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Determination of leaf status of soybean varieties on shading

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Abstract. *Mawarni L, Nisa TC, Napitupulu JA, Karyudi. 2019. Determination of leaf status of soybean varieties on shading. Biodiversitas 20: 615-620.* The shading tolerant soybean varieties could be identified through the character growth such as leaf status. This study was aimed to obtain the shading tolerant of soybean variety based on their seed size and on the leaf status. Design of experiments was a split-plot design with three replications. The level of shading as the main plot with four levels of treatment was without shading, 30%, 50%, and 70% shading. Soybean varieties as the subplot had 4 genotypes namely Anjasmoro, Pangrango, Tanggamus, and Nanti wherein one variety comes with large seed; two come with medium seeds and the other one comes with small seed. The leaf status for determination was the greenness of leaves, the sum of chlorophyll a, chlorophyll b, total chlorophyll a and b, the forms of chloroplasts and leaf area. The soybean varieties with different seed sizes showed the differences only on the leaf area but not on leaf greenness or on the sum of chlorophyll. Nanti variety, having small seed, turns out to have the largest leaf area but is not shade tolerant. The large leaf area may be due to the forming of tetra foliate leaves on Nanti variety. The form of chloroplast of Anjasmoro variety in 70% shading is better than other varieties. Therefore, determining leaf status can identify shading tolerant of soybeans.

Keywords: Chlorophyll, chloroplast, leaf area, shading tolerant, soybean

INTRODUCTION

In the last decade, the cropping system has been rediscovered by scientific research. Monocropping systems have to be revised and may not be the best-performing systems anymore, considering sustainability, income security and nutritional diversity in rural areas. Therefore, intercropping systems offer alternatives for more sustainable agriculture with reduced input, and stabilized yield (Knoerzer et al. 2009) continued the thought of agroecology (Altieri 1998; Gliessman 2016). In this study, intercropping soybean under the oil palm will be simulated in a shading house. Light is shown to affect the orientation of plant chloroplasts, such as in low light intensity, chloroplasts will be collected from two parts, namely the nearest cell wall and the furthest cell wall from light. This causes the leaf color to be greener because the position of the chloroplast is concentrated on the leaf surface (Anderson and Osmond 1987; Salisbury and Ross 1992). Handayani (2003) found that Ceneng (black soybeans) and Pangrango variety consistently tolerant to 50% and 75% shading wherein anatomical character closely associated with the character of tolerance is chlorophyll a while morphological character is the number of productive branches. Trikoesoemaningtyas et al. (2008) resumed the expected value of the low light intensity affected the anatomy and morphology of soybean leaf, i.e. the amount of chlorophyll and size of leaves. Shaded plants will get limited sunlight, even though the light has a very important role in the process of plant growth and development. Low light intensity stresses also result in changes in agronomic, anatomical, physiological, molecular and biochemical characters (chlorophyll, carotene, carbohydrates, and rubisco enzymes) which are related to photosynthetic efficiency so that it will affect the yield of these plants (Soverda et al. 2013).

The general purpose of this study was to obtain a shading tolerant soybean varieties and to identify growth characters (morphological, anatomical, physiological and biochemical) and yield components which are closely associated with the nature of soybean tolerance to shading. In this study, we focused on leaf status. The leaf status for determination was the leaf greenish, the sum of chlorophyll a, chlorophyll b, total chlorophyll, anatomy forms of chloroplasts and leaf area. Globally, the study about this was still rare especially on soybean with various seed size.

MATERIALS AND METHODS

This experiment was conducted used polybag in arranged shading house at the Experimental Farm, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Selangor, Malaysia on latitude 2.986649 and longitude 101.708301.

