

Variation in leaf characters and agronomical traits of Yard-long bean genotypes between *Dimocarpus longan* and *Psidium guajava* stand

FAJRIN PRAMANA PUTRA, FLORENTINA KUSMIYATI, SYAIFUL ANWAR, WIDARYATI¹,
MUHAMAD GHAZI AGAM SAS[▼]

Department of Agriculture, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro. Jl. Prof. Sudarto No.13, Semarang 50275, Central Java, Indonesia. Tel./fax.: +62-24-7474750, ▼email: muhamadghaziagamsas@lecturer.undip.ac.id

Manuscript received: 10 September 2022. Revision accepted: 3 November 2022.

Abstract. Putra FP, Kusmiyati F, Anwar S, Widaryati, Sas MGA. 2022. Variation in leaf characters and agronomical traits of Yard-long bean genotypes between *Dimocarpus longan* and *Psidium guajava* stand. *Biodiversitas* 23: 5752-5758. Yard-long bean (*Vigna unguiculata* subs. *sesquipedalis*) is one of the most familiar horticulture crops. Leaf characteristics such as trichomes, stomata, and chlorophyll content correlated to other traits that influenced tolerance under sub-optimum conditions and component production traits. This study aimed to evaluate the leaf characters and agronomical traits variations of the Yard-long bean genotypes. The study was conducted by Split-plot design, with the primary factor were shading between *Psidium guajava* and *Dimocarpus longan* Lour. stand. The sub-plot was seven genotypes of Yard-long beans. Analyses were performed of multiple components, including the density of trichome, density of stomata, chlorophylls, carotenoids, nitrate reductase activity, flowering times, harvesting times, and production components under shading conditions between *D. longan* and *P. guajava* stands. The density of trichome, density of stomata, total chlorophyll, carotenoid, and Nitrate reductase activity showed the variability responses for each genotype in different shading conditions. One genotype showed early flowering in shaded conditions between *D. longan* than in shaded conditions between *P. guajava*. Two genotypes have the potential to adapt under shade conditions between *D. longan*. The morpho-agronomic characters that have been characterized may be used as potential donors for future hybridization programs to develop superior yard-long bean varieties.

Keywords: Leaf variability, pigment contents, trichomes, vegetable agroforestry

INTRODUCTION

The Yard-long bean (*Vigna unguiculata* subs. *sesquipedalis*) is one of the most familiar horticulture crops commonly known as asparagus bean, Chinese long bean, long-podded cowpea, pea bean, or snake bean (Vivalapali et al. 2014; Pidigam et al. 2021). Yard-long bean is an important vegetable commodity in Indonesia because yard-long beans per capita consumption are almost three times higher than other legumes (Widyawan et al. 2020). The Yard-long bean belonging to the family Fabaceae is a distinctive subspecies of cowpea (*Vigna unguiculata* (L.) Walp.), characterized by its climbing growth habit and long draping pods. This plant was domesticated from cowpea (*Vigna unguiculata* subsp. *unguiculata*), which resulted in the change of traits such as growth habits, longer pods, and larger seed size (Kongjaimun et al. 2012). Yard-long bean is cultivated for its tender pods relished as a vegetable, a good source of vitamins A and C, protein, fiber, and other minerals (Haque et al. 2020). It makes Yard-long beans potentially a functional food that improves health, so they need to be developed. However, the development of Yard-long bean production will not be optimal if the agricultural field is more focused on the cultivation of food crops. In addition to these factors, several factors affecting production are climatic factors and crop management systems such as shade, fertilizing and genetic properties (Blair et al. 2016).

Considering that the Yard-long bean is a C3 plant that is a shade-loving tree, it needs suitable micro-climatic conditions for optimum growth (Blair and Wu 2016). Research on Yard-long beans in shaded conditions has been carried out by Haque and Ahmed (2010) that Yard-long beans have high pod yields of up to 50% shade level, so the Yard-long beans have the potential to be developed with agroforestry systems. Agroforestry is a land use that integrates woody perennials with seasonal crops in the same land management unit (FAO 2015). The integration of vegetables in tree-based systems is known as Vegetable Agroforestry (VAF). It is a potential method that can transform low-production farms into properly managed, diverse, and ecologically robust agroforestry systems along with the provision of micronutrients to the diet of the rural community (Singh et al. 2021).

The main limiting factor for planting between plant stands is the low intensity of sunlight (Bellaloui et al. 2012; Abdel-Wahab and El-Rahman 2016). This condition will lead to changes in morphology and physiology as a strategy for dealing with stress. Shade conditions caused an increase in chlorophyll-a and chlorophyll-b but reduced the ratio of chlorophyll a/b (Mawarni et al. 2019). In addition, Gonçalves et al. (2001) added shade to increase the carotenoid content of legume leaves. Then the activity of several enzymes of different plant metabolic pathways is regulated by light. Nitrate reductase is an enzyme that regulates nitrogen assimilation in plants, regulated by light

intensity changes. Although the response of nitrate reductase activity under different light-intensity conditions is not the same in each species, in general, its activity is higher in light conditions than in dark conditions (Bellaloui et al. 2012). Besides, leaf characters have comprehensive functions to know

plant traits because leaf characters correlate with other traits, such as tolerance against herbivore insects, tolerance under sub-optimum conditions, and production traits. Studies in recent decades have demonstrated that trichomes have a more role in plants, especially in environmental interaction. Trichomes act as light reflectors under high light intensity conditions, so plants do not experience stress. Trichomes also have chemical defense functions as they accumulate large amounts of bioactive chemicals in many plants (Herawati et al. 2019). The intensity of sunlight also affects the formation of tissues or organs that protect plants against the stress of solar radiation. Changes in leaf characteristics in shading conditions will impact Yard-long bean yields.

