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Research article

Association between chlorophyll stability and drought tolerance in Robusta coffee

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Abstract

Importance of the work: Chlorophyll stability under water deficit stress might be a promising criterion for drought tolerance selection in Robusta coffee.
Objectives: To investigate the effect of water deficit on chlorophyll stability and photosynthesis in Robusta coffee, as well as to assess the drought tolerance of Robusta coffee genotypes and the relationship between chlorophyll stability and drought tolerance in Robusta coffee.
Materials & Methods: In hydroponics experiments, six Robusta genotypes were evaluated to compare control and water deficit conditions. Total dry matter, chlorophyll density (chlorophyll content per unit leaf area), chlorophyll content (chlorophyll content per plant) and the soil plant analysis development (SPAD) index were all measured. In addition, the net photosynthetic rate (P_n), transpiration rate (E) and maximum quantum efficiency (F_v/F_m) were determined.
Results: Water deficit stress significantly reduced all chlorophyll parameters, photosynthesis and total dry weight parameters. The total dry weight and chlorophyll parameters differed significantly between genotypes. Significant relationships between chlorophyll parameters and P_n, E and F_v/F_m were found in water stress conditions (Pearson's correlation coefficient, r = 0.49* to 0.68**, where * = significant at $p < 0.05$ and ** = significant at $p < 0.01$). The total dry weight of Robusta coffee was related to the chlorophyll content, chlorophyll density and the SPAD index (r = 0.51* to 0.90**). Furthermore, the chlorophyll density had a strong positive relationship with the SPAD index (r = 0.85**).
Main finding: Chlorophyll stability was an important mechanism of drought tolerance in Robusta coffee. The SPAD chlorophyll meter could be used to rapidly assess relative chlorophyll status in coffee genotypes and to select drought tolerance in Robusta coffee.

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Introduction

Coffee is a beverage crop with a high value on the global market and is one of the most important agricultural products in international agricultural trade (DaMatta et al., 2010, 2018). More than 100 coffee species are widely grown in tropical regions around the world; two of the most popular or economically important coffee species are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*), which together account for 99% of all coffee in the world (Silva et al., 2013). Robusta coffee is an economically significant coffee species around the world, with small-scale family farms producing more than 70% of the world's Robusta coffee, which is mostly grown under rainfed conditions with irrigation as an exception (Rossing et al., 2014; DaMatta et al., 2018). Consequently, changes in annual rainfall patterns and quantity could have a significant impact on such activities. Drought has resulted in significant reductions in Robusta coffee yields by around 50% in coffee production areas in Brazil but particularly in Vietnam, the world's second-largest producer (Nguyen and Nguyen, 2018; Semedo et al., 2018). In Thailand, Robusta coffee is an important economic crop. Robusta coffee is most widely grown in the country's south, accounting for 75% of total coffee production; however, the majority of coffee-growing areas are not irrigated (Kiattisin et al., 2016), so farmers must rely on seasonal rainwater for irrigation.

Droughts and climate change are becoming more common and severe and have had a significant influence on the growth and yield of Robusta coffee (DaMatta et al., 2010, 2018; Byrareddy et al., 2021). Selecting and improving drought-tolerant coffee genotypes capable of producing acceptable yields under water deficit is one of the most effective ways to relieve drought stress and improve Robusta coffee yield and quality when water is limited. Coffee breeding for drought tolerance was based on the biomass weight and yield of coffee genotypes tested under water-stress conditions (Silva et al., 2013; Cheserek et al., 2015). However, genotype selection is a critical and difficult process because biomass and yield are quantitative characteristics controlled by multiple genes (Oliveira et al., 2020). Furthermore, environmental effects are frequently involved, particularly genotype × environment interactions (Sixto et al., 2016; Oliveira et al., 2020). This has posed a major barrier to the breeding of coffee (Cheng et al., 2016; Marie et al., 2020). Therefore, the relationship should be investigated between the traits of interest and the drought tolerance of coffee. Thus, the breeding of Robusta coffee to be drought tolerant could use these traits as a supplementary tool.