Materials

The materials are seeds of soybean varieties namely Anjasmoro, Pangrango, Tanggamus, and Nanti with descriptions are listed in Table 1. According to the classification of Balitkabi (*Balai Penelitian Kacangan dan*

Umbian Indonesia) or Iletri (Indonesian Legumes and Tuber Crops Research Institute) (2005), the size of soybeans can be classified into three groups, namely small seed (<10 g/100 seeds), medium seed (10-12 g/100 seeds), and large seed (13-18 g/100 seeds). Anjasmoro was soybean variety with large seed, Pangrango and Tanggamus were with medium seeds and Nanti was with small seed.

This variety was screened from early experiment with the result that Anjasmoro and Pangrango were variety consistently tolerant to shading but Tanggamus and Nanti were not tolerant. Based on grain yield per plant (g), it was found that the variety of Nanti had a small tolerance index while Pangrango was able to tolerate 70% shading, and Anjasmoro variety is able to tolerate 30% and 50% shading (Mawarni 2011). Reasons for variety selection also adjusted for time of flowering (R1) which was similar and all varieties had determinate development type. Soil media was the mixture of topsoil, sand, compost (with volume ratio 3: 3: 2), which was put in polybag with diameter 25 cm long 35 cm (40 x 21 cm folded) in shelter houses made of wood with black polypropylene plastic gauze according to treatment, added with NPK compound fertilizer (15: 15: 15), insecticide, fungicide, herbicide, and supplied with chemicals for histological investigation.

Instrumentation

The instrumentation used in this study were the farming tools, 25 kg scales, beaker glass for measuring the amount of watering, thermometers for measuring air temperature, 4 mm sieve and laboratory tools such as ovens, microtomes for conducting histological investigations and Transmission Electron Microscopy (TEM) Olympus BZ for describing histological of leaf anatomy especially the chloroplast. Other instruments were Minolta Chlorophyll meter SPAD-502 to measure the greenness of leaves in the field, Spectrometer to measure the amount of chlorophyll a and b, Lux meters (range 0-200,000 Lux and accuracy ± 2%) to measure the intensity of the sun, LI-6200 XT (Portable Photosynthesis System) to measure simultaneously the amount of light received by leaves (μ mol m-2 s-1), leaf temperature (ºC), CO_2 concentration (ppm), relative humidity (%), net photosynthesis (μ mol m^{-2} s^{-1}), stomatal resistance (s cm^{-1}), stomatal conductance (cm s^{-1}) and air temperature (ºC) and LICOR Leaf Area Meter Model Li-3100 for measuring leaf area. In this paper, we focused on studying the leaf greenish, the sum of chlorophyll a, chlorophyll b, total chlorophyll, forms of chloroplast, and the leaf area.

Making shelter houses. Supporting logs were used as pillars for shelter houses. Each shelter was 3 m x 1.5 m tall and 1.5 m high above the ground. Three types of black polypropylene plastic gauze were connected to get 30%, 50% and 70% light transmission. Each main plot or between blocks was 2 m apart to avoid shadows between shelters.

Preparation for polybags. The soil was dried first for about 5 days and then sieved with a 4 mm sieve while cleaning from dirt. Soil media (volume ratio of topsoil: sand: compost = 3: 3: 2) was mixed evenly using a hoe, and put in each polybag as much as 5 kg. Then all polybags were arranged in shading house according to the treatment.

Planting soybeans. A day before planting, Furadan was given as much as 0.2 g per hole to prevent the attack of seed flies. In each polybag, soybean seed was planted with ± 2 cm deep and covered with soil.

Nurture. Watering was done manually with as much as 500 ml of water/polybag/day except on raining day as the water was enough. NPK compound fertilization (15: 15: 15) was given only 30 days after planting as much as 2 g/polybag. After 24 days of planting, the plants are inoculated with UPM R48 3 ml of solution per polybag. The solution was dripped around the root neck using a millimeter pipe. Pest prevention was carried out by spraying Decis 2.5 EC insecticide with a dose of 0.7 ml/liter of water starting at 2 weeks after planting and it was repeated every 2 weeks to 1 week before harvest. Prevention of disease was done by spraying Dithane M-45 fungicide with a dose of 1 g/liter of water starting at 3 weeks after planting and it was repeated every 2 weeks to 2 weeks before harvest. Weeds in the shelter were carefully sprayed with the mixture of Gramoxone 45 WP and 75 ml/14 L of water while the weeds outside were trimmed with a lawn machine regularly.

