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Physio-Biochemical Characteristics of Prope Legitimate Seedlings of 13 Cocoa Clones Under Drought Stress

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ABSTRACT

The tolerant seedlings are determined by their physiological and biochemical responses. This study aimed to determine stomatal density, relative water content, and proline in prope legitimate seedlings under drought stress. The research was carried out at the Indonesian Coffee and Cocoa Research Center in July 2018 – February 2019, arranged in a randomized split-plot experimental design with six replications. The main plot was soil moisture content, and the sub-plot was the cocoa prope legitimate seedlings. The differences of mean values were tested using analysis of variance, followed by the DMRT and T-student test at 5 % level, analysis of the relative decrease in stomatal density and relative water content, proline content ratio, and the dendrogram analysis. The results showed that the cocoa seedlings under drought experienced significant changes in relative water and proline content but not stomatal density. Drought decreased in the relative water content of <50% and increased proline content in the seedlings. The relative water and proline content divided the prope legitimate seedlings into two groups. The prope legitimate seedlings from KW 516, KW 641, Scavina 06, KKM 22, KW 617, and ICCRI 03 clones were drought-tolerant.

Keywords: Cocoa; Drought; Physio-biochemical; Prope legitimate-seedlings

ABSTRAK

Sifat toleran bibit terhadap cekaman kekeringan salah satunya ditentukan berdasarkan tanggap fisiologi dan biokima. Penelitian bertujuan untuk mengidentifikasi densitas stomata, kandungan air relatif, dan kandungan prolin bibit propelegitim terhadap cekaman kekeringan. Penelitian dilaksanakan di Pusat Penelitian Kopi dan kakao Indonesia, Jember, Indonesia mulai bulan Juli 2018 sampai dengan bulan Februari 2019, menggunakan rangcangan percobaan petak terbagi teracak lengkap dengan 6 ulangan. Petak utama adalah kadar lengas air, anak petak adalah bibit propelegitem kakao. Perbedaan nilai tengah diuji menggunakan analisis ragam, dilanjutkan uji DMRT dan T-student pada taraf 5 %, analisis penurunan relatif terhadap karakter densitas stomata dan kandungan air relatif, rasio kandungan prolin, serta analisis dendrogram. Hasil penelitian menunjukkan bahwa bibit kakao pada kondisi kekurangan air signifikan berubah pada kandungan air relatif dan kandungan prolin, tetapi tidak signifikan pada densitas stomata. Kekeringan menyebabkan penurunan kandungan air relatif < 50 % dan meningkatkan rerata kandungan prolin pada seluruh populasi bibit propelegitim kakao. Karakter kandungan air relatif dan prolin membagi bibit propelegitim dalam 2 kelompok. Bibit propelegitim asal klon KW 516, KW 641, Scavina 06, KKM 22, KW 617, dan ICCRI 03 terindikasi bersifat toleran kekeringan.

Kata kunci: *Kakao; Kekeringan; Fisio-Biokimia; Bibit Propelegitim*

INTRODUCTION

The tolerant rootstocks under drought are very useful in breeding cocoa seedlings, as it is part of the seedling directly related to environmental factors, such as water deficit, nutrient deficit, and stress conditions (Sodre & Gomes, 2019). Tolerant rootstock allows for the development of tolerant cacao grafting seedlings to reduce the damage of drought on cocoa plants, including decreased yield, increased pest and disease attacks, and seedling mortality (Gateau-Rey et al., 2018; Santhyami et al., 2018; Yoroba et al., 2019). Cocoa rootstock can be derived from generative propagation (seeds), cuttings, and tissue culture. Rootstock can be produced using controlled pollination or prope

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legitimate seeds from open pollination of a female parent whose identity is identified and a male parent growing close together. The cocoa rootstock is generally obtained from prope legitimate seeds that are easily and quickly produced into seedlings (Zasari et al., 2020).

Evaluating the physiological and biochemical characteristics of prope legitimate seedlings under drought stress helps obtain tolerant prope legitimate seedlings. Physio-biochemical characteristics of water status are often used to determine species susceptible to drought stress. Characteristics such as stomata density, relative water content (RWC), and osmolyte compounds can be used to evaluate plant tolerance to drought stress. The physiological and biochemical characteristics of seedlings are associated with the drought tolerance mechanism (Zhang et al., 2018; Zakariyya et al., 2017; Setyawan et al., 2018).

