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Anatomical and Agronomic Responses of Arabica Coffee Seedlings (*Coffea arabica*) to Addition of Nitrogen

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ABSTRACT

The role of nitrogen in plant growth is well known, but there have been few studies on the response of nitrogen fertilizers to changes in plant anatomy and agronomy. This study aims to observe the response of the tissue and organs of arabica coffee seedlings to adding nitrogen. Nitrogen is used in the urea fertilizer form. This research used a single factor of CRD with five replications. 4 levels were tried without urea fertilizer as a control (N1), dosage of urea fertilizer 15 g urea.plant^{-1} is equivalent to 6,9 g of nitrogen (N2), dosage of urea fertilizer 30 urea.plant^{-1} is equivalent to 13,8 g of nitrogen (N3), and dosage of urea fertilizer urea.plant^{-1} is equivalent to 20,7 g of nitrogen (N4). After application, the plants are incubated for 30 days to allow time for the fertilizer to be absorbed by the plants. The analysis result stated that the chlorophyll content was very significantly different, while other agronomic variables were not significantly different. Less adaptation period was thought to be the cause of the various agronomic variables being not significantly different. This is reinforced by the response that arises in plant tissue. The addition of urea fertilizer responded to the thickness of the mesophyll tissue, the size of the epidermal cells, and the regularity of the lower epidermal cells. The result of this research concluded that the response of plants to the treatment had not yet reached the organ level but was still at the tissue level.

Keywords: Anatomical responses, Arabica coffee seedlings, Nitrogen

ABSTRAK

Peranan unsur nitrogen terhadap pertumbuhan tanaman telah banyak diketahui, namun kajian tentang respon pemberian pupuk nitrogen terhadap perubahan anatomi dan agronomi tanaman belum banyak dilakukan. Penelitian ini bertujuan untuk mengamati respon jaringan dan organ bibit kopi arabika terhadap penambahan unsur nitrogen. Unsur nitrogen yang digunakan dalam bentuk pupuk urea. Penelitian ini menggunakan RAL satu faktor dengan 5 kali ulangan. Terdapat 4 taraf yang dicobakan, yaitu kontrol/tanpa pupuk urea (N1), dosis pupuk urea 15 g/tanaman atau setara dengan 6,9 g N (N2), dosis pupuk urea 30 g/tanaman atau setara dengan 13,8 g N (N3), dan dosis pupuk urea 45 g/tanaman atau setara dengan 20,7 g N (N4). Pasca aplikasi, dilakukan inkubasi pada tanaman selama 30 hari untuk memberikan waktu agar pupuk terserap oleh tanaman. Hasil analisis menyebutkan kandungan klorofil berbeda sangat nyata, sedangkan variabel agronomi lainnya berbeda tidak nyata. Masa adaptasi yang kurang lama diduga menjadi penyebab berbagai variabel agronomis yang diamati berbeda tidak nyata. Hal ini diperkuat dengan respon yang timbul pada jaringan tanaman. Perlakuan penambahan urea memberikan respon terhadap ketebalan jaringan mesofil, ukuran sel epidermis dan keteraturan sel epidermis dalam. Hasil penelitian ini dapat disimpulkan bahwa respon tanaman terhadap perlakuan belum sampai pada tingkat organ, melainkan masih di tingkat jaringan.

Kata kunci: Respon anatomi, Bibit kopi arabika, Nitrogen

INTRODUCTION

Nitrogen is an essential macronutrient that plants need. The primary function of nitrogen in stimulating the vegetative period is numerous (Senoaji & Praptana, 2013), such as increases in cell division and enlargement, main constituent of chlorophyll (Pramitasari et al., 2016), induced growth (Pramanik & Bera, 2013) and elongation of new shoot, and helps water absorption (Munawar, 2011). The research of (Sudradjat et al., 2014) stated that applying of nitrogen fertilizer affected plant height, number of leaves, and stem diameter of palm oil seedlings. Nitrogen also plays a role in compiling organic compounds, amino acids, and proteins that plants need. Nitrogen can be lost through volatilization and leaching up to 20-55% of the fertilizer applied (Jimenez et al., 2017). Nitrogen is an element with high mobility, and it is needed in large quantities, so farmers often add nitrogen through fertilization.