Table 1. Description of soybean varieties (Balitkabi 2016)

Description	Anjasmoro	Pangrango	Tanggamus	Nanti
Source	Mass selection from pure strain Mansuria	Crossing local variety of Lampung x Davros in 1983	Derivate selection of Kerinci x No. 3911	Single cross of Dempo variety and No. 3623
Potential production (ton/ha)	2.03-2.25	1.4-2.0	1.22	1.24
Color of leaf	Green	-	-	Dark green
Development type	Determinate	Determinate	Determinate	Determinate
Time of flowering (days)	35.7 - 39.4	±40	35	35
Sharp of leaf	Ovale	-	-	Lanceolate, medium size
Weight of 100 seeds	14.8-15.3 g	± 11 g	11 g	10 g, oval shape

Methods

A split-plot factorial design was used in this study with three replications. The treatment consisted of (i) the level of shading as the main plot with 4 levels of treatment i.e. without shading, 30% shading, 50% shading, and 70% shading; (ii) soybean varieties as the subplot with four varieties, i.e. Anjasmoro, Pangrango, Tanggamus, and Nanti. All data were collected from soybean leaves samples at 75% R1 stadia (Fehr et al. 1971). The sum of chlorophyll in the leaf on the field was measured with Chlorophyll Minolta SPAD-502 meter on samples 6 mm^2 on three places, i.e. top, middle and bottom leaves then was averaged (Chang and Robinson 2003; Parry et al. 2014; Yuan 2016). The sum of chlorophyll a and chlorophyll b from leaf tissue was measured using Spectrometer with Method of Coombs (Coombs et al. 1985). The leaf tissue for histological leaf anatomy was conducted with Method of Spence (Spence 2001) consisting of fixation, vacuum, dehydration, infiltration, embedding, sectioning with microtome at 10-12 mm, mounting and staining. Leaf area in mm^2 was measured on all leaves in the samples, which were taken and immediately measured with Leaf area meter LICOR LI-3100. Data were analyzed by ANOVA (F test) at the test level of 5%, followed by a DMRT test (Gomez and Gomez 1984) at test level 5%. The data was processed by SAS and Microsoft Excel program.

RESULTS AND DISCUSSION

The greenness of leaves

Table 2 showed the greenness of leaves of soybean by using SPAD-502 at various levels of shading and varieties were not significantly affected.

The sum of chlorophyll a, chlorophyll b, and total chlorophyll

The investigation continued to calculate the sum of chlorophyll a, chlorophyll b and total chlorophyll with a spectrometer to more clearly determining leaf status. The shading treatment causes a real difference to the sum of chlorophyll a and total chlorophyll. The varieties led to a noticeable difference to the sum of chlorophyll a, chlorophyll b and total chlorophyll.

Differences in shading levels showed a significant effect on chlorophyll a and total chlorophyll a and chlorophyll b. However, varieties and interactions between shading and variety levels did not significantly affect chlorophyll a and total chlorophyll a and chlorophyll b. Differences in the level of shading, variety, and interaction of shade and variety levels did not significantly affect the sum of chlorophyll b. Further tests were listed in Table 3.

The highest sum of chlorophyll a was found in the non-shade treatment followed by 50 and 70% shade levels which were not significantly different from 30% shade. While, the shade level of 30% showed a smallest number of chlorophyll a but was not significantly different with 50 and 70% shade. A similar phenomenon occured in the total sum of chlorophyll a and chlorophyll b.