Based on this information, developing a shade-tolerant Yard-long bean genotype is necessary. So far, no Yard-long bean variety is tolerant to shade. Yard-long bean development can be done through a plant breeding process. The primary breeding objective is to increase the pod yield per hectare by increasing the number of pods per plant (Kusmiyati et al. 2021) and to improve other attributes such as photo-insensitivity (Sundari 2009), resistance/tolerance to biotic stresses (Sas et al. 2021), terminal drought tolerance, and wide adaptation to different agroclimatic conditions (Lestari et al. 2019). The assembled Yard-long bean genotype needs to know the leaf character and agronomic character so that it can be used to conclude that the plant is tolerant or susceptible to shade. So, Yard-long bean production will be developed through the Vegetable Agroforestry (VAF) system. This study aimed to find information on the Yard-long bean genotype that was adaptive to the Vegetable Agroforestry (VAF) system based on the characteristics of the leaves and leaf pigment.

MATERIALS AND METHODS

Location of study

The study was conducted at *Undip Agrotechno Park*, Departement of Agriculture, Universitas Diponegoro, Semarang, Central Java, Indonesia. The field site's

elevation was 193 m (above sea level) with an altitude of 7° 03'18" S and a longitude of 110° 26'26" E. The illustration of the research area is presented in Figure 1.

Materials

The genetic materials used in this study included genetic material from the Department of Agriculture, Universitas Diponegoro, Indonesia, with seven genotypes of F7 (seven generations) (Table 1).

Research design

The experiment was laid out in a Split-plot Design with two factors. The main factor is shading between the *Psidium guajava* L. stand (S1) and the *Dimocarpus longan* Lour. stand (S2). The sub-factor is seven genotypes of Yard-long. The genotypes used in this study were Yard-long bean genotypes consisting of seven F7 lines.

Each experiment unit is a 6 x 1 m² plot with 24 plants per plot. The soil was treated with 20 tons. ha⁻¹ manure two weeks before planting. The succeeding fertilizations were carried out once two weeks using NPK Mutiara 16:16:16 100 kg. ha⁻¹. Pest and plant diseases were controlled mechanically and chemically using insecticide (profenofos 2 ml. L⁻¹ and carbofuran 17 kg. ha⁻¹).

Procedures

Plant epidermal imprints

The epidermal of the plant, such as stomata and trichome attributes, were collected by rapid imprinting technique (Inamdar and Patri 1969). The abaxial leaf surface was taken first and washed using tap water. Then it was dried, and the transparent nail polish was applied uniformly with a brush on the surface. It was then dried at room temperature for approximately 20 minutes. The nail polish imprints were placed on glass coverslips and photographed under a microscope (Kusmiyati et al. 2018).

Table 1. Material genetics in this study

Genotypes	Notes
KP-6718-1-1-10	KP-U-67 x KP-H-18
KP-6718-1-1-3	KP-U-67 x KP-H-18
KP-6718-1-1-4	KP-U-67 x KP-H-18
KP-6718-1-1-5	KP-U-67 x KP-H-18
KP-6718-3-2-13	KP-U-67 x KP-H-18
KP-6796-3-4-10	KP-U-67 x KP-H-1916
KP-6796-3-4-7	KP-U-67 x KP-H-1916

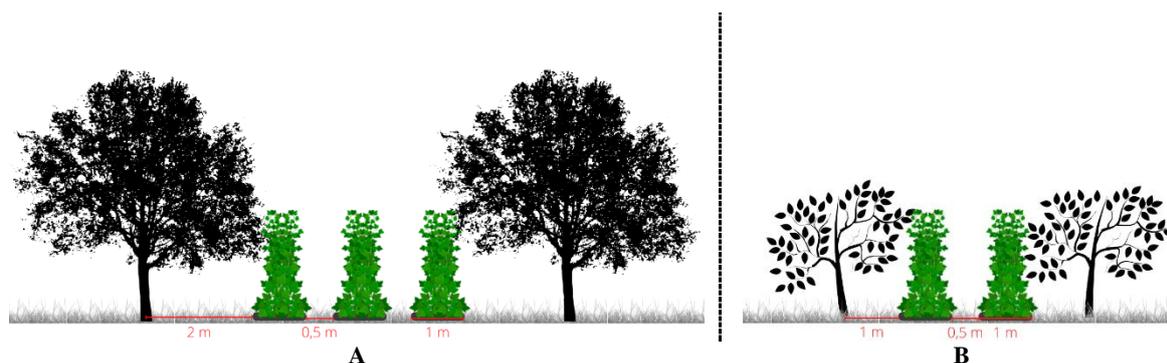


Figure 1. Yard-long bean genotypes between (A) *Dimocarpus longan* and (B) *Psidium guajava* stand

Chlorophyll content and carotenoid analysis

Observation of chlorophyll and carotenoid content ($\text{mg}\cdot\text{g}^{-1}$) in leaves was determined according to Lichtenthaler and Wellburn (1983). Observations were made with a spectrophotometer at a wavelength of 646 nm, 663 nm, and 470 nm. Chlorophyll and the carotenoid level were counted with the following formula:

$$\text{Chlorophyll-a} = -0.00281 \times \lambda_{646} + 0.01221 \times \lambda_{663}$$

$$\text{Chlorophyll-b} = 0.02013 \times \lambda_{646} - 0.00503 \times \lambda_{663}$$

$$\text{Carotenoid} = (1000 \times \lambda_{470} - 3.27 \times \text{Chlorophyll-a} - 104 \times \text{Chlorophyll-b}) / 229$$