Adequate nitrogen fertilizer in planting media greatly affects the seedling phase. Seedlings become one of the factors that determine the success of plant productivity, including arabica coffee cultivation. Generally, nitrogen deficiency in the arabica coffee seedlings causes the symptoms of stunted growth, such as leaf chlorosis, leaf rolling which started from the oldest leaves, and shoot death (Supriadi, 2017). Nitrogen deficiency also decreases chlorophyll content and productivity (Ferreira et al., 2016). The excess of nitrogen fertilizer in plants generally causes the leaves to become dark green, lengthening the vegetative period, delaying the generative period of the plant, and delaying the ripening process. Another negative effect of excess nitrogen is that the plant tissue becomes weak, making it susceptible to pest attacks (Senoaji & Praptana, 2013).

The addition of nitrogen fertilizer was attempted according to plant needs. The excess of nitrogen can cause plant stress. The plant's response to stress is not directly at the organ level but also affects the tissue. Mesophyll tissue is the initial tissue that has the potential to receive stress directly, considering the position of this tissue is right under the epidermal cell. This is the important tissue because it is where the photosynthetic process occurs. The perfectly differentiated mesophyll tissue is composed of palisade tissue (as supported tissue) and sponge tissue. (Damayanti et al., 2015) stated that the mesophyll tissue in older leaves shows the differentiation into palisade and sponge tissue with larger intercellular cavities. The presence of stress in the plant causes this tissue differentiation to be disrupted. (Ningsih et al., 2016) stated that leaf mesophyll tissue resulting in tissue culture treated with paclobutrazol in high concentrations caused the differentiation of mesophyll tissue to be more likely to resemble palisade tissue. This is also supported by the discovery of chlorophyll in large quantities. The research of (Alponsin et al., 2017) stated that the thickness of the mesophyll tissue in *V. korinchense* varies at various altitudes, which is thought to be due to the abiotic stress of nutrient deficiency as the soil height increases.

The research regarding nitrogen fertilizer for various crops has been widely studied. However, there has yet to be much discussion regarding the impact of nitrogen application on plant anatomy. This impact needs to be discussed if the treatment is given to annual crops such as coffee, cocoa, and others. This is because the productivity life of plants can reach tens of years, so the growth stage is also slower than the other kinds of crops. The impact caused by adding of nitrogen fertilizer cannot be seen immediately in plant morphological changes, but it is still at a tissue level. Studies on anatomical changes that occur at the tissue level due to the addition of fertilizer have not been widely revealed. Therefore, it is necessary to study the addition of nitrogen fertilizer and its response to the anatomy and agronomics of coffee seedlings so that the quality of coffee seedlings is obtained.

METHODOLOGY

This research was conducted in the greenhouse of the Faculty of Agriculture, Jember University, from August until November 2020. The materials used were 5th month old Komasti variety arabica coffee seedling, urea fertilizer, topsoil, 35 x 35 cm polybag size, chlorophyll meter SPAD 502, and ruler. The experiment was designed using a completely randomized design with five replications. The factor tested was the dosage of element N (urea fertilizer) applied to 5th month old Arabica coffee seedlings. The urea fertilizer dosages tested were four levels, namely control/without urea fertilizer (N1), dosage of urea fertilizer 15 g urea.plant^{-1} or equivalent to 6,9 g N (N2), dosage of urea fertilizer 30 g urea.plant^{-1} or equivalent to 13,8 g N (N3), and dosage of urea fertilizer 45 g urea.plant^{-1} or the equivalent of 20,7 g N (N4). Therefore, it needs 20 experimental units.

