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Local Soybean (*Glycine max* (L)) Stomatas' Morphological And Anatomic Response In 3rd Vegetation Stage Towards Light Intensity Sress

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Abstract. The aim of this research is to know the morphological and anatomic stomatas's response of local soybean varieties in a 3rd vegetative stage towards light intensity stress. The experiments were arranged in a single factor Completely Randomized Design (CRD) with the treatment consisting of: without shade and with shade of 50, 60, 70, and 80% which repeated three times. The results showed that light intensity affects stomata length, diameter, and number of stomatal. The use of 50% shade which produces a daily average light intensity of 18,360 lux shows the number, length, and diameter of stomata that are not different from the controls. This local soybean cultivation can be done in shade intensity 50-70%.

1. Introduction

One effort to reduce soybean imports dependence which currently reac 260% requirement [1], is by utilize dry land for soybean extensification. Nevertheless, there are some obstacles such as soil acidity, low cation exchange capacity, and P deficiency, potential for drought stress [2], and low sunlight intensity. This can happen because some land is only available in forest plantation and plantations itself, and also in agroforestry system. Highly intensity of light is greatly determining nutrients role as a barrier for plant growth, however the role is not too strong under low light intensity conditions [3]. However, there are not many studies which discussed about levels of sunlight intensity that control leaf tissue growth including morphological response and stomata anatomy.

The stomata hole greatly determines balance between loss and water retrieval through opening-closing mechanism of the stomata hole [4]. By initiation of abscisate acid levels [5], stomata play an important role in preventing air bubbles formation between xylem transport tissue when the potential of water is low. Its closing stomata hole as dehydration response thus preventing water loss [6, 7]. Furthermore, in sufficient water supply conditions through the mechanism of regulating holes volume by stomatal cells [8], there will be a recovery of water loss and retrieval in which air bubbles in the tissue become re-filled [5]. The stomatal volume pits is determined by the length and diameter of stomata; while the amount of stomata per certain extent of leaves will greatly determine the plant ability to control the balance of the water loss and retrieval. The three variables that stomata had will ultimate determine the plants ability to grow.

This study aims to determine the effect of shade level or sunlight intensity on morphological and anatomical responses that represented in number, length, and diameter of local soybean stomas in the third vegetative stage.

2. Materials and methods

The experiment was conducted at Green House and Agrokompleks Laboratory of Agriculture Faculty of Universitas Muhammadiyah Sidoarjo with height 4.42 m asl.

The local soybean seeds of Gepak Kuning varieties and soil from soybean field are obtained from Balitkabi - Department of Agriculture, Malang, East Java. The seeds are washed with 50% alcohol for 5 seconds and rinsed with sterile water 3 times. After being drained, seeds are planted onto planting medium surface (soil and compost 8:1 v/v) in a 20x20 cm polybag. Polybag placed under the shade of paranet with the percentage of shade 50%, 60%, 70%, 80%, and no shade (control). The 50% shade is a level of shade on the intensity of sunlight by paranet up to 50% against normal conditions. The 50% shade intensity level is also a level that has been tested to produce some shade resistant soybean varieties. Planting media watered every day with sterile water in the morning (at 8:00 pm) and afternoon (at 15:00).

The experiments were arranged in Completely Randomized Design (CRD) with replicates three times to obtain 15 experimental units. The observed variables were the number of stomata per m², stoma cell length, and stoma holes width as measured when the soybean was 18 days after pla4ing (DAP) and performed by using a microscope with 400 times magnification. The data obtained were analysed by ANOVA followed by 5% LSD test. As the supporting data, we also observed the micro climate representation such as light intensity with light meter, environmental temperature (inside and outside), leaf temperature with infrared thermometer, and pH of planting medium. Observations were made from the beginning of 1 DAP until the 18th day at 07.00, 12.00, and 15.00 WIB.

3. Results and discussion

The result of variance analysis (5% level of F tes) showed that shade intensity influence the amount, length and diameter of soybean stomata stems in 3rd vegetative phase or 18th DAP with mean of each variable shown in Table 1 and the representation of diameter and length stomata for each treatment are shown in Figure 1.

