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Growth Enhancement in Three Arabica Coffee Clones by Frequent Watering in Seedling Stage

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Abstract

Coffee cultivation in Indonesia generally only relies on rainfall as a source of water. Drought that occurs in the dry season inhibits growth, reduces yields, and even death of plants. Not all plants are equally resistant to drought conditions. Drought-resistant coffee clones usually develop several resistance mechanisms, including the formation of specific compounds to protect cells and tissues from damage caused by drought stress. Therefore, the objective of this study was to investigate the influence of watering interval on the seedling growth of three clones of Arabica coffee. This study applied intervals of watering every two, four and six days as first factor and three Arabica coffee clones as second factor, namely Andungsari 2K, S 795, and Andungsari 1. The experimental design used a factorial completely randomized design where every treatment combination had three replications. This study on Arabica coffee nursery lasted for three months. Results obtained in this study demonstrated that combination of watering every four and six days on Andungsari 2K clone had the best values on several observed variables. Number of stomata, leaf thickness, fresh root weight, and root dry weight were larger for watering every six days on the Andungsari 2K clone, meanwhile leaf number, stem diameter, fresh and dry weight of leaves were larger for watering every four days on Andungsari 2K clone. This study showed that there was an interaction between watering intervals and Arabica coffee clones on plant growth. Treatment combination of watering every six and four days on Andungsari 2K clone significantly affect number of stomata, fresh root weight, and root dry weight especially for watering every six days on Andungsari 2K clone.

Keywo rds: drought, stress, watering interval, *Coffea arabica*

INTRODUCTION

Arabica coffee is cultivated in the highlands of many parts of Indonesia as recommended at 1,000-1,700 m above sea level (asl.). Below 1000 m asl., the growth of this type of coffee is disturbed by leaf rust disease (Baon, 2016). Nonetheless, Arabica coffee like highland plantation crops totally depends on rainfall as a main source of water for coffee cultivation especially in facing drought problem which is closely related to high air temperature. Drought is a term that refers to plants experiencing water shortages due to limited water in planting media (Dewi *et al.*, 2019). Therefore, when it occurs in dry season, it may cause lack of water for plants, eventually it may affect all aspects of plant growth, including physiological, biochemical, anatomical and shape. Drought inhibits growth and reduces yields which greatly affects coffee production. Condition of plants under drought stress may result in decrease of shoot growth and increase of root growth (Santosa *et al.,* 2016).

The long drought that once occurred in Indonesia had caused losses for coffee plantations in East Java including decreased production and death of young plants in 1982. The decline in production in those government owned plantations due to long drought reached 34-68% with death rates ranged 5.4-7.5%. In 1991 several coffee plantations in East Java a long dry season also took place. The impact of the dry season in 1991 was quite serious as revealed from many of coffee plants' canopies dried up, especially those in the lowlands. Abdoellah (1997) stated that occurrence of long dry season has become more unpredictable.

According to Salisbury & Ross (1995), drought stress that occurs on plants can be interpreted as all changes in unfavorable environmental conditions that might reduce or destruct the growth or development of plants. Symptoms of damage of coffee plants due to drought stress in the field can be observed from the wilting and yellowing of leaves in the early stages and drying of leaves and branches (dieback) if drought stress continues. If the drought is very serious, the plants could die particularly coffee plants in seedling stage (Nur, 1992). According to Dominghetti *et al.* (2016), the groundwater field capacity below 74.6% can be dangerous for coffee seedlings, especially those derived from somatic embryogenesis and cuttings. Anticipation to reduce the impact of drought stress has been carried out by coffee growers. The best solution is by watering or irrigation (Abdoellah, 1997). Irrigation for coffee may be carried out in Indonesia but requires high costs, especially for land with less water. Another easy and efficient way to deal with the drought in Indonesia is by using tolerant coffee clones to drought stress.

King'oro *et al.* (2014) investigated the tolerance level of one type of Robusta coffee seedlings and nine types of Arabica coffee seedlings against drought. The results showed that there was a decrease in the number of leaves, number of internodes, plant height, percent of root biomass and crowns due to drought stress for all genotypes. The study also revealed that the tall type of Arabica coffee seedlings had better resistance to drought stress.