Drought in plants is indicated by decreased stomatal density to maintain turgor stability or water potential in leaf tissue. Stomata plays a role in regulating plant water use. Stomatal density is considered a reasonably effective indicator to determine the tolerance of plants (Bertolino et al., 2019). The relative water content indicates the water status in the plant, which reflects the balance between the water supply to the leaves and the transpiration rate. Plant water status can be important for selecting species or cultivars tolerant to drought stress (Ahmad et al., 2018). Drought-stressed plants synthesize osmolyte compounds that enable plant cells to cope with dehydration by maintaining turgor, buffering against Reactive Oxygen Species, and maintaining redox homeostasis. Proline is an osmoprotectant compound that protects cell membranes and metabolic processes from damage due to stress (Zegaoui et al., 2017).

This study aimed to identify changes in the stomatal density, relative water content, and proline content of 13 cocoa clones of prope legitimate seedlings under drought as information that can make it easier to get tolerant rootstock. Changes in the characteristics of stomatal density, relative water content, and proline content were used as indicators of drought tolerance in cocoa seedlings (Zakariyya et al., 2017; Ahmad et al., 2018; Meher et al., 2018; Setyawan et al., 2018).

MATERIALS AND METHODS

Experimental site

This research was conducted at the greenhouse of Kaliwining experimental garden, Indonesian Center for Coffee and Cocoa Research, Jember, East Java, Indonesia, located 45 m above sea level. The study was conducted in July 2018 – February 2019. The average temperature during the study period was 25 – 30 °C, and the humidity was 59.6 – 89.3%.

Clone source

Plant materials were prepared from the propagation of prope legitimate seed clones, namely KW 516, KW 617, KW 641, ICCRI 03, TSH 858, Sca 06, MCC 02, KEE 02, KKM 22, ICS 60, Sul 01, Sul 02, and Sul 03. Clones are plants obtained from vegetative and asexual propagation with identical characteristics to their parents. The genetic material is superior clones, namely KW 516, KW 641, Sulawesi 01, Sulawesi 03, and KKM 22 showed tolerant growth performance, while clone ICS 60 was classified as drought sensitive (Setyawan & Susilo, 2017; Zakariyya et al., 2017).

Experimental design

A split-plot design with 2 factors and 6 replications was implemented to determine the effects of variable levels under drought. The main plot was the soil moisture content, consisting of 100% and 25%, while the subplot was 13 prope legitimate cocoa seedlings, including KW 516, KW 617, KW 641, ICCRI 03, TSH 858, Sca 06, MCC 02, KEE 02, KKM 22, ICS 60, Sul 01, Sul 02, and Sul 03. All tested plants were propagated from prope legitimate seedlings from 13 clones that were grown in polybags with a size of 15 cm x 25 cm containing 1300 g media consisting of topsoil, sand, and compost with a ratio of 2:1:1 (v/v), filled with 2 g of fertilizer and sprayed with pesticides until 2 months old. Determining soil water content is often conducted using the gravimetric method (Usowicz et al., 2017). The soil water content treatment was controlled manually by adding water to the soil media every 5 days until the seedlings were 5 months old (Setyawan et al., 2018).

Measurement of stomatal density, RWC, and proline content

The physiological and biochemical characteristics observed include stomatal density obtained based on the results of the imprint technique (Zakariyya et al., 2017). The stomata of the abaxial part of the leaf were observed under a microscope with 10 times magnification, and the density was counted. RWC was determined at the Agronomy Laboratory of Puslitkoka Jember, Indonesia (Zasari et al., 2020).

Proline content was measured using the method carried out by Bayat & Moghadam, (2019). 0.5 g fresh leaves were extracted with 5 ml sulfosalicyclic acid and then centrifuged at 3000 rpm. The supernatant was added with glacial acetic acid and ninhydrin, incubated at 100 °C for 1 hour and cooled. Next, add 4 ml of toluene and shake for 15-20 seconds. Absorption at 520 nm was read by a spectrophotometer.