In water deficit conditions, the coffee plant's leaf water potential decreased and abscisic acid production increased, signaling the closure of stomata and lowering of the transpiration rate of the coffee plant (Menezes-Silva et al., 2015; Silva et al., 2018). Subsequently, intercellular CO_2 concentrations decreased and mesophyll resistance increased, resulting in lower photosynthesis rates (DaMatta and Ramalho, 2006; Menezes-Silva et al., 2017; Wang et al., 2018). Consequently, coffee plant growth was affected and yields were reduced (Dias et al., 2007; Farooq et al., 2009). Thus, the high photosynthetic capacity of coffee genotypes under water deficit conditions is an important mechanism of drought tolerance in coffee (Menezes-Silva et al., 2017; Santos et al., 2021; Arunyanark et al., 2022). Therefore, identifying drought-tolerant genotypes of Robusta coffee might be aided by research into the physiological characteristics associated with the photosynthetic efficiency of plants. Plant photosynthetic capacity is closely related to chlorophyll content in the leaves (Banks, 2018). In many crop plants, chlorophyll stability in leaves is closely related to photosynthetic capacity and the ability of plants to maintain chlorophyll density and chlorophyll content under water deficit conditions has been proposed as a drought-tolerant mechanism (Arunyanark et al., 2008; Aryal et al., 2015; Monteoliva et al., 2021). However, there has been little published research on the relationship between leaf chlorophyll status and drought tolerance in Robusta coffee. Furthermore, the leaf chlorophyll content can be measured easily and quickly using the SPAD chlorophyll meter reading (Arunyanark et al., 2009; Dong et al., 2019), making it suitable as an effective, indirect tool in the selection of plant genotypes, where a large number of samples needs to be assessed non-destructively.

Chlorophyll stability in the leaves might be related to drought tolerance in coffee and so could be used in Robusta coffee breeding for drought tolerance. Thus, the objectives of the current study were to investigate the effect of water deficit on chlorophyll stability and photosynthesis in Robusta coffee, as well as to assess the drought tolerance of Robusta coffee genotypes and the relationship between chlorophyll stability and drought tolerance in Robusta coffee.

Materials and Methods

Experimental design and treatments

From September to October 2018, the experiment was carried out at the Tropical Vegetable Research Development

Center (TVRDC) at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, under greenhouse conditions with a natural photoperiod. The experiment was set up as a split plot in a randomized complete block design with four replications. The main plot had two water regimes: the control condition and the water deficit condition. The subplots consisted of six Robusta coffee genotypes: SC05, PP01, PP05, SKE06 and TPO17 (which are all widely grown in Thailand, particularly in Southern Thailand, but have not yet been tested for drought tolerance) and FRT141 (a drought-tolerant genotype evaluated by Nestlé Agricultural Services in Chumphon Province, Thailand).

All coffee seedlings were grown from rooted stem cuttings in polyethylene bags with 2 kg of soil mixed with coir, rice husk and cow manure (2:1:1:1). The seedlings were transplanted to a hydroponic system with half-strength Hoagland solution at pH 5.8 and an air pump when they were aged 6 mth. In this experiment, each genotype and treatment had 16 plants. The nutrient solution and pH levels were maintained daily by adding nutrient solution and adjusting the pH with HCl. The plants were given 2 wk to adjust to the hydroponic conditions before the water deficit treatments began (0 days after the trial began). Throughout the experiment, the control condition was a half-strength Hoagland nutrient solution (osmotic potential, Ψ_{sol} = -0.035 MPa). To generate two levels of water deficit stress, water deficit conditions were imposed using polyethylene glycol (PEG6000; BASF Co. Ltd.; Germany). The solution was 5% PEG (Ψ_{sol} = -0.07 MPa) for the first week and then increased to 9% PEG (Ψ_{sol} = -0.14 MPa) in stepwise succession for the second week (14 d after the trial began). Similar PEG solutions have been used to induce drought stress in Eucalyptus, as expressed in a variety of plant responses, including osmotic adjustment (Utkhao and Yingjajaval, 2015). After the water stress ended, stressed coffee plants were evaluated for recovery by removing the coffee plant from the solution and thoroughly rinsing the roots to remove the polyethylene glycol solution before transplanting it into a nutrient solution under control conditions for 20 d (34 d after the trial began).

