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# Prope legitimate rootstocks determine the selection criteria for drought-tolerant cocoa

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**Abstract.** *Zasari M, Wachar A, Susilo AW, Sudarsono. 2020. Prope legitimate rootstocks determine the selection criteria for drought-tolerant cocoa. Biodiversitas 21: 4067-4075.* Drought tolerant cocoa seedlings are needed for the success of cocoa cultivation under drought conditions. Prope legitimate rootstock is feasible to be used in the selection of drought-tolerant cocoa seedlings. This objective of this study was to determine the selection criteria and tolerance of prope legitimate cocoa rootstocks in response to drought stress. This study was conducted in the greenhouse of Kaliwining experimental garden, Indonesian Center for Coffee and Cocoa Research, Jember, Indonesia. Plant materials were arranged in a split-plot design with 2 factors and 6 replications. The main plot was the soil moisture content, i.e 100%, and 25%, while the subplots were 13 prope legitimate cocoa rootstocks. The result showed that root fresh weight was the best characters for the selection of drought tolerance in prope legitimate cocoa rootstock. Based on the value of stress susceptible index and clustering analysis divided the tested prope legitimate rootstock into two groups. The group for tolerant genotype was consisted of ICCRI 03, ICS 60, TSH 858, KKM 22, KW 641, KW 516, and MCC 02, while the sensitive genotype group was consisted of Sul 01, Sul 03, Sca 06, KEE 02, KW 617, and Sul 02.

**Keywords:** Cocoa, prope legitimate rootstock, selection criteria, drought tolerance

## INTRODUCTION

Climate instability is a serious challenge to the productivity and stability of agricultural crops (Waseem et al. 2011; Kyei-Mensah et al. 2019). Extreme rainfall fluctuations allow the plant to experience a water deficit (Mishraa and Cherkauer 2010; Bari et al. 2016; Medina and Laliberte 2017) which impacts on its final production (Zlatev and Lidon 2012). Cocoa is a very sensitive plant to drought stress (Carr and Lockwood 2011; Amos and Thompson 2015; De Almeida 2016; Gateau-Rey et al. 2018; Santhyami et al. 2018; Yoroba et al. 2019). Drought stress causes seedling mortality, fruit and seed size reduction, and the increase of pest and disease attacks on cocoa (Atayese et al. 2013; Longe and Oyekale 2013; Santos et al. 2014; Ofori et al. 2015; Gateau-Rey et al. 2018).

Drought condition is a threat to cocoa cultivation. Drought tolerant cacao seedling is needed to overcome the threat of drought (Cazares, et al. 2010) as well as the expansion of cocoa plantations to the marginal area with drought conditions as the limiting factor (Setyawan and Susilo 2017; Setyawan et al. 2018). Exploration has been carried out through a number of studies related to the characterization and selection to obtain drought-tolerant cacao seedling (Atayese et al. 2013; Dos Santos et al. 2016; De Almeida et al. 2016; Zakariyya et al. 2017; Setyawan et al. 2018; Zakaryya and Indradewa 2018a, 2018b).

Drought tolerant cacao seedling can be produced through generative and vegetative propagation methods. As one of popular vegetative methods, grafting has been widely used by cocoa farmers in Malaysia, Philippines, or even Indonesia (Sodré and Gomes 2019). In addition to its easiness to practice, the grafting technique produced superior seedling because it can accommodate the combination of two good characteristics from two different parents, i.e rootstock and scion (Pranowo and Wardiana 2016). Rootstock is the lower part of seedling, while scion was the upper part that combined through grafting techniques. Rootstock was combined with the scion in the upper part. The rootstock should be prepared from any varieties with a good adaptation to the lack of water, nutrition, and other environmental stress (Lopez-Ortega et al. 2016; Warschefsky et al. 2016). In general, the propagation technique of cacao rootstock uses a controlled crossbreed seed. However, the use of open pollinated seeds is seemed to be more feasible for rootstock production (Susilo 2015; Zakariyya et al. 2017).

Prope legitimate seed is a term for any seed that produced by mother plant who receive random pollen from one or several clones that grow in the same block. This seed can be used as the source of genotype diversity that is required during the selection of drought-tolerant rootstock. The study of tolerance capability of prope legitimate rootstock should be conducted in the dry field instead of in vitro condition (Atayese et al. 2012; Dos Santos et al. 2016; De Almeida et al. 2016; Setyawan et al. 2018). The success to obtain prope legitimate rootstock is highly dependent on the accuracy of selection criteria, however, there is still limited studies regarding this issue. Therefore, this study aimed to determine the selection criteria and tolerance of prope legitimate cocoa seedling under drought stress conditions.