Arabica coffee seedlings were transplanted into bigger polybags to conduct the application of urea fertilizer. The planting media used is topsoil. The soil to be used is analyzed for the elements N, P, K, ammonium, and nitrate to determine the N content in the soil. Urea fertilizer is applied by dissolving the fertilizer in water and applying it around the plant roots. The plants are then adapted for 30 days for the fertilizer to be absorbed by the plants. Agronomic variables were observed, such as leaf chlorophyll content, the height of plants, number of growing shoots, number of leaves, root to shoot ratio, plant fresh weight, and dry weight. The anatomical variables were observed such as the thickness of the leaf mesophyll tissue, the length of the leaf palisade cells, and the size of the plant epidermis cells. The experimental data were indicated significantly different and were checked by analysis of variance (ANOVA) and then continued using the Duncan Multiple Range Test (DMRT) at a significance level of 95% to determine the differences between treatments.

RESULTS AND DISCUSSION

In general, arabica coffee seedlings are planted in a 10 x 20 cm polybag size. The coffee seedlings were transplanted into bigger polybags 35 x 35 cm with topsoil as planting media to facilitate the application of fertilizer. Considering the treatment that will be applied is nitrogen, it is necessary to know the initial nitrogen level in the media. The results of the pre-treatment analysis of the planting media are presented in table 1.

Table 1. Result analysis of planting media

No	Variable	Analysis Method	Unit	Analysis Result	Status*
1	N total	Kjeldahl	%	0,17	low
2	P_2O_5	SNI 2803:2010	%	0,347	Very low
3	K_2O	SNI 2803:2010	%	0,078	Very low
4	Ammonium	Cawan Conway	mg.g^{-1}	0,003	
5	Nitrate	Salicylic acid	mg.g^{-1}	0,001	

**based on the Appendix 3 criteria for assessing the soil analysis, Balittanah (2009)*

The result analysis of planting media shows that the media contains low nitrogen elements, both actual nitrogen (ammonium and nitrate) and potential nitrogen (N total). This planting media is highly suitable for this experiment with the assumption that the given treatment causes the changes that occur in plants. Various agronomic variables observed, such as plant height, number of leaves, number of growing shoots, fresh weight, dry weight, and root to shoot ratio, showed not significant different in the analysis of variance. The lack of an adaptation period after treatment is thought to be the cause of the agronomic variables not being significantly different. The result of other analyses of variance stated that the leaf chlorophyll content was highly significantly different due to the addition of nitrogen. This is also reinforced by several differences in plant tissue caused by urea treatment, such as the thickness of the mesophyll tissue, the size of the epidermal cells, and the regularity of the plant epidermal cells. This means that the plant's response to treatment has not yet reached the organ level but is still at the tissue level.

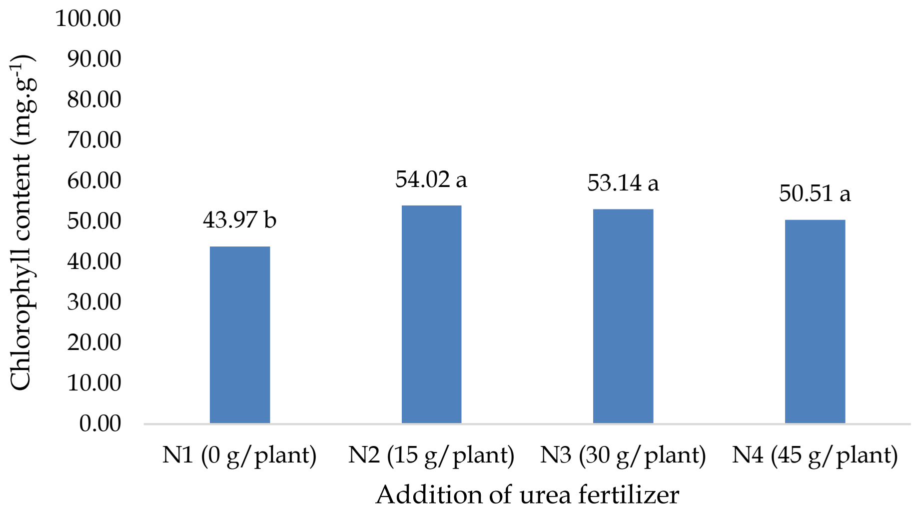


Figure 1. Chlorophyll content in arabica coffee leaves due to treatment with the addition of nitrogen fertilizer. (Note: the number following the same letter indicated that not significantly different in DMRT at a 95% significance level)