Meteorological conditions and measurement of plant water status

Data loggers (WatchDog data-logger 1000 series Micro Stations; Spectrum Technologies Inc.; USA) installed inside the greenhouse were used to record the maximum and average photosynthetic photon flux density (PPFD), maximum, average and minimum air temperature and relative humidity (RH) every 15 min after the seedlings had been transplanted to the hydroponic conditions until the end of the stress period.

The plant water status was determined at two different times in the control and stress treatments: predawn (pd; 0400–0600 hours) and midday (md; 1100–1300 hours), using the first fully expanded leaves of four different plants per genotype. The relative water content (RWC) of the leaf samples was determined. The leaf fresh weight (FW), turgid weight (TW) and dry weight (DW) were measured and calculated using the equation (Barrs and Weatherley, 1962): RWC = (FW - DW) / (TW - DW). RWC was recorded at 0, 14 and 34 d after the trial began.

Measurement of leaf gas exchange

Throughout the experiment, one coffee plant from each replication was randomly assigned to measure the leaf gas exchange rate of the same coffee plant. Data were collected at 0 d, 14 d, 24 d and 34 d after the trial began. Leaf gas exchange measurements were performed on fully expanded leaves (the third and fourth leaves from the top of the plant). During 0830–1030 hours, leaf samples were evaluated using a portable infrared gas analyzer (LI-6400; Licor Inc.; USA) equipped with a broadleaf 2.0 cm^2 fluorometer chamber (LI-6400-40, Licor Inc.; USA). The net photosynthetic rate (P_n) was determined under the following conditions: photosynthetic photon flux density (PPFD) of 1,000 mol/m^2/s (with 10% blue light), reference CO_2 concentration of 400 mol CO_2/m^2/s, leaf chamber temperature of 28–33 °C; and RH of 75%–85%. In addition to providing P_n data, the instrument recorded the transpiration rate (E). Using a pulse amplitude-modulated fluorometer (PAM-2100; Heinz Walz; Germany), the maximum quantum efficiency (F_v/F_m) was determined on the same leaf. The measurements were taken in the dark during 0400–0530 hours. F_v/F_m was calculated using the equation: $F_v/F_m = (F_m - F_o)/F_m$, where F_o is the minimum variable fluorescence and F_m is the maximum variable fluorescence.

Measurement of chlorophyll stability and soil plant analysis development index

One coffee plant from each treatment was chosen at random for each replicate to measure chlorophyll stability, the soil plant analysis development SPAD index and the total dry weight. The SPAD index and chlorophyll content were measured at 0 d, 14 d and 34 d after the trial began. The fully expanded leaves were sampled at the third and fourth leaves at the top of the plant. A chlorophyll meter (model SPAD-502; Minolta Camera;

Japan) was used to measure the SPAD index that was calculated by averaging the results of three measurements on the leaf sheet. One leaf disc was taken from the same leaf sample using a cork borer with a diameter of 0.028 cm^2. Leaf discs were soaked in 4 ml of *N, N*-dimethylformamide and kept in the dark for 24–48 h before determining the chlorophyll content using light absorption with a spectrophotometer. The chlorophyll content per unit leaf area (chlorophyll density) was analyzed following the procedures described by Moran (1981). The chlorophyll density and total leaf area per plant were used to calculate the chlorophyll content (chlorophyll content per plant).

Measurement of total dry weight

The total dry weight of each sampled Robusta coffee plant was collected at 0 d, 14 d and 34 d after the trial began. The same sample was used to get the chlorophyll measurements. In the plant samples, the leaves were removed from the plant. The total leaf area per plant was measured using a leaf area meter (LI–3100, Licor Inc., USA) from a leaf sample. The leaves, stems, branches and roots were separated and dried for 72 hr at 75 °C in a hot air oven. Once the plant had dried, it was weighed and the total dry weight was recorded.