## MATERIALS AND METHODS

### Experimental site, time and plant materials

This study was conducted at the greenhouse of Kaliwining experimental garden, Indonesian Center for Coffee and Cocoa Research, Jember, East Java, Indonesia. The study was conducted from July to December 2018. The average temperature and relative humidity of the greenhouse during the study period were 25-30ºC and 59.6-89.3%, respectively. 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. All tested plants were grown in polybag with a size of 15 cm x 25 cm containing 1300 g soil media until 2 months old.

### Experimental method

The study used a split-plot design with 2 factors and 6 replications. The first factor as the main plot was the soil moisture content, i.e. 100% and 25%, while the second factor as the subplot was 13 prope legitimate cocoa rootstocks, i.e 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. The moisture content was controlled manually by adding a certain amount of water to the planting medium every 5 days until the seedlings were 5 months old (Setyawan et al. 2018). Determination of moisture content following the Gravimetric method (Dobriyal et al. 2012). Several variables that observed at 3 months after treatment were the percent of survived seedlings; the plant height and the root length that was measured using the bar; the stem diameter that was measured using calipers; the number of leaves that were calculated manually; the weight of plant canopy and root (both in term of dry and wet basis) that was weighed by using a digital balance; the area of leaf and root were measured by using ImageJ software, and the root volume was measured following the volumetric method in beaker glass.

Other observed variables were stomatal density and relative water content. Stomatal density was determined through the imprint technique. The sample was selected leaf on the 3rd position from the apical bud. The abaxial part of the leaf was smeared with transparent nail polish as wide as ± 0.5 cm and the allow it to dry. The dry nail polish is assumed to be attached to the epidermis and stomata cells. Next, the dry nail polish was gently removed using sticky tape and then placed on the object-glass for further observation under the microscope. Stomata were observed with a lens magnification for about 10 times and then calculated using software called Image Raster. The relative water content was calculated from the following equation (Pizard et al. 2011) as follow:

$$RWC (\%) = [(Wf - Wd) / (Ws - Wd)] \times 100$$

Where: RWC is relative water content (%), Wf is the leaves fresh weight, Wd is the leaves dry weight, Ws is the leaves turgid weight.

Proline content also measured in present experiment. Proline was measured by using the method of Abraham et al. (2010). The sample was prepared in form of 0.5 g leaves. The leaf was crushed with mortar, then add by 10 ml 3% sulfosalicylic acid and filtered with filter paper. The mixture of filtrate with 2 ml ninhydrin acid and 2 ml glacial acetic acid in a test tube was heated at 96ºC for 60 minutes and then terminated by cooling the solution inside the ice water for about 5 minutes. The product was extracted with 4 ml toluene (99.5%) and then shaken with vortex for 15-20 seconds to form two layers (chromoform). The red top layer containing proline was taken with a pipette and then the absorbance was measured using a spectrophotometer at a wavelength of 520 nm. The content of proline was determined by reading the standard of pure proline.

### Data analysis

Data were processed through analysis of variance by using R software version 3.44. The homogeneity test was performed by using the F-test at a 5% confidence level. Determination of selection criteria referred to variables that showed significant effect on the variety of interactions between genotypes and the environment. Data from the selected variables were processed by relative decrease analysis to distinguish genotype responses. Analysis of the component was carried out to assess the estimation value of inheritance and variability (Syukur et al. 2011). Genotype tolerance was analyzed using stress sensitivity index that was calculated by the formula: $SSI = (1 - Ys / Yp) /(1 - \bar{Y}s / \bar{Y}p)$ (Fisher and Maurer 1978), Ys = average specific genotype of drought stress conditions, Yp = average of an optimum condition genotype, $\bar{Y}s$ = average of all drought stress genotypes, and $\bar{Y}p$ = average of all optimum condition genotypes. The grouping of rootstock genotypes was performed by using a heatmap clustergram analysis.

## RESULTS AND DISCUSSION

### Agronomical characters as the main selection criteria

Selection was an important step in efforts to improve plant genetic quality. Selection based on the mean value of population was the most suitable method for family-level selection in the annual crops (Mayo 1980; Padi et al. 2013). Prope legitimate rootstock was obtained from the seed multiplication with the same female parent. Tolerant characters of cocoa rootstock could be predicted through identification of specific characters as selection characters (Santos et al. 2014; Ofori et al. 2015; Fang and Xiong 2015). Selection character was determined by using certain approach that refers to the influence of various interactions between genotype and environment and relative decrease. Characters with significant interaction variance showed the different intergenotype responses to stress (Mohammadi and Abdulahi 2017; Anshori et al. 2018a; Anshori et al.

