The Effects of Nitrogen Fertilizer Dosages on Anatomical Characters of *Ipomoea batatas* L. Leaf

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Diterima Oktober 2008 disetujui untuk diterbitkan Januari 2009

Abstract

The research was conducted in order to understand the effects of various nitrogen dosages on the anatomical characters of *Ipomoea batatas* leaf, and to find out the most influential dosage on the number and size of leaf anatomical characters. The characters observed consisted of thickness of cuticle, leaf, and palisade, as well as stoma size, and their density within mm^2 of leaf surface. The method applied was experiments arranged in Completely Randomized Design. The results showed that all levels of nitrogen dosage affected the anatomical characters of sweet potato leaf while the most influential dosage was 200 kg urea per ha.

Key words: anatomical characters, nitrogen, *Ipomoea batatas*

Introduction

Ipomoea batatas (sweet potato) is an annual plant that grows on the land surfaces up to several meters long. Almost of the entire parts of the plant can be consumed as these contain sufficient nutrients including vitamins, minerals, carbohydrates, proteins and lipids (Widodo, 1991). Sweet potato is the second most important crops growing in Indonesia following cassava. Its tubers are mostly used as food while its surplus is used as feed and raw materials for industries (Saleh and Hartoyo, 2003). To increase its production, fertilizers such as nitrogen are commonly applied. Nitrogen is very important element for growing and developing plants in which it is used to form organic molecules such as amino acid, enzymes, coenzymes, amides, nucleic acids, nucleotides, proteins, and chlorophylls. The element is required by plants in amount of 16,000 µm of Hoagland's nutrient solution (Taiz and Zeiger, 1998).

The leaf anatomical structure of all plants is similar where it consists of three parts, *i.e.*, epiderm, mesophyll, and vascular bundles. The epiderm is the outermost of leaves that has function to protect the plant from mechanical injuries, pest attack and plant diseases (Fahn, 1982). The environmental condition affects plant growth and development. For example, leaf anatomical characters, but not anatomical structure, such as cuticle thickness, leaf thickness, palisade thickness, stoma size, and number per square leaf area can be influenced by soil fertility (Dickison, 2000). The cuticle thickness is the most important part of the plant because this gives plant protection from diseases. In addition, stoma number and size are more important factors for the plant structural resistance (Goodman, 1986). Plants with low number of stoma (5-15 stoma per mm^2 leaf) and small size (17-23 µm), have been shown to be more resistant to diseases (Juwarno and Samiyarsih, 2002). Study by Dorly (1997), showed that cuticle thickness of *Ipomoea trifida* had the highest correlation with the virulence of *Elsinoe batatas* bacteria, which also caused scab disease to *Ipomoea batatas*. The objectives of this research were to understand the effects of various nitrogen dosages on the anatomical characters of *sweet potato* leaf, and to find out the most influence dosage on the size and number of those anatomical characters.

Material and Method

This research had been conducted in Purwokerto and in the Structure and Development Plant Laboratory, Faculty of Biology, Jenderal Soedirman University from

March to June 2006. The method was experiments with design arranged in Completely Randomized Design. The nitrogen dosages applied consisted of 0, 50, 100, 150, and 200 kg urea per ha with three replicates. The growth media was mixture between soil and cattle manure (2:1). Each of sweet potato plant (25 cm in length) was grown in a plastic pot (20 x 20 cm). The nitrogen fertilizer was applied a week after planting. The plants were taken five weeks after planting in which the fifth leaf samples counted from the tip of the plant were taken. The microscopic observation was done three times for each leaf. The microscope slides were prepared with semi-permanent technique using glycerine medium. The parameters observed included the thickness of cuticle, leaf, and mesophyll, as well as the stoma density and their size. The stoma density was given in number per micrometer square.

Result and Discussion

The anatomical structure of sweet potato leaf can be distinguished into four parts. These were one layer of cuticle, one layer of each upper and lower epiderm, two layers of palisade parenchyma, and one layer of sponge parenchyma cells (Figure 1). The stoma type is paracityc celled which consisted of two kidney-shaped guard cells, and two subsidiary cells (Figure 2).