Statistical analysis

Analysis of variance on all parameters was calculated using a split-plot in a randomized complete block design to evaluate the effect of water deficit stress and genotype variability. For each water condition, the mean ± SE for Robusta coffee plant were calculated separately. In addition, the response of Robusta coffee genotypes to drought stress was assessed using an analysis of variance for each water condition independently. The least significant difference was used in mean comparisons. Simple correlations between chlorophyll parameters, photosynthetic efficiency and the total dry weight of Robusta coffee were calculated. Data from three ages (0 d, 14 d and 34 d after the trial began; n = 18) in both control and water deficit conditions were used to calculate the correlation coefficients.

Results and Discussion

Meteorological conditions and plant water status

The average RH during the experiment was 82.9% (maximum and minimum average values were 95.9% and 64.2%, respectively, data not shown), the average air temperature was 29.2 °C (maximum and minimum average temperatures were 37.0 °C and 24.9 °C, respectively) and the maximum photosynthetic photon flux density was 434–867 mol/m^2/s.

The relative water content of Robusta coffee leaves was investigated at two different times. The results showed no significant difference between the relative water content in the predawn (RWC_{pd}) of 96.5% and 96% for Robusta coffee under control and water deficit conditions at 0 d (Fig. 1A). However, coffee plants in the water deficit condition had lowered RWC_{pd} to 67.2% after 14 d, compared to 95.3 % in the control condition. After 34 d of recovery, the water deficit plant had an RWC_{pd} increase of 93.9%, which was not significantly different from the control condition of 95.9%. Similar results were found in relative water content at midday (RWC_{md}). At 0 d, there was no significant difference in RWC_{md} between Robusta coffee under the control and water deficit conditions, with values of 83.8% and 84.7 %, respectively (Fig. 1B). However, after 14 d, the water deficit coffee plants had reduced RWC_{md} to 61.2 %, compared to 84.2 % in control conditions.

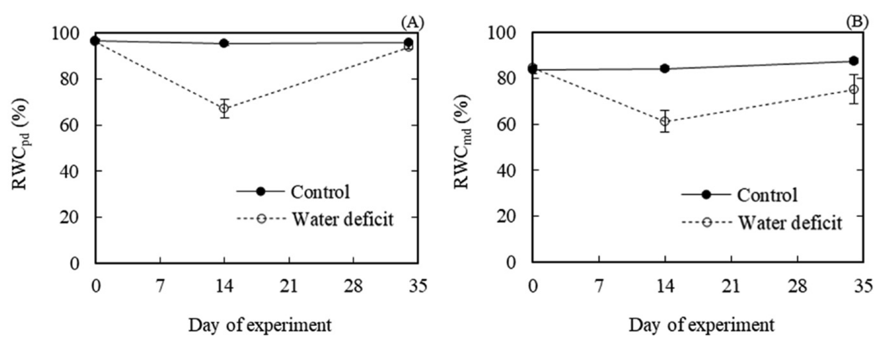

Fig. 1 Effect of water deficit stress on relative water content (RWC) at: (A) predawn (pd); (B) midday (md) of Robusta coffee grown under control and water deficit conditions, where error bars represent ± SE

Water-stressed coffee plants had an RWC_{md} increase of 75.2 % after 34 d, but it remained below the control at 87.6%. According to the findings, water deficit simulations using a PEG solution resulted in Robusta coffee water stress, with a significant reduction in RWC after the water deficit. The findings corroborated another study that demonstrated that a lack of water affected the RWC of Robusta coffee grown in soil, with the RWC responses of *Coffea arabica* and *Coffea liberica* to water deficit being similar (Vu et al., 2018). Due to a prolonged water deficit, water deficit stress was detected in the Robusta coffee plants throughout both predawn and midday. The current study found that RWC_{pd} of water-deficit Robusta coffee could recover after returning to normal conditions; however, RWC_{md} decreased more than RWC_{pd}, suggesting that RWC_{md} recovery might take longer for water-deficit plants.