2019). The analysis of variance in Table 1 showed various interaction variance in all observed characters. The characters of stem fresh weight, stem dry weight, root fresh weight, dry weight, and canopy fresh weight showed a significant interaction variance. Thus, it was the indicator that five mentioned characters were likely to be desired selection criteria.

The tolerance level of plants to drought stress could be estimated based on the magnitude of the relative decrease in various plant growth characters. The calculation of relative decrease was performed only for significant characters. The results of relative decrease analysis (Table 2) showed that there was a decrease in the average value of stem fresh weight, stem dry weight, root fresh weight, root dry weight, and canopy fresh weight in all observed genotypes in response to drought stress. Relative decrease indicated the response level of plant genotype to stress (De Leon et al. 2015; Anshori et al. 2018b, 2019).

Determination of the right selection criteria could improve the efficiency and the accuracy of the selection process (Santos et al. 2014; Ofori et al. 2015; Fang and Xiong 2015; Dos Santos 2016). The right selection criteria were indicated by the possibility to be inherited to the offspring. The proper selection criteria should have a heritability value. The estimation of heritability aimed to evaluate the suitability between genetic potential and phenotypic performance of selected plants. In general, there were two types of heritability values, namely broad sense heritability ($h^2_{bs}$ ) and narrow-sense heritability ($h^2_{ns}$) (Mayo 1980; Islami et al. 2015; Baloch et al. 2016). The broad-sense heritability represented the magnitude of the role of genetic to the phenotypic aspect, so it was often used to determine the selection character (Rubiyo and Sudarsono 2011; Islam et al. 2015; Dos Santos et al. 2016).

The result of the variance component (Table 3) showed that there was the root fresh weight character with the highest estimation on the broad inheritance value ($h^2_{bs}$). The broad inheritance value on root fresh weight character in present study was categorized as high level (Syukur et al. 2011), so that the character of root fresh weight was effectively used in determining selection criteria. The higher broad sense inheritance value might lead to the higher effect of genetics resulting in the phenotypic performances; in this case, the root fresh weight (Mayo 1980; Rubiyo and Sudarsono 2011; Dos Santos et al. 2016). The phenotypic expression of this root character was influenced either by genetic and environmental factors (Padi et al. 2013; Geonaga et al. 2015). Relatively controlled environmental conditions on both climatic and soil factors allowed the genetic factor to influence the maximum expression of root fresh weight.

Genetic variability described the genetic background of the tested genotypes, while the variability of phenotype is oriented towards the physical appearance of the genotypes tested. The level of genetic variability was determined by the genetic variance ($\sigma^2_g$) and the deviation standard of genetic variance ($\sigma_{\sigma_g}$ ), as well as for the variability of phenotype. Both of the genetic variance and phenotype variance of stem fresh weight, root fresh weight, and root dry weight were smaller than twice of standard deviation of genetic and phenotype variances, respectively (Table 3). Thus, the variability of root fresh weight characters was relatively narrow. As predicted, the narrow genetic diversity was directly followed by the narrow phenotypic diversity, because the phenotypic diversity was affected not only by environment but also genetic (Syukur et al. 2010).

In general, the selection process paid attention to characters with a broad genetic variability, however, the determination of selection character should be adjusted to the desired objectives (Susilo 2015; Devy et al. 2018). The improvement of rooting properties would affect the tolerance of the cocoa rootstock. Thus, root fresh weight was appropriate characters chosen as the selection criteria for cocoa prope legitimate rootstock against drought stress. The root weight character was an observed variable that indicated the root growth. One of several approaches to assess plant adaptation was the evaluation of the plant ability to maximize the rooting system under lack of water condition (Atayase et al. 2013; Placide et al. 2014; Setyawan et al. 2018).