Nitrate reductase activity analysis

Observation of nitrate reductase activity was carried out by the method modification of Hageman and Hucklesby. Observations were made with a spectrophotometer at a wavelength of 540 nm. The nitrate reductase activity (NRA) is expressed in $\mu\text{mol NO}_2^- \cdot \text{g}^{-1} \cdot \text{hour}^{-1}$ with the equation:

$$\text{NRA} = \frac{\text{Absorbance sample}}{\text{Absorbance standar}} \times \frac{1000}{\text{Weight leaf sample (g)}} \times \frac{1}{\text{Incubation time}} \times \frac{500}{1000}$$

Evaluation of agronomic traits

The parameters observed consist of flowering time (DAP), harvest times (DAP), pod number per plant, and the pod's weight per plant ($\text{g}\cdot\text{plant}^{-1}$).

Data analysis

Data analysis was carried out on morphological and agronomic parameters. The data obtained were tested using analysis of variance (ANOVA) with a significance level of 5%. Significantly different variables were further tested using the HSD test using STAR software.

RESULTS AND DISCUSSION

Leaf morphology under different shading conditions

Leaf characters have comprehensive functions to know plant characters under several conditions, such as shading conditions. Based on this research, the data of leaf morphology, including stomata and trichomes characters, are obtained as in (Figure 2).

Table 1 shows that the Yard-long genotypes had different responses in shaded conditions under *D. longan* stands and shaded under *P. guajava* stands. It can be seen in all the parameters, including the number of stomata, stomatal density, and trichome density.

Based on the density of stomata formed, the subsurface stomata of the Yard-long bean genotype shaded by *D. longan* ($2.83\text{-}4.19 \text{ stomata}\cdot\mu\text{m}^{-2}$) had significantly lower density than those shaded by *P. guajava* ($4.06\text{-}5.36 \text{ stomata}\cdot\mu\text{m}^{-2}$). This condition is similar to the research by Jumrani and Bhatia (2020), which showed that the stomata density of legumes would decrease if the light intensity conditions were low.

The decrease in stomatal density by 18%-44% occurs in conditions of 35%-75% light intensity (Jumrani and Bhatia 2020). Similar to stomata, the trichomes formed in each genotype of Yard-long beans shaded by *D. longan* stands had a lower density ($0.088\text{-}0.134 \text{ trichomes}\cdot\mu\text{m}^{-2}$), while the genotype of Yard-long beans shaded by stands of *P. guajava* had a higher or denser trichome, the density ranging from $0.130\text{-}0.189 \text{ trichomes}\cdot\mu\text{m}^{-2}$. Based on these results, the density of stomata is influenced by environmental conditions, as well as the density of trichomes which is influenced by environmental influences, where light is a factor that can affect the density of trichomes (Herawati et al. 2019), which increases the density of trichomes in plant leaves. Which is not shaded according to the function of leaf trichomes in avoiding excessive water loss, overheating, and photoinhibition (Armero et al. 2018; Raux et al. 2020).

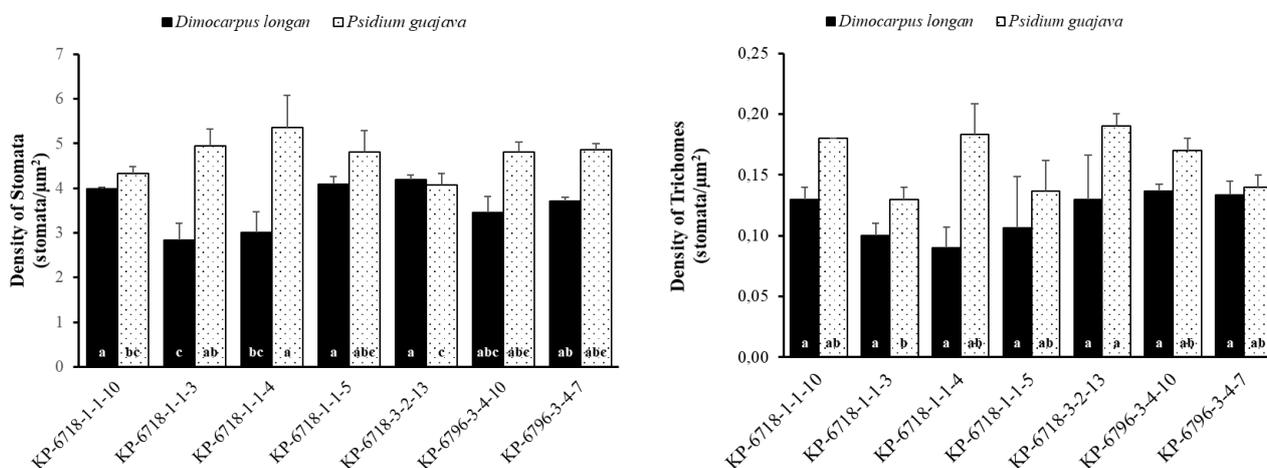


Figure 2. The density of stomata and density of trichomes of Yard-long bean genotypes

Nitrate activities reductase of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

The activity of several enzymes of different plant metabolic pathways is regulated by light. Nitrate reductase is an enzyme that regulates nitrogen assimilation in plants, regulated by light intensity changes (Kaiser and Huber 2001). However, the response of nitrate reductase activity under different light-intensity conditions was not the same in each species. Its activity was generally higher in light conditions than in dark conditions (Morozkina and Zvyagil'skaya 2007).