The results of other research showed that before the drought period the coffee plants produced the same biomass, but after the period of drought for 21 days there was a difference in biomass between the coffee clones tested (Chemura *et al.* 2014). Furthermore, it was stated that root biomass was an important factor in determining coffee varieties which are tolerant to drought stress. Deeper root systems of tolerant clones make it possible to get greater access to water from deeper soil and to maintain more favorable internal water status than drought-sensitive clones (Pinheiro *et al.,* 2005; Achar *et al.,* 2011)

Another investigation conducted by Tesfaye *et al.* (2015) reported that the anatomy of coffee leaf adapted to drought stress conditions. The variables of leaf extension rate and specific area size (approach to calculate leaf thickness) are important indicators in the selection of tolerant genotypes to drought stress. According to Farooq *et al.* (2009), smaller leaf area when plants are experiencing drought stress is a mechanism to reduce water loss to the environment from plant tissue. Melo *et al.* (2014) explains that modification of the upper epidermis and palisade tissue thickness are important variables for selection of Arabica coffee plants which are tolerant to drought stress. Batista *et al.* (2010) added that Arabica Bourbon Amarelo and Catimor have a good tolerance to drought stress due to their thicker cuticle and palisade tissue, larger bundle sheath and higher stomatal density. This causes the varieties to be more efficient in minimizing the rate of transpiration and increasing photosynthetic activity in drought stress conditions.

The agronomic/morphological approach is carried out to correlate the tolerance level of Robusta coffee to drought stress because of its easiness to apply (Anim-Kwampong & Adomako, 2010; Anim-Kwampong *et al*., 2011). Silva *et al*. (2013) argue that combining morphological and physiological characteristics is useful for evaluating the success of the performance of coffee clones in response to drought stress at the nursery stage.

Not all plants are equally resistant to drought conditions. Nur *et al*. (2000) found that BP 308 is resistant to drought condition and nematode attack which is related with its very high rooting density. Nur & Zaenudin (1992, 1995) also found that BP 409, BP 42, and BP 234 clones were more tolerant to drought stress compared to BP 358 and BP 288 clones. The comparison of each Arabica coffee clone is expected to determine the clones that are resistant to drought stress. In addition to being resistant to drought stress, there is also the potential for planting with minimal low water availability. Therefore, the objective of this study was to investigate the influence of watering interval on the seedling growth of three clones of Arabica coffee.

MATERIALS AND METHODS

The research location was in a greenhouse of Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia. This study used three clones, namely Andungsari 2K which has a sturdy crown and quite wide; secondly S 795 which is resistant to nematode; and thirdly Andungsari 1 which has dwarf character (Puslitkoka, 2001). Cutting seedlings of the three Arabica coffee clones were three months old taken from the nursery of ICCRI.

A factorial completely randomized design was used in this study which consisting of watering interval factor and coffee clone factor. Watering interval factor involved every two, four and six days. Meanwhile, coffee clone factor consisted of Andungsari 2K, S 795, and Andungsari 1. Therefore, there were nine treatment combinations with three replications, which resulted in 27 experimental units which consisted of five seedlings.

Growing media consisted of soil, sand, and manure with a ratio of 1:1:1, and mixed until evenly distributed. The planting medium of about 1.5 kg was put into each polybag of 15 cm x 25 cm. Before planting the cutting seedlings were removed from nursery soil, and followed by cleaning te roots from soil attached. In the centre of growing media the seedlings were planted. Coffee seedlings were watered according to the treatment, namely watering at intervals of two days, four days, and six days. Growing medium was watered until reach field capacity.

Observed Variables

Number of stomata has been counted by painting lower surface of leaf using nail polish, then the nail polish template that taken from the leaf surface been observed under light microscope. Leaf thickness was observed by using a caliper at the end of experiment (three months after planting). Plant height, stem diameter, leaf number, fresh and dry weight of shoot and root, leaf area were also recorded.

Data Analysis

Observations of this research were carried out from June to August 2021 until the seedlings were ready to be transplanted. The data obtained were analyzed in stages according to the objectives. The test used is the F test to determine the effect of the treatment given. Then proceed with the Tukey honestly significant difference (HSD) test at the level of = 5% to determine the difference among all treatments.

RESULTS AND DISCUSSION

Height of Arabica coffee seedlings was greater under watering every four days on S 795 clone compared to other treatment combinations of clone and watering interval during this study. Meanwhile, watering every two days on Andungsari 2K clone had the lowest value of plant height compared to other treatment combinations (Table 1). Based on the results obtained, this study showed that plant height was very significantly influenced by the combination of watering interval and Arabica coffee clone. Seedling plant height was higher on S 795 clone than other two clones. Meanwhile, watering interval every four and six days resulted in similar plant height but those two were higher than watering interval of two days.

According to Felania (2017), plant growth is related with meristems that produce new cells, enlarge and undergo differentiation. Cells that are actively dividing can be found in the apical meristem. One of the functions of the apical meristem is as a supporting tissue for plant height growth. Sufficient water can affect the apical meristem in plants because the apical meristem will absorb less water to carry out division. This causes plants with a sufficient amount of water tend to grow taller than plants with a water deficit (Pratiwi & Faizah, 2021).