**Table 1.** Analysis of variance of agronomical characters of prope legitimate cocoa rootstocks under drought stress condition

| Source | PSS (%) | SD (cm) | PH (cm) | NL (leaf) | LA (cm) | FWL (g) | DWL (g) | FWS (g) |
|---|---|---|---|---|---|---|---|---|
| Water content | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ |
| Genotype | $0.21^{tn}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.39^{tn}$ | $0.59^{tn}$ | $0.00^{**}$ |
| Water content.Genotype | $0.29^{tn}$ | $0.23^{tn}$ | $0.97^{tn}$ | $0.46^{tn}$ | $0.07^{tn}$ | $0.13^{tn}$ | $0.35^{tn}$ | $0.00^{**}$ |
| CV (%) | 9.08 | 5.57 | 9.75 | 8.54 | 8.89 | 18.85 | 19.39 | 13.95 |
| | **DWS (g)** | **FWR (g)** | **DWR (g)** | **RA (cm)** | **RL (cm)** | **RV (cm)** | **FWC (g)** | **DWC (g)** |
| Water content | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.03^{*}$ | $0.50^{tn}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ |
| Genotype | $0.05^{tn}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ |
| Water content.Genotype | $0.01^{*}$ | $0.03^{*}$ | $0.02^{*}$ | $0.05^{tn}$ | $0.52^{tn}$ | $0.12^{tn}$ | $0.00^{**}$ | $0.12^{tn}$ |
| CV (%) | 19.09 | 15.82 | 23.35 | 16.61 | 8.35 | 23.57 | 13.03 | 16.01 |

Note: *significantly different at α 5%, ** significantly different at α 1%, $^{tn}$ not significantly different, EN: experimental number, CV: coefficient of variance, PSS: the percentage of survived seedling, SD: stem diameter, PH: plant height, NL: the number of leaves, LA: the leaf area, FWL: the fresh weight of leaf, DWL: the dry weight of leaf, FWS: the fresh weight of stem, DWS: the dry weight of stem, FWR: the fresh weight of root, DWR: the dry weight of root, RA: the root area, RL: the root length, RV: the root volume, FWC: the fresh weight of plant canopy, DWC: the dry weight of plant canopy

**Table 2.** The relative decrease of stem fresh weight stem dry weight, root fresh weight root dry weight, and canopy fresh weight of prope legitimate cacao rootstock genotypes under drought stress condition

| Genotype | Stem fresh weight (g) | | | | Stem dry weight (g) | | | | Root fresh weight (g) | | | | Root dry weight (g) | | | | Canopy fresh weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Environment | | | RD | Environment | | | RD | Environment | | | RD | Environment | | | RD | Environment | | | RD |
| | Opt | Sts | Delta | (%) | Opt | Stress | Delta | (%) | Opt | Stress | Delta | (%) | Opt | Stress | Delta | (%) | Opt | Stress | Delta | (%) |
| KW 516 | 10.82 | 5.38 | 5.44 | 50.32 | 4.90 | 2.39 | 2.51 | 51.30 | 4.19 | 2.76 | 1.43 | 34.21 | 1.38 | 1.13 | 0.25 | 18.04 | 20.88 | 9.30 | 11.57 | 55.44 |
| KW 617 | 9.37 | 5.35 | 4.02 | 42.92 | 4.15 | 2.24 | 1.91 | 46.02 | 4.01 | 2.15 | 1.85 | 46.26 | 1.36 | 0.77 | 0.59 | 43.68 | 18.13 | 10.66 | 7.46 | 41.18 |
| KW 641 | 8.41 | 5.47 | 2.94 | 35.02 | 3.85 | 2.37 | 1.49 | 38.59 | 3.99 | 2.83 | 1.16 | 29.07 | 1.26 | 1.24 | 0.02 | 1.23 | 16.77 | 9.08 | 7.70 | 45.89 |
| ICCRI 03 | 8.17 | 5.24 | 2.94 | 35.90 | 3.94 | 1.98 | 1.96 | 49.70 | 3.00 | 2.30 | 0.70 | 23.20 | 1.10 | 0.86 | 0.24 | 21.49 | 16.83 | 9.49 | 7.34 | 43.60 |
| TSH 858 | 8.81 | 5.55 | 3.26 | 36.96 | 3.78 | 2.43 | 1.34 | 35.57 | 3.52 | 2.53 | 0.99 | 28.24 | 1.09 | 0.82 | 0.27 | 24.58 | 17.05 | 10.25 | 6.80 | 39.91 |
| Sca 06 | 7.99 | 4.85 | 3.13 | 39.25 | 4.01 | 1.85 | 2.16 | 53.87 | 3.66 | 2.42 | 1.24 | 33.96 | 1.20 | 0.80 | 0.40 | 33.39 | 16.60 | 8.39 | 8.21 | 49.45 |
| KEE 02 | 9.34 | 5.48 | 3.86 | 41.35 | 4.77 | 2.19 | 2.59 | 54.19 | 3.99 | 2.47 | 1.52 | 38.13 | 1.46 | 0.95 | 0.50 | 34.42 | 18.68 | 8.85 | 9.83 | 52.61 |
| KKM 22 | 7.76 | 5.93 | 1.83 | 23.63 | 3.59 | 2.43 | 1.16 | 32.26 | 3.77 | 2.90 | 0.86 | 22.91 | 1.27 | 1.14 | 0.13 | 10.43 | 15.81 | 9.64 | 6.17 | 39.01 |
| MCC 02 | 9.93 | 6.26 | 3.67 | 36.99 | 4.56 | 2.46 | 2.10 | 46.09 | 4.39 | 3.15 | 1.24 | 28.27 | 1.64 | 1.12 | 0.52 | 31.87 | 18.79 | 10.30 | 8.48 | 45.15 |
| ICS 60 | 7.19 | 5.14 | 2.06 | 28.56 | 3.82 | 2.14 | 1.68 | 44.02 | 3.26 | 2.39 | 0.88 | 26.84 | 1.17 | 0.90 | 0.27 | 23.18 | 15.21 | 9.61 | 5.60 | 36.83 |
| Sul 01 | 9.51 | 4.70 | 4.81 | 50.54 | 4.78 | 2.14 | 2.64 | 55.30 | 3.92 | 2.52 | 1.41 | 35.85 | 1.47 | 0.82 | 0.64 | 43.85 | 18.72 | 8.86 | 9.86 | 52.67 |
| Sul 02 | 9.01 | 4.61 | 4.40 | 48.87 | 4.41 | 1.81 | 2.59 | 58.89 | 4.07 | 2.08 | 1.99 | 48.96 | 1.29 | 0.76 | 0.54 | 41.52 | 17.59 | 8.28 | 9.31 | 52.92 |
| Sul 03 | 8.62 | 4.96 | 3.66 | 42.51 | 4.45 | 1.76 | 2.69 | 60.48 | 4.25 | 2.41 | 1.84 | 43.26 | 1.62 | 0.82 | 0.80 | 49.29 | 17.34 | 9.66 | 7.67 | 44.26 |