Chlorophyll content can be nondestructively measured by using SPAD (Efendi et al., 2012). Chlorophyll content was very significantly different based on the result of the Duncan test at a 95% significance level. Arabica coffee seedlings without the addition of urea fertilizer or control treatment (N1) produced 43,97 $mg.g^{-1}$ chlorophyll content, while other treatments were able to increase leaf chlorophyll content reached 54,02 $mg.g^{-1}$; 53,14 $mg.g^{-1}$; and 50,51 $mg.g^{-1}$ respectively. This data shows that the addition of nitrogen fertilizer has a significant effect on chlorophyll synthesis in leaves. The optimum addition of urea, which produces the highest chlorophyll content is N2 treatment (15 g $urea.plant^{-1}$). This dosage is more effective and efficient for increasing the chlorophyll content in the arabica coffee seedlings than another. The other dosage needs more urea fertilizer, but it is similar to the chlorophyll content produced.

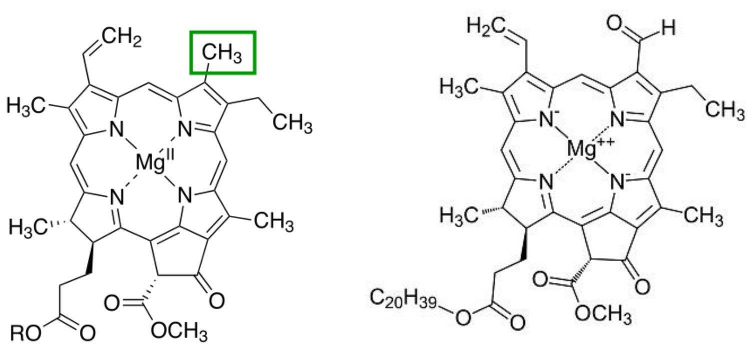


Figure 2. Structure of chlorophyll a (left) and chlorophyll b (right)

Nitrogen plays an important role in plant metabolism, such as the core compound that forms chlorophyll (Hernita, 2012). The structure of chlorophyll consist a core with 4 nitrogen elements which bounded to the magnesium (N_4Mg). The structure of chlorophyll consists of a core with four nitrogen elements bound to the magnesium (N_4Mg). The availability of nitrogen in the plant body determines the synthesis of chlorophyll in the leaves. More chlorophyll in the leaves can increase the rate of photosynthesis, so plant growth is faster ((Pramitasari et al., 2016); (Pramanik & Bera, 2013)). The large amount of chlorophyll contained in plant leaves causes the mesophyll tissue (where chlorophyll is synthesized) to become larger, it means that the layer of mesophyll tissue in the leaves will become thicker. The large amount of chlorophyll contained in plant leaves causes the mesophyll tissue (where chlorophyll is synthesized) to become larger, which means that the layer of mesophyll tissue in the leaves will become thicker. Figure 3 shows that adding urea fertilizer at a dosage of 15 g.plant^{-1} (N2) produces thicker mesophyll tissue than other treatments.

