Malik, M.F.A., M. Ashraf, S. Qureshi and A. Ghafoor. 2006. Utilization of Diverse germplasm for Soybean Yield Improvement. Asian J. Plant Sci. Vol 5. 63-67p

Murti, R.H., A. Puspitasari and S. Mitrowihardjo. 2014. Stability analysis of nine promising clones of tea (*Camellia sinensis*). Agrivita 36(1):81-90p

Mohamed, S.M., E.E. Ali and T.Y. Muhamed. 2012. Study of Heritability and Genetic Variability Among different plant and Fruit Characters of Tomato (*Solanum lycopersicon* L.) Int. J. Sci and Techno. Res. Vol. 1. 30-113p

Nasir, M. 1999. Heritabilitas dan kemajuan genetik harapan karakter agronomi tanaman lombok (*Capsicum annum* L.). Habitat 11 (109) : 1-7p

Pachrudin. 2006. Kajian Beberapa Faktor Yang Mempengaruhi Perkembangan Populasi *Empoasca* sp. Di Kebun Teh Pagilaran, Kabupaten Batang. Tesis. Universitas Gadjah Mada.

Pinaria, A. A. Baihaki, R. Setiamihardja, dan A. A. Darajat. 1995. Variabilitas Genetik dan Heritabilitas

Ranganna, S. 1977. Manual of Analysis of Fruit and Vegetable Product. Tata McGraw-Hill Publishing Company Limited. New Delhi. 69 – 71 p

Rantau, D. E., E. Al Hafiizh., W. Rahman., dan T. M. Ermayanti. 2014. Analisis Ukuran dan Kerapatan Stomata pada *Artemisia annua* L. Hasil Perlakuan

Kolkisin. Prosiding Seminar Nasional XXIII "Kimia dalam Industri dan Lingkungan". Yogyakarta

Sari, N.M. 2015.Wereng daun Empoasca vitis Gothe (Hemiptera: cicadellidae) hama teh di pt pagilaran. Skripsi Fakultas Pertanian. Tidak dipublikasikan

Selvaraj, I.C., P. Nagarajan., K. Thiyagarajan., M. Barathi and R. Rabindran. 2011. Genetic Parameters of Variability, Correlation, and Path Coefficient Studies for Grain Yield and Other Yield Attributes among Rice Blast Disease Resistant Genotypes of Rice (*Oriza sativa* L.). Afr. J. Biotechnol. Vol. 10. 34-3322p

Setiawan, I., T. Hermawan., D.D. Putranto, S. Nurcahyo, dan A.W. Hidayat. 1999. Pengelolaan Hama Pada Tanaman Teh PTP Nusantara VIII (Persero). Lembaga Pendidikan Perkebunan, Kampus Yogyakarta. 22 p

Setyamidjaja, D. 2000. BudidayatehdanPengolahanPascapanen. Penerbitkanisius. Yogyakarta. 154 p

Sreelathakumary, I and L. Rajamony. 2004. Variability, Heritability and Genetic Advance in Chili (*Capsicum annum*. L). J. Tropical Agric. Vol 42. 7-35p

Sriyadi, B. W. Astika, D. Muchtar, Sutrisno. 1991. Seleksi pohon induk teh dari tanaman muda asal biji propellegitim. Zuriat 2 (1).

Straub, C. S., N. P. Simasek, M.R. Gapinski, R. Dohm, E.O. Aikens, S.h Muscella. 2013. Influence of nonhost plant diversity and natural enemies on the potato leafhopper, Empoasca fabae, and pea aphid, Acyrthosiphon pisum, in alfalfa. J Pest Sci 86:235–244

Sudarmadji. 2007. Variasi Genetik, Heritabilitas dan Korelasi Genotipik Sifat-Sifat Penting Tanaman Wijen (*Sesamum Indicum* L.). *Jurnal Littri*, Vol. 13 No. 3, September 2007 : 88 – 92p

Suharsono, M. yusuf, dan A. P. Paserang. 2006. Analisis ragam, heritabilitas, dan pendugaan kemajuan seleksi populasi F2 dari persilangan kedelai kultivar Slamet x Nokonsawon. Tanaman Tropika. 9(2):86-93p

Suprapto dan Narimah Md. Kairudin. 2007. Variasi genetik, heritabilitas, tindak gen, dan kemajuan genetik kedelai *(Glycine max [L.] merill)* pada Ultisol. J. Ilmu Pertanian Indonesia. 9(2): 183-190p

Susiana, E. 2006. Pendugaan Nilai Heritabilitas, Variabilitas dan Evaluasi Kemajuan Genetik Beberapa Karakter Agronomi Genotipe Cabai (*Capsicum annum* L.) F4. Tesis. Institut Pertanian Bogor.

Tong,SM and MG Feng. 2016. A mixture of putative sodium salts of camptothecin and bamboo tar is a novel botanical insecticide against rice planthoppers and stem borers. J Pest Sci 89:1003–1011

Tyagi, S.D. and M.H. Khan. 2011. Correlation, Path-coefficient, and Genetic Advance in Lentil (*Lens culinaris* medik) Under Rainfed Condition. Int. Res. J. Plant Sci. Vol. 2. 191-200p

Vidya, C., S.K. Oommen.and V. Kumar. 2002. Genetic Variability and Heritability of Yield and Related Character in Yard-long Bean. J. Tropical Agric. Vol. 40. 3-11p

Wagiman and B. Triman. 2011. Ketahanan Relatif Enam Belas Nomor Klon Teh Pgl Terhadap Serangan *Empoasca* sp. J. PerlindunganTanaman Indonesia: 17(2): 60–65p

Zhang, Z., C. Zhou, Y. Xu, X. Huang, L. Zhang and W. Mu. 2016. Effects of intercropping tea with aromatic plants on populationdynamics of arthropods in Chinese tea plantations. J Pest Sci 90:227–237

Zhen, Y. 2002. Tea, Bioactivity and Therapeutic Potential. Taylor and Francis, New York. 57 p.