Note: opt: the optimum condition, sts: the stressed condition, delta: the difference of mean obtained from the results of optimum condition minus the stressed condition, RD: the relative decrease of observed variables

**Physiological and biochemical characters of prope legitimate cocoa rootstocks**

Drought stress-induced various plant responses in terms of morphological, physiological, and biochemical characters (Borem et al. 2012). The physiological and biochemical responses of plants at seedling stage could be used to predict the tolerance to drought stress (Santos et al. 2014; Ofori et al. 2015; Fang and Xiong 2015). Relative water content (Lugojan and Ciulca 2011; Anjum et al. 2011a; Ghobadi et al. 2011: Zakaryya et al. 2017), stomatal density ( Zakaryya et al. 2017; Lahive et al. 2018), and proline content (Anjum et al. 2011b; Kishor and Sreenivasulu 2014; Zakaryya and Indradewa 2018a) frequently reported to be selection index for determining plant tolerance to drought stress.

The result showed that relative water content, stomatal density, and leaf proline content had a significant interaction variance in response to drought stress (Table 4). Thus, these characters indicated the various responses under drought stress conditions.

Based on the analysis of relative decreases in Table 5, there was various reduction in term of relative water content and stomatal density of rootstock in response to drought stress, even some genotype did not show a decrease in relative water content and stomata density. The decrease of relative water content and stomata density was indicated the plant responses to water stress (Ghobadi et al. 2011; Zakaryya et al. 2017). All prope legitimate rootstock genotypes showed an elevated leaf proline content (Table 5). In response to osmotic stress such salinity and drought, most plants accumulated proline as natural osmolyte and also for preventing the cell from reactive oxygen species (ROS) (Anjum et al. 2011b; Polavarapu et al. 2014; Zakariyya and Indradewa 2018a).

**Estimation of index and prope legitimate cocoa rootstock tolerance**

Evaluation of drought tolerance characteristics of genotypes can be predicted using susceptible stress index (SSI). SSI values are used to measure the yield stability due to changes in the environment related to the mechanism of resistance and genotype sensitivity (Khayatnezhad and Gholamin 2012; Anshori et al. 2018b). The results of analysis based on the SSI value of root fresh weight and root dry weight characters (Table 6), showed that the prope legitimate cocoa rootstock genotypes were divided into two groups, namely medium tolerant and susceptible to drought stress. Genotypes that had a SSI value of $0.5 < SSI \leq 1$ were indicated to be medium tolerant, while groups of genotypes that had a SSI value of > 1.00 were sensitive (Fisher and Maurer 1978).