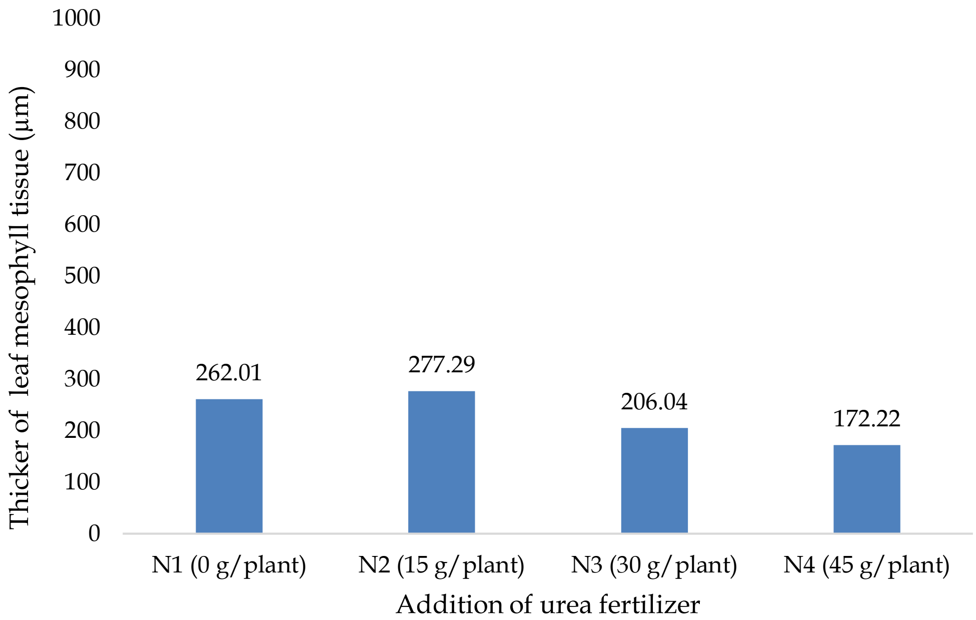
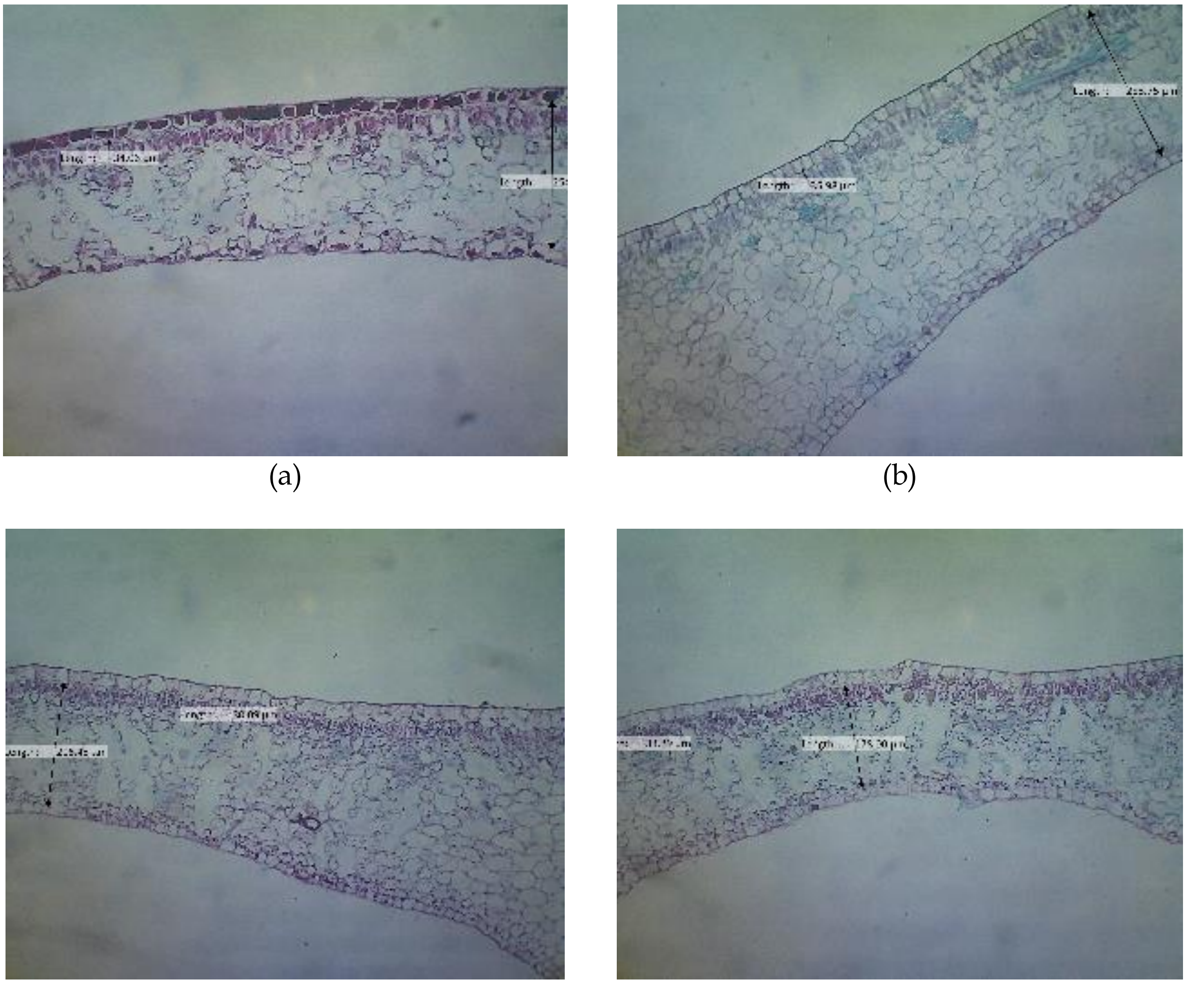