The form of chloroplast

Figure 1 shows the chloroplast to 70% shading (the level of extreme shading) on leaf tissue. The shape of chloroplasts on all varieties looked like damaged at R1 stadia. In Anjasmoro, the variety had relatively better chloroplasts than that in Pangrango and Tanggamus varieties while chloroplasts of Nanti variety was very thin and was not green anymore. Forms of chloroplasts of four varieties showed clear differences. Chloroplasts in Anjasmoro variety still showed large shape (long size ± 2978µm), intact and contained a green substance. The measurement results on chloroplasts at Pangrango variety were still intact but less green and smaller than Anjasmoro but greener than varieties of Tanggamus and Nanti. Meanwhile, chloroplasts in Nanti were small (long size ± 2250 µm), incomplete and green levels were smaller than the other three varieties. This showed that the variety of Anjasmoro was quite tolerant to light reduction while the most intolerant variety was Nanti. These results supported the previous research by Mawarni (2011) which reported that Anjasmoro and Pangrango were varieties consistently tolerant to shading, but Tanggamus and Nanti were not.

Table 2. The greenness of leaves of soybean at various levels of shading and varieties

Variety	Levels of shading				
	0%	**30%**	**50%**	**70%**	**Average**
	unit/6 mm^2				
Anjasmoro	36.83	37.33	37.67	35.83	36.92
Pangrango	32.53	37.70	36.17	35.57	35.49
Tanggamus	34.57	34.33	36.07	36.43	35.35
Nanti	35.03	31.90	35.90	35.13	34.49
Average	34.77	35.22	35.68	35.49	

Table 3. The sum of chlorophyll a, chlorophyll b and total chlorophyll of soybean at various levels of shading and varieties

Treatments	Chl a	Chl b	Total Chl a+b
	mg/g bb		
Levels of shading			
Non (0%)	5.06 a	3.17	8.23 a
30%	2.80 b	2.16	4.96 b
50%	4.33 ab	2.71	7.05 ab
70%	4.21 ab	2.40	6.61 ab
Varieties :			
Anjasmoro	3.93	2.37	6.30
Pangrango	4.68	2.44	7.13
Tanggamus	4.63	2.97	7.61
Nanti	4.01	2.35	6.35

Note: Different letters in the same column and group represent significant at Duncan's multiple range test (p=0.05)