Table 2 shows that the SxG interaction significantly affects nitrate reductase activity based on the analysis of variance. The interaction *D. longan* stands with genotypes KP-6718-1-1-10 and KP-6718-1-1-5 showed high nitrate reductase activity. In the interaction *D. longan* stands with genotypes KP-6718-1-1-10 and KP-6718-1-1-5, the highest nitrate reductase activity was 0.449 $\mu\text{mol NO}_2^- \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ and 0.440 $\mu\text{mol NO}_2^- \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. All genotypes of Yard-long beans gave different responses to nitrate reductase activity under both shade conditions. In conditions shaded by stands of *D. longan*, genotypes KP-6718-1-1-10, KP-6718-1-1-3, KP-6718-1-1-5, KP-6718-3-2-13, and KP-6796-3-4-7 showed higher nitrate reductase activity compared to the shaded condition of *P. guajava* stands (Table 2). The phenomenon is affected because plants respond to low light conditions by increasing plant leaf area through efficient light capture. Then the plant will increase the photosynthesis process, followed by respiration. NADH and NADPH are energy products from photosynthesis and respiration, which are used as energy to reduce NO_3^- to NO_2^- (Latifa et al. 2009). However, the Yard-long bean genotypes KP-6718-1-1-4 and KP-6796-3-4-10 showed lower nitrate reductase activity in the shaded conditions of *D. longan* compared to the shaded conditions of *P. guajava* stands. Plant nitrate reductase activity was generally lower under low light conditions (Bellaloui et al. 2012).

Chlorophyll content and carotenoid of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

The analysis of variance results showed that plant stands (S), Yard-long bean genotype (G), and SxG interactions had a significant effect on leaf pigments such as chlorophyll-b, total chlorophyll, chlorophyll a/b ratio, and carotenoids (Table 3). The interaction between SxG did not significantly affect the levels of chlorophyll-a. However, the shading of plant stands showed significant differences in leaf chlorophyll-a levels. The chlorophyll-a of Yard-long beans genotypes among *P. guajava* stands was higher at 0.0239 $\text{mg} \cdot \text{g}^{-1}$. Then based on the analysis of variance, the genotypes showed a significant difference in the chlorophyll-a levels. The genotype KP-6718-3-2-13 had the highest chlorophyll-a content compared to the other genotypes (Table 3). Post Hoc HSD levels of chlorophyll-b and total chlorophyll showed that the SxG interaction between *D. longan* stands with genotype KP-6718-1-1-5 had high levels of chlorophyll-b and total chlorophyll compared to other treatment interactions, 0.0286 $\text{mg} \cdot \text{g}^{-1}$ and 0.0519 $\text{mg} \cdot \text{g}^{-1}$, respectively. Light intensity changes

affect plant physiology, especially on leaf chlorophyll levels (Liu et al. 2017; Yang et al. 2018). According to Wang et al. (2021), chlorophyll a, chlorophyll b, and total chlorophyll levels in legume leaves were higher at low light-intensity conditions. Chlorophyll is an essential component in the photosynthetic system and can absorb, transmit, and convert light energy (Lazár 2015).

Chlorophyll a and b are the main components of chlorophyll which has different roles. Chlorophyll-a is directly associated with photosynthesis, while chlorophyll-b in photosystem II captures diffused light (Field et al. 2013). Shading stress increases photochromic content proteins in the photosynthetic system, which can bind more chlorophyll and reduce chlorophyll degradation and photooxidation, increasing chlorophyll content to increase the efficient utilization of light energy (Yao et al. 2017).

Based on Post Hoc HSD, leaf carotenoid levels showed that the SxG interaction between *D. longan* stands and KP-6718-1-1-10 had the highest leaf carotenoid content compared to the interactions of other treatments, which was 2.02 $\text{mg} \cdot \text{g}^{-1}$. *D. longan* stands between the Yard-long bean genotypes formed high total leaf chlorophyll. However, it resulted in a low a/b chlorophyll ratio in the Yard-long bean genotype (Table 3). Similar results have been observed in Gonçalves et al. (2001), which is a decrease in chlorophyll a/b in low light conditions. The decrease in chlorophyll a/b ratio was mainly due to greater shading levels causing an increase in chlorophyll-b content compared to chlorophyll-a. Decreasing chlorophyll a/b values is beneficial for increasing plant-level low-light utilization, which increases plant survivability in the shade (Wang et al. 2021)

In addition, leaf carotenoid content increased with the presence of *D. longan* stands among the Yard-long bean genotypes. The total chlorophyll content in almost all Yard-long bean genotypes increased when planted among *D. longan* stands except for the KP-6796-3-4-7 genotype, which did not change the total chlorophyll content. However, the shade provided by *D. longan* decreased the chlorophyll a/b ratio in six-Yard-long bean genotypes except for the KP-6718-3-2-13 genotype. Shade from *D. longan* also increased leaf carotenoid content, especially in three long bean genotypes, namely KP-6718-1-1-10, KP-6718-1-1-3, and KP-6718-1-1-4, by 38.36%, 8.94%, and 12.64%, respectively.