Effect of water deficit stress on photosynthetic efficiency of Robusta coffee

In water deficit stress, Robusta coffee quickly closed the stomata to control the water loss of the plant, resulting in a reduced transpiration rate (E) by the Robusta coffee leaves. The Robusta coffee was grown in a normal nutrient solution in both water conditions at 0 d. The E values were the same under the control and water deficit conditions (Fig. 2A). The Robusta coffee under water deficit conditions was reduced to 0.30 and 0.26 mmol $H_2O/m^2/s$ after being stressed for 7 d and 14 d, respectively. Although the E values increased to 0.64 and 0.40 mmol $H_2O/m^2/s$ after recovery at 24 d and 34 d, they were still lower than the control condition, which had E values in the range 0.98–2.55 mmol $H_2O/m^2/s$ throughout the experiment. The results revealed that the water deficit reduced the rate of transpiration in the Robusta coffee due to the response of stomata to maintain the water balance within the plant by controlling water loss (DaMatta et al., 2018; Roonprapant et al., 2021). However, closing the stomata to reduce the transpiration rate also affected the photosynthesis rate of coffee leaves. Under control and water deficit conditions, there was no significant difference in P_n values at 0 d (Fig. 2B). The P_n values of water deficit plants were lowered to 0.75 µmol $CO_2/m^2/s$ and 0.39 µmol $CO_2/m^2/s$ when the Robusta coffee was stressed for 7 d and 14 d, respectively, increased to 2.57 µmol $CO_2/m^2/s$ and 1.88 µmol $CO_2/m^2/s$ after recovery at 24 d and 34 d, respectively, but remained lower than the control condition, which had P_n values in the range 4.37–6.60 µmol $CO_2/m^2/s$.

In coffee photosynthesis, stomatal inhibition constitutes a major limiting factor (DaMatta et al., 2019; Martins et al., 2019; Dubberstein et al., 2020). The initial mechanism in response to a lack of water is for plants to close their stomata, which limits the passage of CO_2 and water, decreasing the net photosynthetic rate of plants as a result (Cornic, 2000; Roonprapant et al., 2021; Arunyanark et al., 2022). In the current study, during a water deficit period, the Robusta coffee had a reduced net photosynthetic rate. As the severity of the water deficit increased, the rate of photosynthesis declined, with the lowest values near zero or nearly no photosynthesis. Furthermore, even after the Robusta coffee returned to its normal water level, the water deficit continued to impact photosynthesis.

The study at maximum quantum efficiency (Fv/Fm) also found that longer and more severe water deficit stresses tended to decrease Fv/Fm. The Fv/Fm values of the Robusta coffee were 0.78 and 0.75 at 0 d and 7 d, respectively, in a water deficit condition (Fig. 2C), which was similar to the control condition.