Figure 3. the thicker Arabica coffee leaf mesophyll tissue due to the treatment with the addition of nitrogen fertilizer

Mesophyll tissue is tissue in leaves that is located under the epidermal cells. The mesophyll tissue in the leaves differentiates into a layer of palisade parenchyma and sponge tissue (Damayanti et al., 2015). The primary function of mesophyll tissue is as a place of occurrence for photosynthesis. Therefore, chlorophyll is abundant in this tissue. Figure 3 shows that the highest thickness of the mesophyll tissue was produced when urea fertilizer was added at the dosage of 15 g.plant^{-1} (N2). This is in accordance with the highest chlorophyll content in Arabica coffee leaves, which was also produced by adding urea fertilizer in treatment dosage 15 g.plant^{-1} (N2). One of the functions of nitrogen elements is as a constituent of chlorophyll. Therefore, adding nitrogen fertilizer to arabica coffee seedlings can increase the leaves' chlorophyll content. Nitrogen fertilizer at dosages of 30 g.plant^{-1} and 45 g.plant^{-1} resulted in lower mesophyll tissue thickness and chlorophyll content compared to the 15 g.plant^{-1} treatment.



(a) (b) (c) (d)

Picture 4. The difference in the thickness of mesophyll and palisade tissue in arabica coffee leaves is due to the addition of urea fertilizers. (a) N1 (without fertilizer), (b) N2 (15 g.plant^{-1}), (c) N2 (30 g.plant^{-1}), and (d) N4 (45 g.plant^{-1}) at 100x microscope magnification.

Based on tissue histology results, coffee leaves have palisade cells, which are only found under the upper epidermal cell (adaxial side). Such mesophyll tissue is called the dorsiventral type (Dorly et al., 2016). Palisade cells are the cells in the mesophyll tissue that have a tight cell arrangement but still have a few cavities. This is different from sponge tissue, which has many cavities between cells. According to (Damayanti et al., 2015), the function of palisade cells is particular. It is where photosynthetic activities take place This illustrates the large amount of chlorophyll adsorbed to the edges of the palisade cell walls. Chlorophyll is used in photosynthesis to capture sunlight. The more chlorophyll is formed, the more palisade cells are formed so that the mesophyll tissue becomes thicker than the other.