Grouping was used to determine the similarity among tested plant genotypes. There was a need to know which characters that classified the plant population into the group. This information could be used to ease the selection process. There was a number of grouping method, and one of them was clustergram analysis. Clustergram analysis was a multivariate analysis by combining several clusters into a flat dimension. The contrast color could ease to understand in which characters specified to a certain group (Lee et al. 2016). This analysis had carried out on morphological characters (Zimisuhara et al. 2015; Anshori et al. 2018a). As the consequence, the simple and easy understanding related to grouping of genotypes could increase the selection effectiveness (Yuan et al. 2016).

**Table 3.** The component of variance, heritability, and deviation standard of genetic characters in prope legitimate cacao rootstock genotypes under drought stress condition

| Characters | Variance and Standard deviation | | | | Heritability | Variability | |
|---|---|---|---|---|---|---|---|
| | $\sigma_g^2$ | $\sigma_{\sigma_g}$ | $\sigma_p^2$ | $\sigma_{\sigma_p}$ | $h_{bs}^2$ | $2(\sigma_{\sigma_g})$ | $2(\sigma_{\sigma_p})$ |
| Stem fresh weight | 0.07 | 0.25 | 0.33 | 1.52 | 0.21 | 0.49 | 3.04 |
| Root fresh weight | 0.04 | 0.06 | 0.08 | 1.49 | 0.50 | 0.13 | 2.99 |
| Root dry weight | 0.01 | 0.01 | 0.02 | 1.11 | 0.27 | 0.03 | 2.23 |

Note: $\sigma_g^2$: variance of genetic, $\sigma_p^2$: variance of phenotype, $h_{bs}^2$: the broad-sense heritability, $\sigma_{\sigma_g}$ = deviation standard of genetic variance, $\sigma_{\sigma_p}$ = deviation standard of phenotype variance

**Table 4.** Analysis of variance of physiological and biochemical characters of prope legitimate cocoa rootstocks under drought stress condition

| Source | Relative water content (%) | Stomatal density (stomata/cm²) | Leaf proline content (µmol/g) |
|---|---|---|---|
| Water content | $0.00^{**}$ | $0.84^{tn}$ | $0.00^{**}$ |
| Genotype | $0.73^{tn}$ | $0.00^{**}$ | $0.00^{**}$ |
| Water content.Genotype | $0.00^{**}$ | $0.00^{**}$ | $0.00^{**}$ |
| CV (%) | 7.36 | 11.64 | $10.27^{tr}$ |

Note: *significantly different at α 5%, ** significantly different at α 1%, $^{tn}$ not significantly different, $^{tr}$: transformation data (log+1.5), CV: coefficient of variance

**Table 5.** The effect of drought stress on the relative water content, stomatal density and leaf proline content in prope legitimate cocoa rootstocks