Table 2 shows that treatment combination of watering every four days on the Andungsari 2K clone had the highest average value compared to other treatment combinations during this study. While the treatment combination of watering every two days on clone Andungsari 1 had the lowest value compared to other treatment combinations.

The stem diameter showed that the highest value was found in the P2K1 treatment combination, which was watering every four days for the Andungsari 2K clone compared to other treatment combinations (Table 2). Overall, the combination of stem diameter treatments showed a very significant different effect, this was because there was a slight water stress enough to cause slow or cessation of cell division and enlargement if the plant experienced very heavy water stress, differentiation of new organs and expansion of organs that had already been developed. some will be

Table 1. Effect of watering interval (2, 4, and 6 days) on plant height of Arabica coffee clones (Andungsari 2K, S 795, and Andungsari 1) during the first 12 weeks after planting

Watering interval (days)	Clone	Plant height (cm) Weeks after planting					
		2	4	6	8	10	12
2	Andungsari 2K	33.5 a	34.6 a	35.6 a	36.4 a	36.7 a	37.7 a
	S 795	42.5 e	43.7 d	44.3 d	45.1 d	45.4 d	46.3 c
	Andungsari 1	40.4 de	42.3 cd	43.2 d	43.7 d	43.8 cd	44.8 bc
4	Andungsari 2K	36.5 bc	39.0 bc	39.2 bc	39.9 bc	40.7 bc	41.5 ab
	S 795	47.9 f	50.0 e	50.8 e	51.7 e	52.5 e	53.4 d
	Andungsari 1	39.2 cd	41.1 cd	42.3 cd	42.8 cd	43.5 cd	44.5 bc
6	Andungsari 2K	34.5 ab	36.1 ab	36.9 ab	37.6 ab	38.6 ab	40.3 a
	S 795	46.4 f	47.9 e	48.7 e	50.1 e	51.1 e	51.9 d
	Andungsari 1	38.9 cd	40.0 c	42.0 cd	42.9 cd	44.1 cd	45.4 c
HSD		2.88	3.32	3.49	3.50	3.84	3.81

Note: Means within a column not followed by the same letter differed significantly at the 0.05 level.

Table 2. Effect of watering interval (2, 4, and 6 days) on stem diameter of Arabica coffee clones (Andungsari 2K, S 795, and Andungsari 1) during the first 12 weeks after planting

Watering inteval (days)	Clone	Stem diameter (mm) Weeks after planting					
		2	4	6	8	10	12
2	Andungsari 2K	5.1 cd	5.5 bc	5.9 cd	6.0 b	6.2 de	6.5 cd
	S 795	4.6 abc	5.1 ab	5.3 ab	5.4 a	5.5 ab	5.7 a
	Andungsari 1	4.3 a	4.7 a	5.1 a	5.3 a	5.1 a	5.7 a
4	Andungsari 2K	5.4 d	5.8 c	6.2 d	6.4 c	6.5 e	6.8 d
	S 795	4.8 bc	5.3 b	5.6 bc	5.7 ab	5.8 bcd	6.1 abc
	Andungsari 1	4.5 ab	5.0 ab	5.4 ab	5.6 ab	5.7 bc	5.9 ab
6	Andungsari 2K	4.8 bc	5.3 b	5.8 bcd	5.9 b	6.0 cd	6.3 bc
	S 795	4.8 bc	5.2 ab	5.5 abc	5.6 ab	5.7 bc	5.9 ab
	Andungsari 1	4.7 abc	5.0 ab	5.5 abc	5.6 ab	5.7 bc	6.0 ab
HSD		0.5	0.5	0.4	0.43	0.5	0.5

Notes: Means within a column not followed by the same letter differed significantly at the 0.05 level

affected first. Provision of maximum capacity water in accordance with field capacity provides good growth, this can be seen in stem diameter (Kurniawan, 2017).

Table 3 shows that treatment combination of four days watering interval on the Andungsari 2K clone had the highest number of leaves compared to other treatment combinations during this study, on the other hand watering every 2 and 6 days on S 795 clone had the lowest value compared to other treatment combinations. Meanwhile, at the end of this study watering every two days on Andungsari 1 clone had the lowest average value compared to other treatment combinations. This results revealed that sufficient level of water availability in coffee plants of Andungsari 2K when four days interval of watering. For plants, water functions as a solvent, namely to dissolve the nutrients available in the soil which are then used for the photosynthesis process. With sufficient availability of nutrients, photosynthesis takes place well and a large amount of photosynthate is produced, eventually among these photosynthates are then used for leaf formation (Nugraha *et al*., 2014).