Another factor that causes changes in the thickness of mesophyll tissue is the presence of abiotic stress, one of which is deficiency such as nitrogen and phosphorus. (Alponsin et al., 2017) stated that in such conditions, the mesophyll tissue (palisade and sponge cells) becomes thicker to defend itself. The thickening of mesophyll tissue is closely related to the CO_2 fixation process that occurs, so the impact is disruption of photosynthesis. Similar research was conducted by (Cambaba et al., 2018), which stated that mesophyll tissue thickens when salinity increases in planting media. In this research, the addition of urea fertilizer at the dosage 30 g.plant^{-1} (N3) and 45 g.plant^{-1} (N4) allegedly showed that it had not become a stress for the plant, so the mesophyll tissue did not become thicker (figure 5).

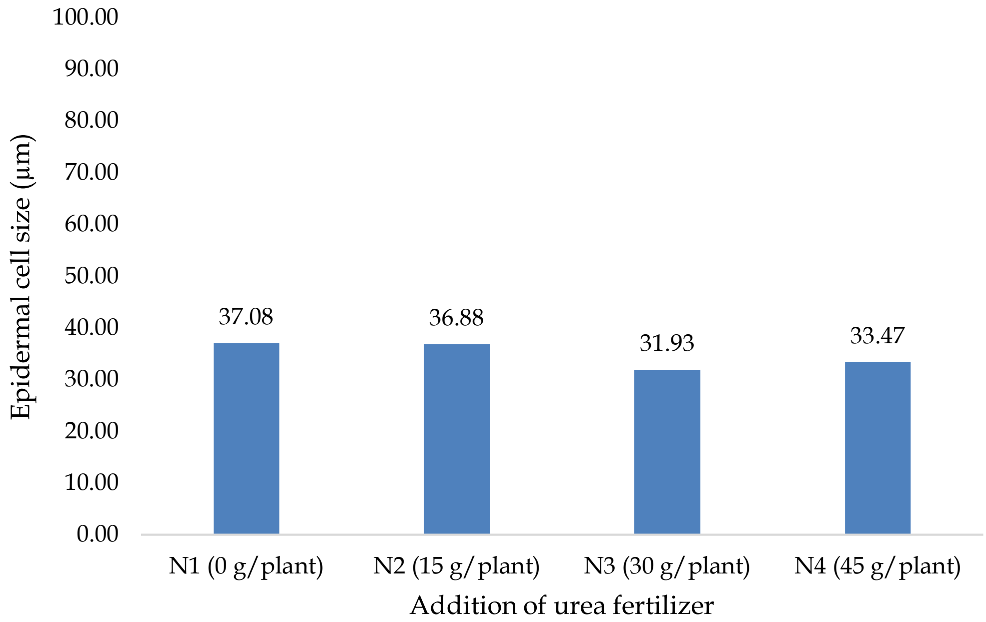
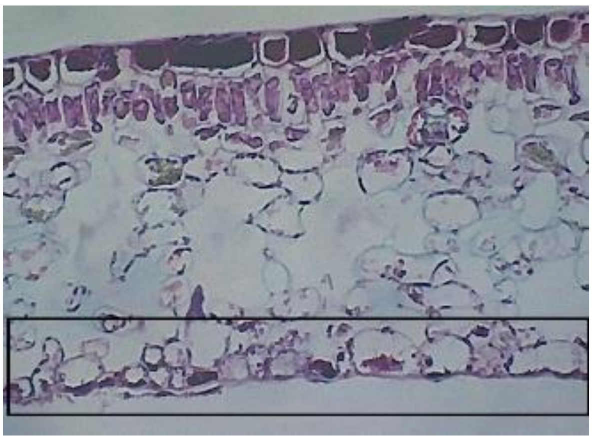
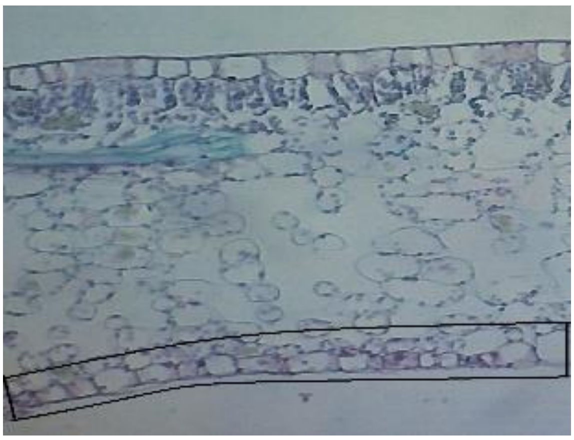