Data analysis

Analysis of variance using R software version 3.44. Homogeneity was tested using the F test at a 5% confidence level. The diversity of prope legitimate genotype responses was analyzed using the t-student test. At the same time, the relative decrease (RD) in relative water content,stomatal density, and the proline content ratio (PR) were measured (Zasari et al., 2020):

RESULTS AND DISCUSSION

Stomatal density, RWC, and proline content of prope legitimate seedlings under drought

The experimental results showed that drought causes changes in the stomatal density, RWC, proline content in cacao prope legitimate seedlings, as shown in Table 1. Drought has a significant effect on RWC and proline content, but the stomatal density tend to be the same. Plants have several adaptation mechanisms to survive and grow in water deficits, including changes in physiological and biochemical responses (Kunikullaya G et al., 2018). Drought resistance in plant species including cocoa is generally through adjustments to physiological characteristics including reduced stomata opening, transpiration, etc. (Seutra Kaba et al., 2021) and biochemical characteristics including increased proline (Janani et al., 2019). The mechanism of resistance in cocoa plants is influenced by genotype and growing environment (Lahive et al., 2019).

Table 1. Physio-biochemical characteristics of prope legitimate seedlings under drought stress

Characters	Soil Water Content	
	100 %	25 %
Stomatal density (stomata/cm^2)	113.95 ± 10.52 a	113.51 ± 10. 45a
Relative water content (%)	67.62 ± 7.87 a	52.02 ± 7.69 b
Proline content (μmol/g)	4.07 ± 2.68 b	94.12 ± 59.37 a

Remark: Means followed by same letters in the same row are not significantly different based on DMRT at a 5% significance level

The performance in stomatal density, RWC, and proline content of prope legitimate seedlings under drought are presented in Table 2. In general,

Table 2. Physio-biochemical characteristics of prope legitimate genotypes seedlings at different levels of soil water content

Prope legitimate seedlings	Stomatal density (stomata/ cm2)			Relative water content (%)			Proline content (µmol/g)		
	100 %	25 %	T-test	100 %	25 %	T-test	100 %	25 %	T-test
KKM 22	104.32± 22.4	110.84±12.8	tn	67.25±20.3	53.13±18.1	**	5.74±1.2	99.86±3.1	**
ICCRI 03	127.78±18.6	119.76±16.7	tn	63.60±17.4	57.17±18.2	*	2.52±0.1	136.45±21.5	**
ICS 60	106.75± 3.6	105.85±12.2	tn	68.23±16.7	50.96±14.8	**	2.10±0.6	55.18±23.8	*
TSH 858	119.49± 8.1	121.48±9.1	tn	69.38±17.6	47.46±14.4	**	1.50±0.2	38.60±15.0	*
MCC 02	110.65± 21.9	117.61±14.0	tn	67.98±16.5	50.73±17.6	**	2.43±0.5	145.86±11.2	**
KW 641	113.5± 12.3	103.37±8.6	tn	66.78±16.2	54.48±19.9	**	4.08±0.8	129.04±4.12	**
Scavina 06	92.34±16.2	123.81±27.5	*	67.43±16.0	53.35±12.8	**	3.48±0.5	34.08±11.0	*
KW 516	121.85± 13.8	112.66±12.2	tn	65.50±17.2	54.19±20.3	**	5.88±0.4	35.86±3.2	**
Sulawesi 01	123.34±6.9	118.69±14.4	tn	67.19±17.1	49.71±17.0	**	3.17±0.2	18.64±0.9	**
KEE 02	109.17±13.6	108.68±19.3	tn	68.29±14.9	49.29±18.5	**	4.146±1.2	124.02±2.2	**
Sulawesi 03	132.63±13.6	114.80±8.5	*	72.04±22.4	50.94±17.2	**	11.69±2.1	46.55±13.2	*
KW 617	99.97±19.4	107.46±9.9	tn	68.65±15.0	55.86±14.6	**	4.37±0.3	217.07±12..1	**
Sulawesi 02	119.53±14.8	110.59±4.6	tn	66.77±18.7	49.03±18.6	**	1.76±0.1	142.45±6.2	**

Remarks: * significant at α 5%, ** significant at α 1%; tn non-significant

Table 3. Relative decrease in stomatal density and relative water content and increase in proline content of prope legitimate seedling under drought stress