Table 2. Nitrate reductase activity of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

Treatment	Nitrate reductase activity ($\mu\text{mol NO}_2^- \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)		Average
	<i>P. guajava</i> (S1)	<i>D. longan</i> (S2)	
KP-6718-1-1-10	0.245 e	0.449 a	0.347
KP-6718-1-1-3	0.242 e	0.387 b	0.314
KP-6718-1-1-4	0.255 e	0.205 f	0.230
KP-6718-1-1-5	0.167 g	0.440 a	0.303
KP-6718-3-2-13	0.222 f	0.261 e	0.242
KP-6796-3-4-10	0.356 cd	0.205 f	0.281
KP-6796-3-4-7	0.258 e	0.317 d	0.288
Average	0.249	0.323	(+)

Gonçalves et al. (2001) stated that leaf carotenoid levels in Tonka Bean increased in low light conditions. Plants that experience stress will increase antioxidant levels in their cells, and the carotenoids are one type of antioxidant produced by plants. However, some experts say leaf carotenoid levels will decrease in low-light conditions because plants adapt well to environmental stresses, especially low light (Zhu et al. 2017; Fu et al. 2022).

Flower and harvest time of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

Figure 3 shows that all the Yard-long bean genotypes between the stands of *D. longan* and *P. guajava* had different responses to flowering and harvest time. Generally, the majority of Yard-long bean genotypes among *D. longan* stands had longer flowering times, but only the KP-6796-3-4-10 genotype showed faster flowering times when planted between *D. longan* stands.

The variation of time to harvest can be affected by flowering time because flowering time influences maturity duration, which is early flowering lines also mature early (Mallikarjuna et al. 2019). Flowering time has strong pleiotropic effects on yield traits (Zhang et al. 2012). In general, whether seeds or fruits are gathered, flowering is a need for crop production. Differential environmental cues and endogenous factors, such as genetic and physiological responses, can control crop flowering (Blümel et al. 2015; Bukucu et al. 2020).

Variations in the response of each genotype are also based on differences in their genetic construction. The phenomenon of KP-6796-3-4-10 showed a genetic effect on avoidance in shaded conditions. It is possible that KP-6796-3-4-10 has a mechanism to deal with stress by accelerating its life cycle. Maggio et al. (2018) state that plants use the stress-avoidance strategy when under stress, which causes decreased vegetative growth, premature plant senescence, early flowering, and other symptoms.

Pod number and pod weight of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

Figure 4 shows that all the Yard-long bean genotypes between the stands of *D. longan* and *P. guajava* had different responses to pod numbers per plant and pod weight per plant. Based on Figure 4, we know KP-6796-3-4-10 has a higher pod number and pods weight per plant than other genotypes on both conditions shading by tree stands. Most genotypes have similar responses against shading by tree stands; KP-6718-113, KP-6718-1-14, KP-6718-1-1-5, KP-6718-3-2-13, and KP-6796-3-4-10 showed decreasing pods production, namely pods number per plant and pods weight per plant. However, KP-6718-1-1-10 and KP-6796-3-4-7 showed increasing pod production in the condition that was more shaded. The phenomenon of KP-6718-1-1-10 and KP-6796-3-4-7 showed a genetic effect to adapt in shaded conditions.

Table 3. Chlorophyll content of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

Treatment	Chlo a	Chlo b	Chlo Total	Chlo a/b	Carotenoid (mg.g ⁻¹)
 mg.g ⁻¹				
Plant stand (S)					
<i>Psidium guajava</i> (S1)	0.0239 a	0.0183 b	0.0421 b	1.32 a	1.72 b
<i>Dimocarpus longan</i> (S2)	0.0235 b	0.0226 a	0.0461 a	1.09 b	1.84 a
CV (%)	0.48	0.37	0.11	0.48	0.10
Genotype (G)					
KP-6718-1-1-10	0.0237 ab	0.0216 d	0.0452 d	1.17 d	1.74 d
KP-6718-1-1-3	0.0236 ab	0.0226 b	0.0462 b	1.06 f	1.87 b
KP-6718-1-1-4	0.0237 ab	0.0218 c	0.0455 c	1.12 e	1.85 c
KP-6718-1-1-5	0.0234 b	0.0255 a	0.0490 a	0.93 g	2.01 a
KP-6718-3-2-13	0.0239 a	0.0170 f	0.0409 f	1.40 b	1.57 f
KP-6796-3-4-10	0.0238 ab	0.0165 g	0.0403 g	1.45 a	1.65 e
KP-6796-3-4-7	0.0237 ab	0.0179 e	0.0416 e	1.32 c	1.75 d
CV (%)	0.68	0.40	0.26	0.96	0.31
Plant stand x genotype (SxG)					
S1 x KP-6718-1-1-10	0.0238 a	0.0164 l	0.0402 i	1.45 b	1.46 j
S1 x KP-6718-1-1-3	0.0238 a	0.0198 f	0.0436 e	1.20 e	1.79 d
S1 x KP-6718-1-1-4	0.0239 a	0.0185 g	0.0425 f	1.29 d	1.74 f
S1 x KP-6718-1-1-5	0.0236 a	0.0224 e	0.0460 d	1.05 f	2.01 b
S1 x KP-6718-3-2-13	0.0238 a	0.0174 j	0.0412 h	1.37 c	1.60 h
S1 x KP-6796-3-4-10	0.0241 a	0.0155 m	0.0396 j	1.55 a	1.65 g
S1 x KP-6796-3-4-7	0.0239 a	0.0177 i	0.0416 g	1.35 c	1.77 e
S2 x KP-6718-1-1-10	0.0235 a	0.0268 b	0.0502 b	0.88 h	2.02 a
S2 x KP-6718-1-1-3	0.0235 a	0.0255 c	0.0489 c	0.92 g	1.95 c
S2 x KP-6718-1-1-4	0.0235 a	0.0251 d	0.0486 c	0.94 g	1.96 c
S2 x KP-6718-1-1-5	0.0233 a	0.0286 a	0.0519 a	0.81 i	2.01 b
S2 x KP-6718-3-2-13	0.0239 a	0.0166 k	0.0405 i	1.43 b	1.55 i
S2 x KP-6796-3-4-10	0.0236 a	0.0174 j	0.0410 h	1.35 c	1.65 g
S2 x KP-6796-3-4-7	0.0235 a	0.0181 h	0.0417 g	1.30 d	1.73 f
CV (%)	0.68	0.40	0.26	0.96	0.31