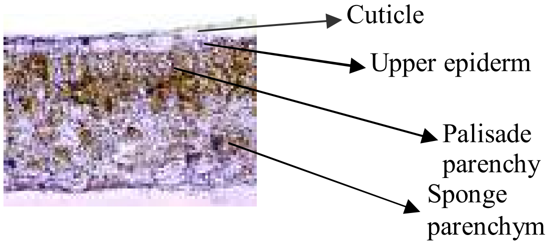

Figure 1. Cross section of sweet potato leaf (400X)

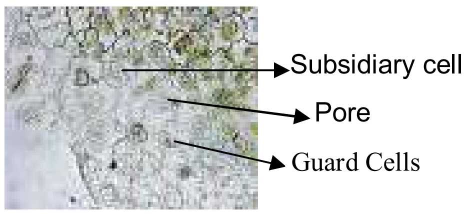

Figure 2. Stoma of sweet potato (100X)

The cuticle thickness of the upper and lower epiderm was influenced by the nitrogen dosages. The dosage effects were statistically very significant for both cuticle thickness ($P < 0.01$). The thickness of the leaf, and palisade, as well as the stoma size, and their density were also affected by level of nitrogen dosages. The analysis of variance for all data observed was presented in table 1.

Table 1. Summary of the analysis of variance for all data observed.

Source of Variance	Degree of freedom	F calculated						F table	
		Cuticle thickness	Cuticle thickness b	Leaf thickness	Palisade thickness	Stoma size	Stoma density	0.05	0.01
Treatment	4	31,89**	65,57**	17,98**	13,45**	43,17**	40,86**	3,48	5,99
Error	10								
Total	14								

** = highly significant

The results of Least Significant Difference test showed that nitrogen dosages affected all variables observed. The greater the nitrogen dosage was the thicker the cuticle, the leaf, and the palisade. As for stoma size, the higher the nitrogen dosage was the smaller it was. The stoma size and density were influenced by soil fertility and plant health (Dickison, 2000). Major element such as nitrogen that contributes to soil fertility has an effect on the leaf structure which in turn affects the stomata. The plant health is correlated to cuticle thickness, size and density of stomata which builds up plant structural defense. The greatest cuticle thickness, the smallest stoma size and the highest stomata density have been shown to form the best plant structural defence (Goodman *et al.*, 1986). Results of stoma density showed that the increasing nitrogen concentration up to 150 kg urea/ha was followed by large increase in stoma density (60.66-255.19%) (Table 2). Nitrogen dosage of 200 kg urea/ha was capable of decreasing the stoma density (14.54%), however, this was greater (203.66% increase) when compared to control (Figure 3). The decrease showed by nitrogen dosage of 200 kg might be due to the capacity of the plant in utilizing nitrogen. A dosage of 150 kg apparently showed to be the optimum dosage. Higher dosage than 150 kg might result in disturbance of stoma cell division that leads to lower stoma generation. Stoma arises through differential divisions in the protoderm. After several divisions of a given protodermal cell, one of these becomes a stoma precursor. The cell division might be influenced by the product of photosynthesis.

Table 2. Average data of all variables observed

Average Dosage (kg/ha)	**Upper Epiderm Cuticle Thickness (µm)**	**Lower Epiderm Cuticle Thick-ness (µm)**	**Leaf Thickness (µm)**	**Palisade Thickness (µm)**	**Stoma Size (µm)**	**Stoma Density (number/ mm^2)**
0	2.50 ±0.00	1.33 ±0.14	188.16±10.29	76.94±2.09	32.49±0.83	3.66±0.66
50	2.67± 0.15	2.14± 0.20	199.05± 3.80	87.33± 3.91	28.33± 0.83	5.88± 0.69
100	3.06± 0.00	2.50± 0.00	201.38± 6.47	88.61± 2.92	25.83± 0.83	10.44±1.66
150	3.08± 0.04	2.56± 0.05	205.49± 4.22	91.16± 2.18	24.72± 0.96	13.00±0.87
200	3.33± 0.17	2.74± 0.08	233.88± 7.91	92.50±3.00	22.77± 1.92	11.11±1.01