| Genotype | Relative water content (%) | | | | Stomatal density (stomata/cm²) | | | | Leaf proline content (μmol/g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Environment | | | RD | Environment | | | RD | Environment | | | RI |
| | Opt | Sts | delta | (%) | Opt | Sts | delta | (%) | Opt | Sts | delta | (%) |
| KW 516 | 65.50 | 54.16 | 11.35 | 17.32 | 121.85 | 112.66 | 9.19 | 7.54 | 5.88 | 35.11 | 35.86 | 83.60 |
| KW 617 | 55.86 | 65.50 | -9.65 | 0.00 | 107.46 | 121.85 | -14.40 | 0.00 | 4.37 | 213.16 | 217.07 | 97.99 |
| KW641 | 68.65 | 55.86 | 12.79 | 18.63 | 99.97 | 107.46 | -7.48 | 0.00 | 4.09 | 126.96 | 88.85 | 95.40 |
| ICCRI 03 | 54.48 | 68.65 | -14.17 | 0.00 | 103.37 | 99.97 | 3.40 | 3.29 | 2.52 | 99.41 | 136.45 | 98.15 |
| TSH 858 | 66.78 | 54.48 | 12.29 | 18.41 | 113.50 | 103.37 | 10.13 | 8.92 | 1.50 | 45.04 | 38.60 | 96.11 |
| Sca 06 | 57.17 | 66.78 | -9.61 | 0.00 | 119.76 | 113.50 | 6.26 | 5.23 | 3.48 | 28.51 | 34.08 | 89.79 |
| KEE 02 | 63.60 | 57.17 | 6.43 | 10.12 | 127.78 | 119.76 | 8.02 | 6.27 | 4.15 | 122.89 | 124.02 | 96.65 |
| KKM 22 | 47.46 | 63.60 | -16.14 | 0.00 | 121.48 | 127.78 | -6.29 | 0.00 | 5.74 | 98.85 | 99.86 | 94.25 |
| MCC 02 | 69.38 | 47.46 | 21.92 | 31.60 | 119.49 | 121.48 | -1.99 | 0.00 | 2.43 | 145.31 | 145.86 | 98.33 |
| ICS 60 | 53.35 | 69.38 | -16.03 | 0.00 | 123.81 | 119.49 | 4.31 | 3.48 | 2.10 | 103.17 | 55.18 | 96.19 |
| Sul 01 | 67.43 | 53.35 | 14.08 | 20.89 | 92.34 | 123.81 | -31.47 | 0.00 | 3.17 | 18.30 | 18.64 | 83.00 |
| Sul 02 | 49.29 | 67.43 | -18.14 | 0.00 | 108.68 | 92.34 | 16.35 | 15.04 | 1.76 | 142.41 | 142.45 | 98.76 |
| Sul 03 | 68.29 | 49.29 | 19.00 | 27.82 | 109.17 | 108.68 | 0.49 | 0.45 | 11.69 | 44.00 | 46.55 | 74.87 |

Note: opt: the optimum condition, sts: the stressed condition, delta: the difference of mean obtained from the results of optimum condition minus the stressed condition, RD: the relative decrease of observed variables, RI: the relative increase of observed variables

**Table 6.** Tolerance characteristic of prope legitimate cocoa rootstock on the drought sensitivity index on root fresh weight and root dry weight characters

| Genotype | Root fresh weight (g) | | | | Root dry weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Optimum | Stress | SSI | Criteria | Optimum | Stress | SSI | Criteria |
| KW 516 | 4.19 | 2.76 | 1.00 | Medium tolerant | 1.38 | 1.13 | 0.60 | Medium tolerant |
| KW 617 | 4.01 | 2.15 | 1.35 | Susceptible | 1.36 | 0.77 | 1.46 | Susceptible |
| KW 641 | 3.99 | 2.83 | 0.85 | Medium tolerant | 1.26 | 1.25 | 0.04 | Tolerant |
| ICCRI 03 | 3.00 | 2.30 | 0.68 | Medium tolerant | 1.10 | 0.86 | 0.72 | Medium tolerant |
| TSH 858 | 3.52 | 2.53 | 0.83 | Medium tolerant | 1.09 | 0.82 | 0.82 | Medium tolerant |
| Sca 06 | 3.66 | 2.42 | 0.99 | Medium tolerant | 1.20 | 0.80 | 1.12 | Susceptible |
| KEE 02 | 3.99 | 2.47 | 1.11 | Susceptible | 1.45 | 0.95 | 1.15 | Susceptible |
| KKM 22 | 3.77 | 2.90 | 0.67 | Medium tolerant | 1.27 | 1.13 | 0.35 | Tolerant |
| MCC 02 | 4.39 | 3.15 | 0.83 | Medium tolerant | 1.64 | 1.12 | 1.07 | Medium tolerant |
| ICS 60 | 3.26 | 2.39 | 0.78 | Medium tolerant | 1.17 | 0.90 | 0.78 | Medium tolerant |
| Sul 01 | 3.92 | 2.52 | 1.05 | Susceptible | 1.46 | 0.82 | 1.47 | Susceptible |
| Sul 02 | 4.07 | 2.08 | 1.43 | Susceptible | 1.29 | 0.76 | 1.39 | Susceptible |
| Sul 03 | 4.25 | 2.41 | 1.26 | Susceptible | 1.62 | 0.82 | 1.65 | Susceptible |