Note: The numbers in the same column followed by the same letter are not significantly different in the HSD Test at a significant level of 5%

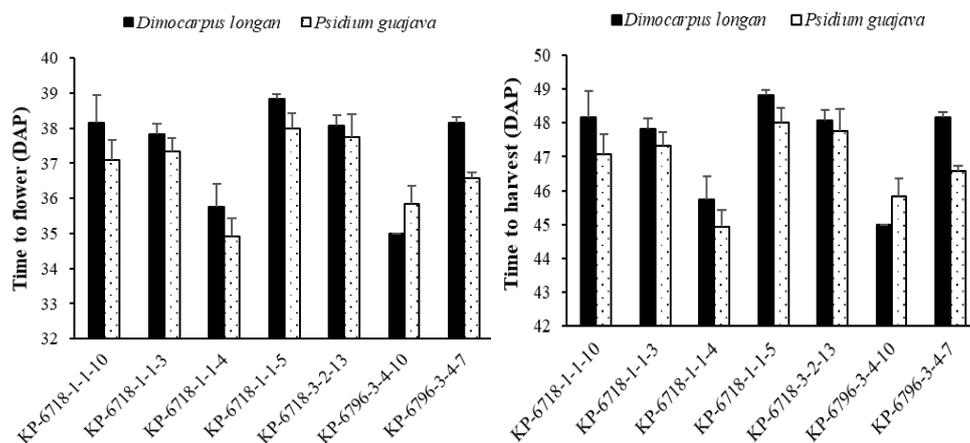


Figure 3. Flower and harvest time of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

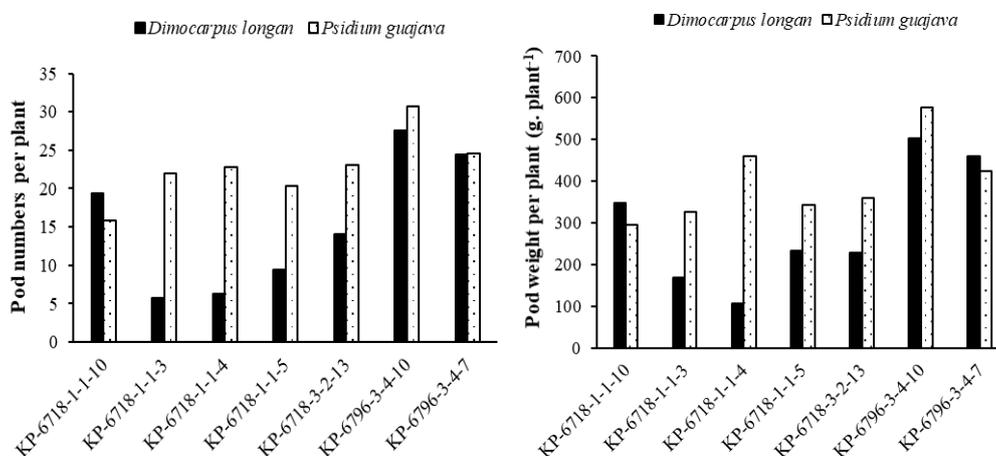


Figure 4. Pod number and pod weight of Yard-long bean genotype between *Dimocarpus longan* and *Psidium guajava* stand

In conclusion, seven Yard-long beans genotypes have variability in leaf characters. The yard-long bean genotype showed low stomatal density and trichome density among *D. longan* stands. Chlorophyll-b levels in all Yard-long bean genotypes increased among *D. longan* stands, causing a decrease in the chlorophyll a/b ratio. Genotype KP-6718-1-1-10 has a fast harvesting age but gives relatively high pod production when planted between stands of *D. longan*. The genotype KP-6796-3-4-7 gave a longer harvest time but high pod production when planted between stands of *D. longan*. Genotypes KP-6718-1-1-10 and KP-6796-3-4-7 are the potentials to be registered as new varieties. These are suitable to be developed in Agroforestry or shaded conditions. KP-6796-3-4-10 has character early flowering and harvesting in shaded conditions with good production traits.

ACKNOWLEDGEMENTS

Thanks are conveyed to the Ministry of Research, Technology and Higher Education, Indonesia, in

accordance with the Research Funding Agreement and Community Service for Fiscal Year 2021.