Table 1:Mean and standard deviation of shade intensity influence towards morphological and anatomy of soybean plant 18 DAP *) (number of stomata per mm² at lower surface of leaves, diameter and length of leaf stomata (mμ))

Anatomy Descripction	Р0	P1	P2	Р3	P4	LSD 5%
Number of stomata	37.33 ± 5.06	32.70 ± 4.28	29.5 ± 0.66	25.33 ± 3.44	24.20 ± 4.44	7.09
	c	bc	ab	A	a	
Diameter of Stomata	10.98 ± 0.62	9.59 ± 1.31	8.73 ± 1.21	$8,\!47\pm1.31$	$\textbf{7.21} \pm \textbf{1.15}$	2.09
	c	bc	ab	ab	a	
Length of Stomata	15.77 ± 0.55	16.53 ± 0.74	16.96±0.97	18.22 ± 1.89	19.35 ± 1.74	2.36
	a	ab	ab	bc	a	

^{*}The numbers followed by the same letter on the same line show no significant difference in 5% LSD test. P0 = no shade, P1 = shade 50%, P2 = shade 60%, P3 = shade 70% and P4 = shade 80%.

The microclimate that accompanies each treatments is shown in Table 2. From Table 1 it appears that there is no significant difference (5% LSD test) in the number of stomata per mm² at the underside of leaf and diameter and length of stomata between control (P0) and shade 50% (P2) treatments. In 60%, 70% and 80% shade treatment, the light intensity obtained by the plants are 17.056 lux, 16.039 lux and 14.999 lux respectively, giving soybean plants responses which related to morphological adaptation and stochastic anatomy were contrast with control and 50% shade treatment. In 80% shade treatment (Figure 1), stomata length is highest (19.35 mμ) compared to lower shade

level which decreased to smallest average in the control plant stomata (15.77 mµ). That fact is the opposite in terms of number and diameter of stomata which getting smaller with the increasing of shade intensity. This phenomenon is the response of soybean plants up to third stage towards shade that aims to optimize the resources which obtained from the environment either in a water form, CO₂, or nutrients for its growth. Stomatal density depends on atmospheric CO₂ concentration, climate change, and water availability [9]. Stomatal responses in dimensions and densities in leaf tissues are closely related to hydrological conditions [10] in which stomata play a role in water control. The movement of water through evapotranspiration which among them is through stomata, is strongly influenced by solar radiation and environment temperature [11], both of them in this study were represented by light intensity and temperature under shade.

Table 2: Mean of shade effect toward micro climate on soybean plants *)

2				,
P0	P1	P2	P3	P4
26.366	18.360	17.056	16.039	14.999
32. 1	29.9	29.2	28.5	27.7
31.0	29.2	28.6	28.1	27.4
	26.366	26.366 18.360 32. 1 29.9	26.366 18.360 17.056 32.1 29.9 29.2	26.366 18.360 17.056 16.039 32. 1 29.9 29.2 28.5

^{*)} The plant micro climate2s measured for 18 days, with three measurements per day ie morning, noon and afternoon. P0: no shade, P1: 50% shade, P2: 60% shade, P3: 70% shade, P4: 80% shade. The pH of media is 7.0 and the mean temperature outside the auspices of 31°C.

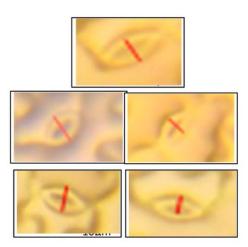


Figure 1: Diameter of soybean plant stomata (with 400 time magnification) on %, 50%, 60%, 70%, and 80% of shade intensity.

The combination of low light levels and high CO₂ concentrations can increase the utilization of photosynthetic light [12, 13] and increase the efficiency of light use in photosynthesis [14]. Photosynthesis can be increased by increasing concentration of CO₂ on shade tolerant species [15], including local soybean varieties that used in this study. An embodiment of this character is the inverse relationship between stomata length (on P0 and P1), and diameter and number of stomata (at P3-P5 treatment) Although no measurements of CO₂ concentrations are observed around the canopy,

but the conditions around the plant will resemble the general condition in the tropics on the forest floor with high CO₂ concentrations [16]. The high concentration of CO₂ can increase carbon uptake by increasing the efficiency of photosynthesis under dynamic light conditions [17, 14]. Even with high CO₂ concentrations, it has a potential to increase the rate of relative photosynthesis in light conditions flecks (below the low-medium intensity paranet) compared to steady state condition (control) [18].

4 Conclusion

Shading intensity significantly affected the number, diameter, and length of soybean stromata on the third stage vegetative growth. The number and diameter of stomata increased and the length of stomata decreased by decreasing shading intensity. The implication of this research is that the local soybean cultivation can be done in shade intensity 50-70%.

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