Table 4 shows that the fresh leaf weight obtained from treatments combination four days watering intervals with clone Andungsari 2K had the highest value which was not significantly different from watering every two days on clone Andungsari 2K (Table 4). Meanwhile, watering every six days for Andungsari 1 clones had the lowest leaf fresh weight and significantly different from other treatment combinations. Based on these results of leaf fresh weight, it could be seen that there was a very significant interaction between clones and watering intervals that occurred on this variable. It seems that watering every four days provide soil water which is enough for the plant need. The water content in the leaves is influenced by the amount of water absorbed by the plant. The availability of water in plants affects plant growth where excess or lack of water may interfere metabolic processes in plant (Nurjanaty *et al.,* 2019).

Table 4 shows that the value of fresh root weight obtained from the combination of treatments at watering intervals of once every 6 days with clone Andungsari 2K had the highest average value nonetheless it was not significantly different from watering interval every 2 days on Andungsari 2K clone 4-day watering interval on clone Andungsari 1 and 6-day watering interval on clone S 795. Based on the results of this study, in general, the interaction between watering intervals and clones had very significant interactions. The interaction of

Table 3. Effect of watering interval (2, 4, and 6 days) on leaf number of Arabica coffee clones (Andungsari 2K, S 795, and Andungsari 1) during the first 12 weeks after planting

Watering inteval (days)	Clone	Leaf number					
		Weeks after planting					
		2	4	6	8	10	12
2	Andungsari 2K	14,3 c	16,1 e	18,1 e	18,5 e	19,1 e	17,1 c
	S 795	6,1 a	6,8 ab	7,0 ab	6,9 ab	7,7 ab	7,5 a
	Andungsari 1	6,5 a	7,9 b	8,3 b	7,9 ab	8,7 ab	6,0 a
4	Andungsari 2K	14,8 c	16,9 e	18,5 e	19,1 e	20,5 e	18,7 c
	S 795	7,7 ab	7,8 b	7,7 ab	7,3 ab	8,0 ab	7,0 a
	Andungsari 1	9,0 b	10,5 c	11,3 c	11,4 c	11,7 c	11,6 b
6	Andungsari 2K	13,3 c	14,2 d	14,1 d	15,9 d	17,3 d	17,7 c
	S 795	6,9 a	6,3 a	6,2 a	6,3 a	7,1 a	6,3 a
	Andungsari 1	7,3 ab	8,1 b	8,2 b	8,4 b	9,1 b	8,0 a
HSD		1,9	1,4	1,5	1,7	1,9	2,2

Notes: Means within a column not followed by the same letter differed significantly at the 0.05 level

Table 4. Effect of watering interval (2, 4, and 6 days) on leaf and root fresh weight, stomata density, leaf area and leaf thickness of Arabica coffee clones (Andungsari 2K, S 795, and Andungsari 1) 12 weeks after planting

Watering inteval (days)	Clone	Leaf fresh weight (g)	Root fresh weight (g)	Stomata density (mm^{-2})	Leaf area (cm^{2})	Leaf thickness (mm)
2	Andungsari 2K	15.1 d	8.22 de	351 c	27.3 ab	0.44 ab
	S 795	6.6 ab	5.31 ab	317 ac	30.4 abc	0.43 aa
	Andungsari 1	7.6 ab	4.81 a	282 ab	43.6 d	0.52 cd
4	Andungsari 2K	17.1 d	7.27 cd	329 bc	27.5 ab	0.40 a
	S 795	7.7 ab	6.51 bc	271 a	35.1 c	0.48 bc
	Andungsari 1	12.6 c	8.00 de	300 ab	32.1 bc	0.52 cd
6	Andungsari 2K	14.7 cd	8.70 e	358 c	25.1 a	0.43 ab
	S 795	6.1 a	8.48 de	311 abc	31.9 bc	0.50 c
	Andungsari 1	8.6 b	5.53 ab	278 a	31.8 bc	0.56 d
HSD		2.4	1.36	49	5.6	0.05

Notes: Means within a column not followed by the same letter differed significantly at the 0.05 level

this treatment combination is due to the rapid response of plants to drought is stomata closure, then in the long term there is a change in growth with increasing root growth. In addition, the response of plant roots in searching for water sources is characterized by elongation of the roots (Mastur, 2017).