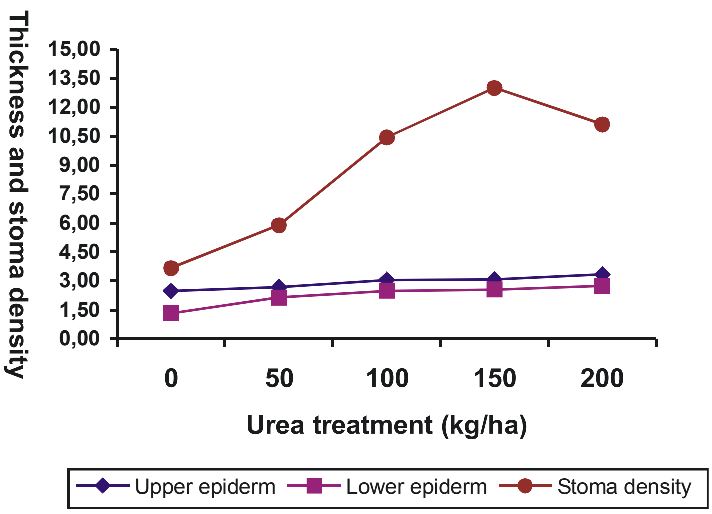

Figure 3. Changes in cuticle thickness of upper and lower epiderm, and stoma density of sweet potato leaf in relation of various dosage of nitrogen (upper and lower epiderm cuticle thickness were given in µm, stoma density was in number/ mm^2)

The cuticle, palisade, and leaf thickness were influenced by nitrogen dosage in which these were thicker as the dosage increased (Figure 4). Nitrogen is required by plants for synthesis of many macromolecules such as proteins and nucleic acids (Taiz and Zeiger, 1998). Plants absorbing nitrogen provided by fertilizer most probably synthesize more macromolecules that contribute to their development of cuticle, palisade, and mesophyll. Thus, increasing nitrogen dosage might provide greater availability of this element.

The nitrogen dosage treatments influenced all variables observed. Nitrogen was used by plant for building up chlorophylls in which these play a crucial role in photosynthesis process resulting in photosyntates. These, then, were used by plants for building up plant body (Taiz and Zeiger, 1998; Turmudhi, 2002). Nitrogen dosage applied led to increasing cuticle, leaf and palisade thickness as well as stoma density to increase, but decreasing stoma size (Table 2).

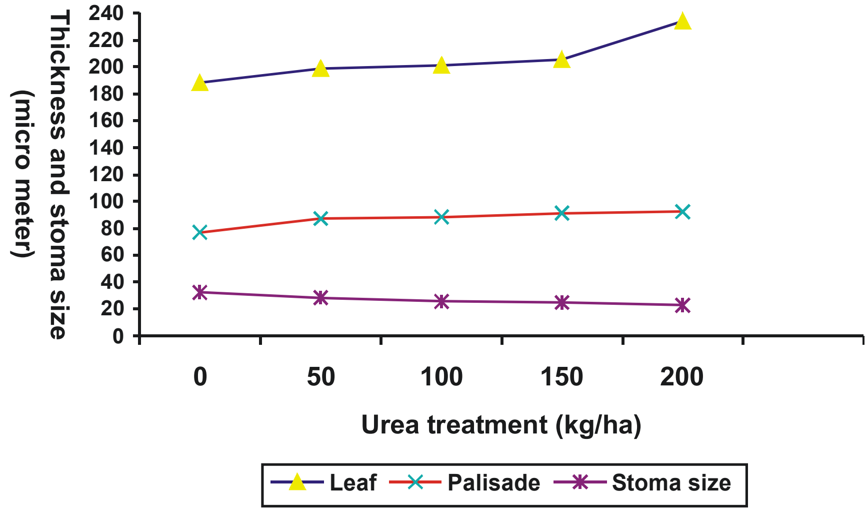

Figure 4. Changes in leaf thickness, palisade thickness and stoma size of sweet potato leaf based on the nitrogen dosages

Conclusion

It was concluded that the nitrogen dosage affected the anatomical leaf characters of sweet potato. Nitrogen concentration at 200 kg urea per ha was the most influential dosage on the thickness of upper and lower epiderm cuticles, mesophyll, palisade, stoma density and the stoma size. The first three characters showed significantly increasing thickness while stoma density decreased in 200 kg dosage. Stoma size decreased following increase of dosage application.

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