REFERENCES

- Abdel-Wahab TI, El-Rahman RA. 2016. Response of some soybean cultivars to low light intensity under different intercropping patterns with maize. *Intl J Appl Agric Sci* 2 (2): 21-31. DOI: 10.11648/j.ijaas.20160202.11.
- Armero JG, Pericas MF, Mulet PA, Conesa MA, Martin C, Galmes J. 2018. The ratio of trichomes to stomata is associated with water use efficiency in *Solanum Lycopersicum* (tomato). *Plant J* 96: 607-619. DOI: 10.1111/tpj.14055.
- Bellaloui N, Smith JR, Gillen AM, Fisher DK, Mengistu A. 2012. Effect of shade on seed protein, oil, fatty acids, and minerals in soybean lines varying in seed germinability in the early soybean production system. *Am J Plant Sci* 3: 84-95. DOI: 10.4236/ajps.2012.31008.
- Blair MW, Wu J. 2016. Editorial: Food legume diversity and legume research policies. *Crop J* 4: 339-343. DOI: 10.1016/j.cj.2016.09.001.
- Blair MW, Wu X, Bhandari D, Bhandari D, Zhang X, Hao J. 2016. Role of legumes for and as horticultural crops in sustainable agriculture. In: Nandwani D. (eds). *Organic Farming for Sustainable*, Springer International, Switzerland. DOI: 10.1007/978-3-319-26803-3_9.
- Blümel M, Dally N, Jung C. 2015. Flowering time regulation in crops-what did we learn from Arabidopsis? *Curr Opin Biotechnol* 32: 121-129. DOI: 10.1016/j.copbio.2014.11.023.