Note: SSI: susceptible stress index

The results of the clustergram analysis (Figure 1) showed that there was two groups of prope legitimate cocoa rootstocks. This grouping was made based on the pattern of color intensity on the selection characters supported by the susceptible stress index. The first group was divided into subgroup 1 which consists of several genotypes, namely ICCRI 03, ICS 60, and TSH 858, while subgroup 2 which consists of KKM 22, KW 641, KW 516, and MCC 02. The second group was divided into subgroup 1 which consists of two genotypes, namely Sul 01 and Sul 3, while subgroup 2 which consists of Sca 06, KEE 02, KW 617, and Sul 02. The first group was the group that was indicated to be tolerant rootstock genotypes. The second group was the group that was indicated to be sensitive rootstock genotypes. In general, the first group showed the relative decrease trend of root fresh weight and root dry weight was lower than the second group. Genotypes responded to water deficit conditions by decreasing root weight (Paez-garcia et al. 2015). The lower relative decrease, the more tolerant the stress. Tolerant genotype was characterized by the plant ability to survive and remain productive under stress conditions (Cazares et al. 2010; Rukundu et al. 2014). Tolerant genotypes usually possessed a deep rooting system with greater number of root hair in drought conditions. The large root volumes could be able to absorb more water so that they could survive in the water shortages condition (Paez-garcia et al. 2015; Setyawan et al. 2018).

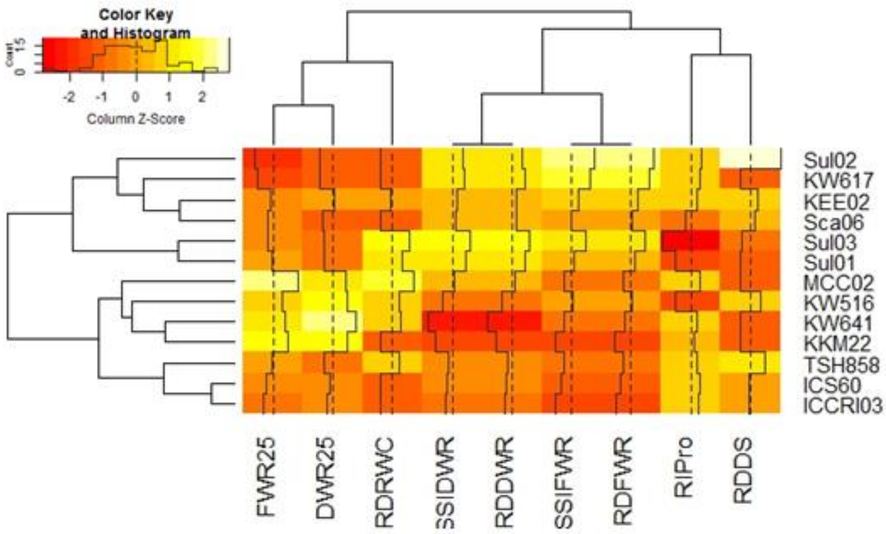


**Figure 1**. The clustergram heatmap of 13 prope legitimate cacao rootstock genotypes under drought condition based on 9 observed variables, i.e FWR25: root fresh weight in stressed condition, DWR25: root dry weight in stressed condition, RDRWC: relative decrease of water content, SSIDWR: Susceptible stress index of root dry weight, RDDWR: relative decrease of root dry weight, SSIFWR: Susceptible stress index of root fresh weight, RDFWR: relative decrease of root fresh weight, RIPro: relative increase of proline content, and RDDS: relative decrease of stomatal density

The reduction of relative water content and stomatal density including increased in prolin content of tolerant and sensitive genotypes were various responses (Figure 1). In general, Genotypes responded to water deficit conditions by decreasing relative water content (Medeiros et al. 2012; Zakaryya et al. 2017) and stomatal density (Zhao et al. 2015; Zakaryya et al. 2017). The plant water status does not only depend on the water availability in plant tissue, but also the stomatal conductivity for opening and closing activities (Ghobadi et al. 2011; Anjum et al. 2011b). The reduction of relative water content and stomatal density were highly influenced by genetic factors (Yang and Mio 2010). Increased proline production was plant adaptation strategy to survive in drought conditions (Medeiros et al. 2012). Proline accumulation was the plant response to drought stress in order to reduce cell damage (Anjum et al. 2011b; Polavarapu et al. 2014). The level of proline accumulation depended on genotype, growing stage, stress level, and other environmental conditions (Wang et al. 2015).

In conclusion, the root fresh weight was the best criterion to select the drought-tolerant prope legitimate cocoa rootstock. The drought-tolerant prope legitimate cocoa rootstocks were ICCRI 03, ICS 60, TSH 858, KKM 22, KW 641, KW 516, and MCC 02, while the sensitive prope legitimate cocoa rootstocks were Sul 01, Sul 03, Sca 06, KEE 02, KW 617, and Sul 02.

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