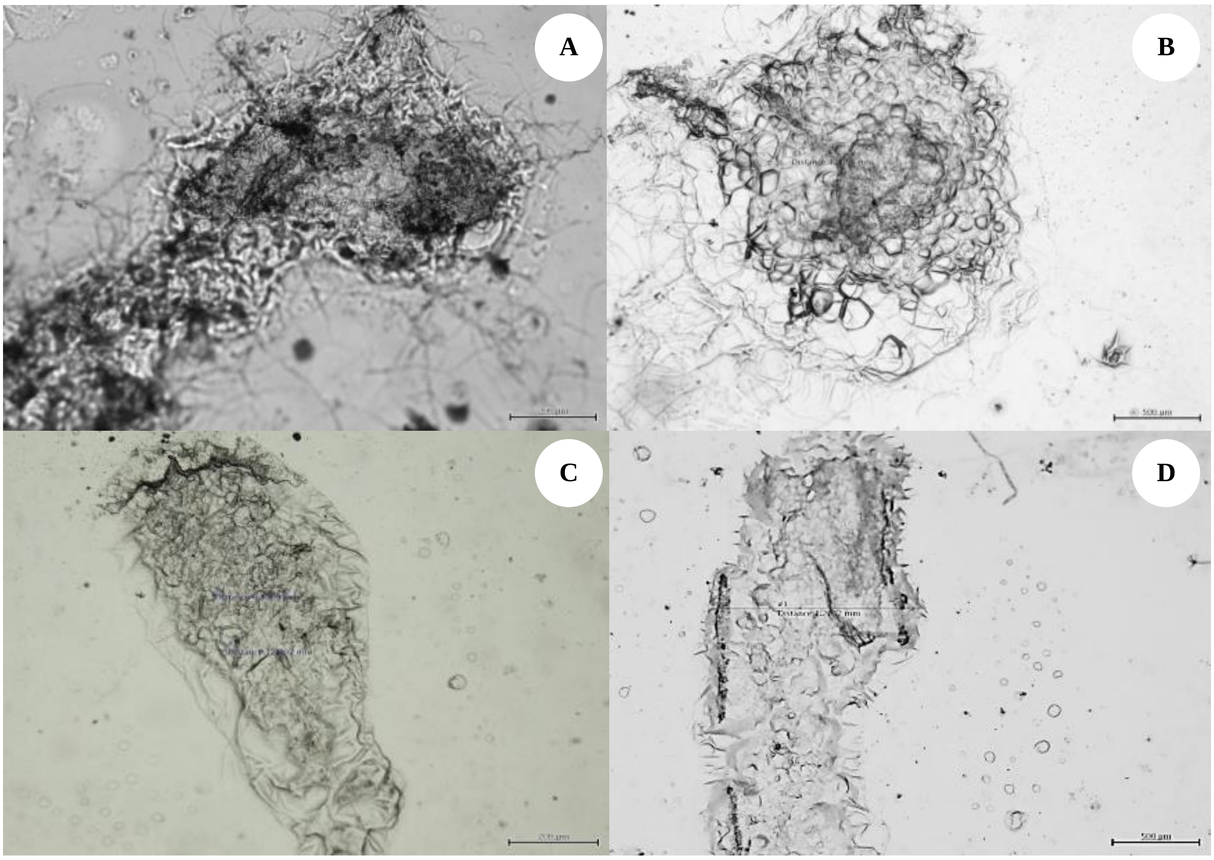

Figure 1. Shapes of chloroplasts in four soybean varieties. A. Anjasmoro, B. Pangrango, C. Tanggamus, D. Nanti to 70% shading at R1 stadia with a TEM in magnification of 1000 x

Table 4. Leaf area of soybean at various levels of shading and varieties

Variety	Levels of shading				
	0%	30%	50%	70%	Average
			cm^2		
Anjasmoro	29.44	35.79	33.93	34.48	33.41 bc
Pangrango	30.53	36.54	32.91	30.59	32.64 c
Tanggamus	20.40	36.18	44.46	33.49	33.63 bc
Nanti	42.65	43.10	47.05	48.74	45.39 a
Average	33.75	37.90	39.59	36.83	

Note: Different letters in the same column represent significant at Duncan's mutiple range test (p=0.05)

Leaf area

Plant leaf area at R1 stage was significantly affected by variety but was not significant affected by the levels of shading and the interaction of the two factors. Table 4 shows that the Nanti variety has the largest leaf area, while, Anjasmoro, Pangrango and Tanggamus varieties have no significantly different.

Discussion

Overview of leaf tissue for histological evidence caused by shade is the shape of chloroplasts (Tsukaya 2003; Kume 2017). Therefore, in this study, the shape of chloroplasts was studied and was associated with the greenness of the leaves and the amount of chlorophyll. Shade-resistant plants can regulate leaves according to the direction and intensity of light so that the shade conditions direct the chloroplasts to muster close to the epidermal layer, consequently leaf color is greener. Experiments with Iris leaves grown at varying light intensities indicate that the chloroplast is green and the number of stomata decreases with decreasing light intensity (Nobel and Long 1985).