Clones	Relative Water Content (%)		Stomatal Density (stomata/cm2)		Proline Content (µmol/g)	
	RD (%)	Criteria	RD (%)	Criteria	PR	Criteria
KW 516	17.32	Medium	7.54	low	6.1	Low
KW 617	18.63	Medium	0.00	low	48.67	very high
KW 641	18.41	Medium	8.92	low	30.58	Medium
ICCRI 03	10.12	Low	6.27	low	53.09	very high
TSH 858	31.60	High	0.00	low	8.79	Low
Scavina 06	20.89	Medium	0.00	low	24.7	Medium
KEE 02	27.82	Medium	0.45	low	28.91	Medium
KKM 22	21.00	Medium	0.00	low	16.39	Medium
MCC 02	25.37	Medium	0.00	low	59.04	very high
ICS 60	25.30	medium	0.84	low	25.23	Medium
Sulawesi 01	26.01	medium	3.77	low	4.87	Low
Sulawesi 02	23.71	medium	7.48	low	79.86	very high
Sulawesi 03	31.94	high	13.44	low	2.98	Low

Remarks: RD = relative decrease, PR = the proline ratio

drought stress decreased RWC t and increased proline content in all tested seedling genotypes. The decrease in stomatal density was shown by some seedling genotypes. The response to drought is influenced by the stability of turgor in leaves by various interrelated mechanisms, such as stomatal density, distribution, size, sensitivity, and proline content (Kardiman & Ræbild, 2018).

he relative decrease in stomatal density, RWC, and increase in proline content of prope legitimate seedling populations are presented in Table 3. Drought stress significantly reduced RWC and increased proline accumulation, but had no effect on the relative stomatal density. Previous studies stated that the response of stomatal density, RWC, and proline content to drought was influenced by genotype (Medina & Laliberte, 2017; Zakariyya et al., 2017; Dzandu et al., 2021).

The stomatal density on seedling leaves did not change due to drought. Stomata are involved in regulating water circulation or controlling water balance and gas exchange in leaf tissue . Stomatal density is thought to have no direct effect on the ability of seedlings to maintain water status in the tissue, but the distribution, size and sensitivity of stomatal are mechanism that affect the rate of water loss through the leaf tissue (Hasanuzzaman et al., 2019; Bertolino et al., 2019).

The stomatal density is limited by stomata size, but interactions between stomatal density and size can also cause differences in gas exchange rates. Plants with small amounts of large stomata tend to have higher water use efficiency than those with large numbers of small stomata (Kardiman & Ræbild, 2018). Loss of water in plants due to drought indicates a decrease in leaf water potential. The RWC level decreases due to increased transpiration rate and decreased water absorption by roots. RWC is an indicator of water status in plant tissues (Bayat & Moghadam, 2019).

Proline accumulation significantly increased in the seedling population, except for seedlings from KW 641 and ICS 60. Lahive et al. (2019) state that genotype is one of the determining factors for the physiological and biochemical response of cocoa seedlings. The Plants exposed to drought have mechanisms to restore redox homeostasis causing the accumulation of non-enzymatic antioxidants, including proline. Accumulating antioxidants is useful for maintaining cellular water homeostasis and preventing damage due to the presence of reactive oxygen species (ROS) (Moreno-Galván et al., 2020). Drought affects the composition of the solute concentration (osmolyte), like proline in cells to maintain water potential stability, including proline (Zegaoui et al., 2017). Proline was found to accumulate in the leaf tissue of cocoa seedlings under drought stress (Zakariyya et al., 2017).

The relative decrease in stomatal density, RWC and the proline content ratio of prope legitimate seedling under drought

The physio-biochemical responses of prope legitemate seedlings to drought can be seen from the results of a decrease and relative increase in the characteristics. The relative decrease in stomatal density and RWC, as well as the increase in the proline of prope legitimate seedlings are presented in Table 3. The relative decrease in stomatal density and RWC in most of the seedling populations was low, namely <50%. The prope legitimate seedlings of KW 617, TSh 858, Scavina 06, KKM 22, and MCC 02 did not show a decrease in the stomatal density. The response of plants to water deficit conditions is characterized by a decrease in stomatal density (Lahive et al., 2021), but the rate of water loss depends on the type of stomata (Bertolino et al., 2019).