- Bukucu SB, Sutyemez M, Kefayati S, Paizila A, Jighly A, Kafkas S. 2020. Major QTL with pleiotropic effects controlling time of leaf budburst and flowering-related traits in walnut (*Juglans regia* L.). *Sci Rep* 10: 15207. DOI: 10.1038/s41598-020-71809-x.
- FAO [Food and Agriculture Organization of the United Nations]. 2015. Agroforestry. <https://www.fao.org/forestry/agroforestry/80338/en/>
- Field KJ, George R, Fearn B, Quick WP, Davey MP. 2013. Best of both worlds: simultaneous high-light and shade-tolerance adaptations within individual leaves of the living stone *Lithops aucampiae*. *PLoS ONE* 8: e75671. DOI: 10.1371/journal.pone.0075671.
- Fu X, Chen J, Li J, Dai G, Tang J, Yang Z. 2022. Mechanism underlying the carotenoid accumulation in shaded tea leaves. *Food Chem* 10 (4): 1-10. DOI: 10.1016/j.fochx.2022.100323.
- Gonçalves CFDE, Marenko RA, Vieira G. 2001. Concentration of photosynthetic pigments and chlorophyll fluorescence of mahogany and tonka bean under two light environments. *Rev Bras Fisiol Veg* 13 (2): 149-157. DOI: 10.1590/S0103-31312001000200004.
- Haque MM, Ahmedz J. 2010. Morpho-physiological changes and yield performance of Yard-long bean under reduced light levels. *J Sher-e-Bangla Agric Univ* 4 (2): 88-93.
- Haque MS, Azad A, Saha NR, Islam MM. 2020. Analysis of yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*) genetic diversity using RAPD markers. *Asian J Biotechnol* 12: 127-135. DOI: 10.3923/ajbkr.2020.127.135.
- Herawati MM, Kasmiyati S, Kristiani EBE. 2019. The effect of shading on density and size of glandular trichomes in *Artemisia cina* tetraploid, the source of anti-cancer artemisinin. *J Phys Conf Ser* 1524: 1-6. DOI: 10.1088/1742-6596/1524/1/012113.
- Inamdar JA, Patri RC, 1969. A new technique for making plant epidermal imprints using various domestic adhesives. *J Microsc* 90 (3): 269-272. DOI: 10.1111/j.1365-2818.1969.tb00711.x.
- Jumrani K, Bhatia VS. 2020. Influence of different light intensities on specific leaf weight, stomatal density photosynthesis and seed yield in soybean. *Plant Physiol Rep* 25 (2): 277-283. DOI:10.1007/s40502-020-00508-6.
- Kaiser WM, Huber SC. 2001. Post-translational regulation of nitrate reductase: Mechanism, physiological relevance and environmental triggers. *J Exp Bot* 52: 1981-1989. DOI: 10.1093/jexbot/52.363.1981.
- Kongjaimun A, Kaga A, Tomooka N, Somta P, Vaughan DA, Srinives P. 2012. The genetics of domestication of yardlong bean, *Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*. *Ann Bot* 109: 1185-1200. DOI: 10.1093/aob/mcs048.
- Kusmiyati F, Anwar S, Herwibawa B. 2021. Agronomic character evaluation of F3 Yard-long bean progenies. *AIP Conf Proc Intl Conf Life Sci Technol* 2353: 030019. DOI: 10.1063/5.0052844.
- Kusmiyati F, Sutarno, Sas MGA, Herwibawa B. 2018. Mutagenic effects of gamma rays on soybean (*Glycine max* L.) germination and seedlings. *IOP Conf Ser: Earth Environ Sci* 102: 012059. DOI: 10.1088/1755-1315/102/1/012059.
- Lazár D. 2015. Parameters of photosynthetic energy partitioning. *J Plant Physiol* 175: 131-147. DOI: 10.1016/j.jplph.2014.10.021.
- Lestari MW, Arfarita N, Sharma A, Purkait B. 2019. Tolerance mechanisms of Indonesian plant varieties of Yard-long beans (*Vigna unguiculata* subsp. *sesquipedalis*) against drought stress. *Indian J Agric Res* 53 (2): 223-227. DOI: 10.18805/IJARe.A-369.
- Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans* 11 (5): 591-592. DOI: 10.1042/bst0110591.
- Liu X, Rahman T, Song C, Su B, Yang F, Yong T. 2017. Changes in light environment, morphology, growth and yield of soybean in maize-soybean intercropping systems. *Field Crops Res* 200: 38-46. DOI: 10.1016/j.fcr.2016.10.003.
- Maggio A, Bressan RA, Zhao Y, Park J, Yun DJ. 2018. It's hard to avoid avoidance: Uncoupling the evolutionary connection between plant growth, productivity and stress "tolerance". *Intl J Mol Sci* 19: 3671. DOI: 10.3390/ijms19113671.
- Mallikarjuna BP, Viswanatha KP, Samineni S, Gaur PM. 2019. Association of flowering time with phenological and productivity traits in chickpea. *Euphytica* 215 (77): 1-8. DOI: 10.1007/s10681-019-2397-2.
- Mawarni L, Nisa TC, Napitupulu JA, Karyudi. 2019. Determination of leaf status of soybean varieties on shading. *Biodiversitas* 20 (2): 615-620. DOI: 10.13057/biodiv/d200243.
- Morozkina EV, Zvyagilskaya RA. 2007. Nitrate reductases: Structure, functions, and effect of stress factors. *Biochemistry* 72 (10): 1151-1160. DOI: 10.1134/s0006297907100124.
- Pidigam S, Thuraga V, Pandravada SR, Natarajan S, Adimulam S, Amarapalli G, Nimmarajula S, Venkateswaran K. 2021. Genetic Improvement of Yard-long Bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* (L.) Verdc.). In: Al-Khayri JM, Jain SM, Johnson DV. (eds). *Advances in Plant Breeding Strategies: Vegetable Crops*. Springer, Cham. DOI: 10.1007/978-3-030-66969-0_10.
- Raux PS, Gravelle S, Dumais J. 2020. Design of a unidirectional water valve in *Tillandsia*. *Nature* 11: 396. DOI: 10.1038/s41467-019-14236-5.
- Sas MGA, Wahyu Y, Syukur M, Hidayat P. 2021. Morpho-agronomy performance, seed nutrition content, and *Aphis craccivora* resistance of cowpea (*Vigna unguiculata*) genotypes. *Biodiversitas* 22 (10): 4320-4326. DOI: 10.13057/bio div/d221024.
- Singh T, Sheera A, Rout S. 2021. Vegetable cultivation in agroforestry. In: Khare N, Rout S, Sahoo G, Ray M, Prusty M, Prusty AK. (eds) *Agroforestry: Prospective, Strategies and Future Aspect*. Taran Publication, New Delhi.
- Sundari T. 2009. Morphological and physiological characteristics of shading tolerant and sensitive mungbean genotypes. *Hayati* 16 (4): 127-134. DOI: 10.4308/hjb.16.4.127.
- Vivalapali, SK, Celine VA, Vahab AM. 2014. Assessment of genetic divergence in among Yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*[L.] Genotypes. *Legume Genomics Genet* 5 (1): 1-3. DOI: 10.5376/LGG.2014.05.0001.
- Wang Y, Huang R, Zhou Y. 2021. Effects of shading stress during the reproductive stages on photosynthetic physiology and yield characteristics of peanut (*Arachis hypogaea* Linn.). *J Integr Agric* 20 (5): 1250-1265. DOI: 10.1016/S2095-3119(20)63442-6.
- Widyawan MB, Hasanah A, Taryono, Alam T, Sayekti RS, Pramana AAC, Wulandari RA. 2020. Multivariate analysis unravels genetic diversity and relationship between agronomic traits, protein, and dietary fiber in yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* Verdc.). *Biodiversitas* 21: 5662-5671. DOI: 10.13057/biodiv/d211211.
- Yang F, Feng LY, Liu QL, Wu XL, Fan YF, Raza MA, Cheng YZ, Chen JX, Wang XC, Yong TW, Liu WG, Liu J, Du JB, Shu K, Yang WY. 2018. Effect of interactions between light intensity and red-to-far-red ratio on the photosynthesis of soybean leaves under shade condition. *Environ Exp Bot* 150: 79-87. DOI: 10.1016/j.envexpbot.2018.03.008.
- Yao XD, Zhou HL, Zhu Q, Li C H, Zhang HJ, Wu JJ, Xie FT. 2017. Photosynthetic response of soybean leaf to wide light-fluctuation in maize-soybean intercropping system. *Front Plant Sci* 8: 1695. DOI: 10.3389/fpls.2017.01695.
- Zhang ZZ, Wang K, Guo L, Zhu YJ, Fan YY, Cheng SH, Zhuang JY. 2012. Pleiotropism of the photoperiod-insensitive allele of Hd1 on heading date, plant height and yield traits in Rice. *PLoS ONE* 7 (12): e52538. DOI: 10.1371/journal.pone.0052538.
- Zhu H, Li X, Zhai W, Liu Y, Gao Q, Liu J, Ren L, Chen H, Zhu Y. 2017. Effects of low light on photosynthetic properties, antioxidant enzyme activity, and anthocyanin accumulation in purple pakchoi (*Brassica campestris* ssp. *Chinensis* Makino). *PLoS ONE* 12 (6): 1-17. DOI: 10.1371/journal.pone.0179305.