Zhang et al. (2016) stated that the higher levels of grana formation in plant chloroplasts appear to be related to the total chlorophyll content. The formation of grana may only be a means of achieving a higher density of light and therefore a more efficient collection of light quanta. In this study, the greenness of the leaves did not show a difference either due to increased shade or differences in responsiveness between varieties. Similarly, there was no difference in chlorophyll content between varieties. The difference in the amount of chlorophyll was due to difference in the level of shade wherein the treatment without shade showed the highest result of chlorophyll a and total chlorophyll. In this study, the difference of total chlorophyll was due to difference of chlorophyll a from chlorophyll b. Chlorophyll a is the primary photosynthetic pigment while chlorophyll b is the accessory pigment that collects energy and passes it on to chlorophyll a. Chlorophyll a is the reaction center of the antenna array of core proteins while chlorophyll b regulates the size of the antenna (Trikoesoemaningtyas 2008).

Figure 2. The formation of tetrafoliate in soybean due to shade

Although, the amount of chlorophyll was not significantly different between varieties, the shape of chloroplast in 70% shade shoed that the Nanti variety has grana in chloroplasts which is not visible anymore. Shade-resistant plants can regulate and direct the leaves according to the direction and intensity of light so that in the shade conditions, they can direct the chloroplasts to congregate near the epidermal layer, consequently, the leaf's color becomes greener. The ability of plants to adapt to shady conditions is determined by their ability to be able to carry out photosynthesis normally in low light conditions. Solar radiation affects the condition of chloroplasts. Chloroplasts will congregate on the side of the closest cell wall and farthest from the radiation. This condition causes the leaves to appear green in shade conditions because the chloroplasts clump on the leaf surface (Kume et al. 2016). However, from this study, the greenness of the leaves did not have a significant effect on the level of shade, variety, and combination (Table 3).

The increase in leaf area as a distinctive feature in shaded plants shows an interesting thing. The leaf area of the plant is not different due to the level of shade. The difference is in the ability of the variety, where the Nanti variety shows the largest leaf area. Nanti variety, with small seeds, turns out to have the largest leaf area but Nanti is included in non-shade tolerant. The large leaf area may be due to the Nanti variety forming tetrafoliate leaves at the bottom of the plant as the characters of shaded soybean plants which should be trifoliate in normal conditions (Figure 2). The purpose of plants forming leaves of tetrafoliate is to increase leaf area as reported by Hidayat (1988). But not all leaves become sources and even become sinks just because they are shaded, as explained by Sonnewald and Rfernie (2018).

In the future, it is possible to see damage to chloroplasts as a result of shade from chloroplast damage in the amount or amount of chlorophyll compared to calculating the greenness of leaves. Studies of soybean leaves in the shade (Yang et al. 2018) found that Chl a, Chl a + b, and Car content was different significantly between normal (N), low light (L), and shade (S) treatments. Plants under treatment N and L each showed minimum and maximum values. Furthermore, Chl a, Chl b, Chl a + b, and Car content under treatment L increased by an average of 50.13%, 122.75%, 63.32%, and 43.06%, proteins that differently accumulated were identified significantly to be rich in chloroplasts. Given that photosynthesis occurs in chloroplasts, chlorophyll biosynthesis and light reactions directly affect photosynthetic capacity. Wu et al. (2018) further showed that (i) leaf area, leaf anatomical structure, and newly developed photosynthetic function of leaves is regulated by systemic irradiation signals from adult leaves; (ii) decreasing cell size and cell number are the main causes of smaller and thinner leaves in the shade; and (iii) sugar can act as a substance signal candidate to regulate the leaf area systemically.

This study also uses seed size as a basis for seed selection. According to seed size depending on varieties (Tables 1, 2 and 3), in this study, the greenness of leaves, chlorophyll a, chlorophyll b and the total chlorophyll of soybean at various varieties were not significant, except for leaf area, so the size of the seeds did not determine leaf status. Meanwhile, El-Din et al (2016) reported, on beans, there is a clear positive increase of plant vegetative growth parameters, chlorophyll, the content of N, P, and K in leaf and the number and quality of seed yield which is obtained by sowing large seeds compared to other seed sizes, meaning that the size of seeds determines their growth.

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