Figure 5. Epidermal cell size due to addition of nitrogen fertilizer

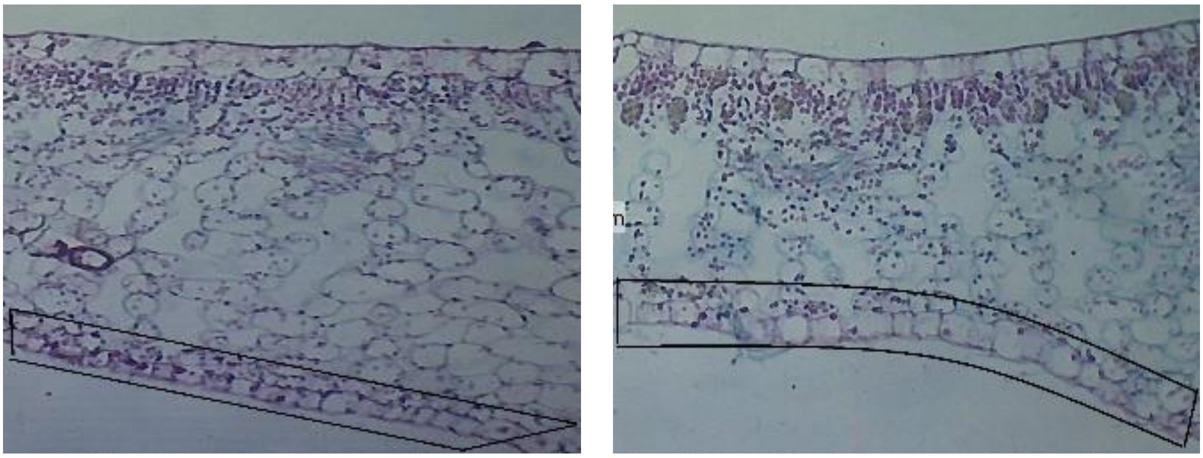
The addition of urea fertilizer also affects the size of plant epidermal cells. The result of the research showed that the gradual addition of nitrogen could increase the size of epidermal cells. According to (Fahmi et al., 2010) adding nitrogen to plants can accelerate the process of changing carbohydrates into proteins, which are used to construct plant cell wall. The addition of nitrogen also plays a role in increasing membrane stability (Xin et al., 2014) and increasing the lignin content of plants (Allison, 2012). Figure 5 shows that the more urea fertilizer added, the larger the size of the epidermal cells produced. The larger size of the epidermal cells provides a regular effect on the lower epidermal cells. The cross-section of leaf tissue in Figure 6 shows that the epidermal cells in the plant are more organized as the dosage of urea fertilizer is increased. An increase in the size of the epidermal cells causes the lower epidermal cells to appear more regularly compared to the control (without the addition of urea fertilizer).



(a)



(b)



(c) (d)

Figure 6. The regularity of lower epidermal cells (showed in the black box) due to the addition of urea fertilizer: (a) N1 (without fertilizer), (b) N2 (15 g.plant^{-1}), (c) N2 (30 g.plant^{-1}), and (d) N4 (45 g.plant^{-1}) at 100x microscope magnification

CONCLUSION

The addition of urea fertilizer gave a very significantly different response to the leaf chlorophyll content variable and was not significantly different from other observed agronomic variables. Adding urea fertilizer changed anatomical variables such as leaf mesophyll tissue, leaf epidermal cell size, and the regularity of Arabica coffee leaf epidermal cells. The treatment applied still responds to the tissue level.

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