Results presented in Table 4 show that the number of stomata obtained from treatment combinations at watering intervals of once every six days with Andungsari 2K clone has the highest value, however it is not significantly different from watering every two days on the same clone, or watering every two days on S 795 clone, watering every four days on Andungsari 2K clone), watering every six days on S 795 clone, but significantly different from the other treatment combinations. In this study, it can be seen that the number of stomata was significantly different to the treatment and watering interval, with the highest total number of stomata found in watering every six days on the Andungsari 2K clone (Table 4). The results of this study support the research result about the influence of wet-dry seasons and shade level on leaf stomata density of seedlings coffee accessions under nursery conditions (Kufa & Burkhardt, 2011). They found that stomata density was in the range of 200-370 mm^{-1}. Among others, water availability also can directly affect the movements of stomata (Wintgens, 2004; Wrigley, 1988). Stomata open slightly in response of the beginning of drought stress. Accordingly, water will be withdrawn from the epidermis into the mesophyll tissues, resulting in stomatal opening and water passes out of the guard cells as drought stress continues. Consequently, the stomata close progressively with increasing drought stress (Martin *et al*., 1993) and when the coffee leaves start to wilt result in the closure of stomata (Kanechi *et al*., 1995). Moreover, they also reported that under normal conditions, a temporary decrease in stomatal aperture could be encountered, as midday stomatal closure is common in plants growing under tropical conditions. In this case, it can be concluded that if plants are faced with drought stress conditions, there is a response made by plants, namely plants will regulate the degree of stomata opening to inhibit water loss through transpiration where in this process the stomata play a role in capturing CO_2 from the air which is needed for photosynthesis (Mansfield *et al*., 2003).

Table 4 also shows that the leaf area obtained from treatment combinations of watering intervals of once every two days with clone Andungsari 1 has significantly higher when compared to other treatments based on 5% Tukey HSD test. There is interaction between treatments and watering intervals was significantly different. Leaf area is one of the components of growth that changes due to treatment on plants experiencing drought stress (Mahdya *et al*., 2020). Therefore, to pass through drought stress, plant need water for growth by enlargement of cells that may increase leaf area (Ruminta *et al.,* 2017) . Therefore, based on the present findings it can be concluded that watering intervals of two days may overcome drought stress compared to watering intervals of four and every six days.

The results of Tukey HSD further test (Table 4), showed that the mean value of leaf thickness obtained from the combination of treatments at watering intervals of once every six days with Andungsari 1 clone had the highest which was not significantly different from interval watering every two and four days for clone Andungsari 2K, four days watering interval for S795 and Andungsari 1 clones.

In this research, there is a significant interaction between Arabica coffee clone and watering interval. It can be seen that in leaf thickness there is an interaction between clone and watering interval, which is very significantly different where the highest value of leaf thickness is found in watering every six days on Andungsari 1 clone (Table 4). Watering every six days has a good relationship on leaf thickness where the leaves get water for a long time compared to watering every two or four days. Leaf thickness is one of the important parameters in plant resistance to drought. At watering every six days the leaves will lose much water and get less water supply. The lack of water availability affect leaf thickness where water loss due to transpiration can be controlled by the cuticle resistance of the epidermis and the boundary layer. There is a role in the upper epidermis, palisade, lower epidermis, stomata density in maintaining moisture conditions in plants. In addition, one of the causes of increased water loss due to drought is the leakage of the network membrane. This study is in line with the work of Zakariyya (2018) which explains that the criteria for dry-resistant cocoa clones can be determined by the ability of plant cells to maintain turgidity through water osmotic stress so that plants do not experience excessive water deficit conditions that cause dehydration.

Drought-resistant coffee clones usually develop several resistance mechanisms, including the formation of specific compounds to protect cells and tissues from damage caused by drought stress. Judging from plant morphology, drought stress causes damage to root hairs because cells in root hairs are damaged which causes impaired absorption of water and nutrients. Plants respond to lack of water by reducing the conductivity of their stomata. This reaction is carried out by plants to reduce excessive water loss due to transpiration. When a plant lacks water, it becomes smaller than a normal plant. Changes in plant growth are caused by metabolic changes related to plant physiology (Widodo *et al.*, 2015). Nonetheless, it is necessary to conduct a more in-depth study of watering intervals and clones in order to obtain more optimal results against drought stress.

CONCLUSIONS

There was an interaction between watering intervals and Arabica coffee clones on growth. Treatment combination of six days watering interval on Andungsari 2K clone had the best value on Arabica coffee growth including number of stomata, fresh root weight, and oven-dried root weight. Meanwhile treatment combination of four days watering interval on Andungsari 2K clone gave the best of leaf number, stem diameter, fresh and dry leaf weight.

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