The highest relative decrease in the RWC was in Sulawesi 03 seedlings, which was 31.94%, and the lowest was in ICCRI 03 seedlings, which was 10.12%. Under drought stress, prope legitimate seedlings showed a RWC of <60%. Wilted plants generally lose water content reaching > 50%. The RWC level indicates a measure of the water deficit in the leaves, which is influenced by decreased water absorption and increased water loss (Zegaoui et al., 2017; Dzandu et al., 2021).

Drought caused an increase in proline content of the leaves of prope legitimate seedlings, ranging between 4.87 – 79.86 times the initial weight. Seedlings of the Sulawesi clone 02 showed the highest increase in proline content (79.86), while Sulawesi 03 clone showed the lowest increase in

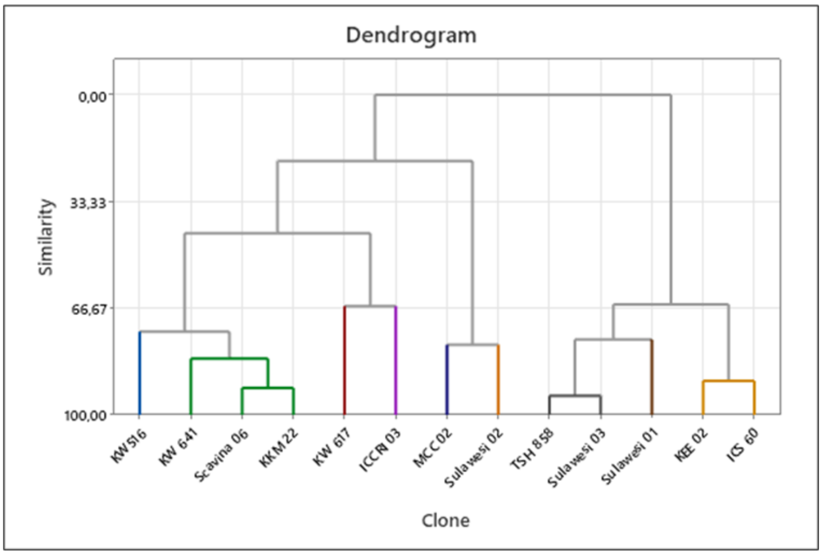

Figure 1. Dendrogram of prope legitimate seedlings based on relative decrease in relative water content and increase in proline content

proline content (2.98). Osmo-protectants such as proline are mostly found in the cytoplasm and not in the vacuole. Proline accumulation is a strategic mechanism for plants when exposed to drought stress to reduce injury to cell (Janani et al., 2019). Cocoa plants accumulate proline due to water deficit (Zakariyya et al., 2017). Increased proline production in leaf cells is one of the plant defense mechanisms against water deficit conditions (Bandurska et al., 2017; Niether et al., 2020).

Grouping of prope legitimate seedling based on physio-biochemical characters under drought

The grouping of seed genotypes based on RWC and proline under drought is presented in Figure 1. The results of the dendrogram analysis found that prope legitimate seedlings were divided into 2 main groups. Cluster I consist of 3 sub-groups, and cluster II consist of 2 sub-groups. Group I include seedlings of KW 516, KW 641, Scavina 06, KKM 22. KW 617, ICCRI 03, MCC 02, and Sulawesi 02 clones, which tend to have a moderate reduction in relative water content and a very high accumulation of proline. Group II include seedlings of TSH 858, Sulawesi 03, Sulawesi 01, KEE 02, and ICS 60 clones, which have moderate RWC reduction but low proline accumulation.

CONCLUSIONS

Population of cocoa prope legitimate seedlings under water deficit conditions showed significant changes in RWC and proline content, but not significant in stomatal density. Drought decreased the RWC of <50% but increased the proline content of cocoa prope legitimate seedlings. Prope legitimate seedlings were divided into 2 groups based on the character of RWC and proline content. The prope legitimate seedlings of KW 516, KW 641, Scavina 06, KKM 22, KW 617, and ICCRI 03 clones were indicated drought-tolerant

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