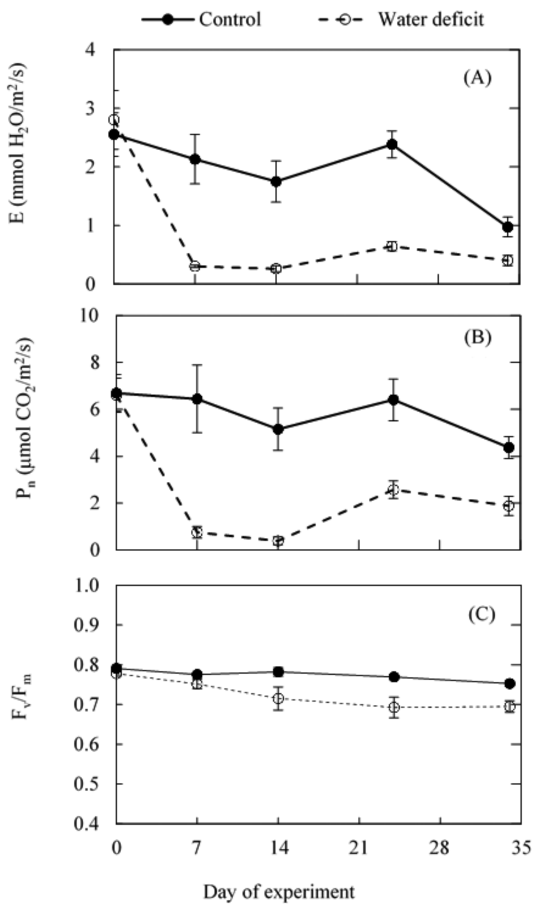

Fig. 2 Effect of water deficit stress on: (A) transpiration rate (E); (B) net photosynthetic rate (P_n); (C) maximum quantum efficiency (F_v/F_m) of Robusta coffee grown under control and water deficit conditions, where error bars represent ± SE

The Fv/Fm value reduced to 0.72 when the water deficit reached 14 d, which was different from the control condition. The reduction in Fv/Fm persisted through the recovery period of 24 d and 34 d when the Fv/Fm values of 0.69 and 0.70 differed from the control condition of 0.75 and 0.79. Fv/Fm was a sensitive indicator of coffee plant photosynthetic performance, representing the potential quantum efficiency of PSII and is a key indicator of the physiological status of the photosynthetic apparatus. As a result, both stomatal and nonstomatal mechanisms may have played a role in the water stress-induced reduction in the net photosynthetic rate (Maxwell and Johnson, 2000; Farooq et al., 2009; Tounekti et al., 2018). Even after restoring normal watering to the coffee plants, the effects of the water deficit lasted for some time before returning to normal. The effect of water deficit on the Fv/Fm values for Robusta coffee have been reported in other coffee species, such as *Coffea arabica* and *Coffea liberica* (Vu et al., 2018).

Effect of water deficit stress on chlorophyll stability of Robusta coffee

There was no significant difference in chlorophyll density between the Robusta coffee grown under the control and the water deficit conditions at 0 d and 14 d (Fig. 3A). The Robusta coffee had chlorophyll densities of 3.64 g/m^2 and 3.53 g/m^2 in the water deficit condition, respectively. The chlorophyll density of the stressed coffee was lowered to 3.01 g/m^2 at 34 d, which was lower than the control condition, which had chlorophyll densities of 3.40 g/m^2, 3.53 g/m^2 and 3.80 g/m^2at the three ages, respectively. At 0 d, the chlorophyll contents of the Robusta coffee under control and water deficit conditions were 0.24 g/plant and 0.29 g/plant, respectively, (Fig. 3B) and not significantly different. On the other hand, the water deficit plants had a reduced chlorophyll content to 0.09 g/plant at 14 d, compared to 0.31 g/plant in the control conditions. After 34 d of recovery, the stressed coffee had a minor increase in chlorophyll content to 0.13 g/plant; however, it was still lower than the control at 0.53 g/plant. Furthermore, the SPAD index was affected by a water deficit. The SPAD index did not differ between the control and water deficit conditions at 0 d and 14 d (Fig. 3C) for these two days, the Robusta coffee had a SPAD index of 44.50 and 44.40 in water deficit, respectively. The SPAD index of the stressed coffee was lowered to 39.38 at 34 d, which was lower than the control, which had SPAD index values of 45.11, 47.12 and 46.49 at the three ages, respectively.

Water deficit affected Robusta coffee chlorophyll stability, including the chlorophyll density and total chlorophyll content, as well as the SPAD index. The reductions in chlorophyll density and the SPAD index were almost similar and was lower than the control at 34 d, while the total chlorophyll content per plant decreased to lower than the control at 14 d. In addition, water deficit has been shown to reduce chlorophyll content and density in a variety of plants (Falqueto et al., 2017; Mihaljević et al., 2021). Several studies have found an association between the chlorophyll content, photosynthesis and PSII response in water-stressed plants (Banks, 2018; Wang et al., 2018; Liang et al., 2019). Furthermore, Santos et al. (2021) found a relationship between photosynthetic efficiency and genes linked to drought tolerance in Robusta and Arabica coffee.

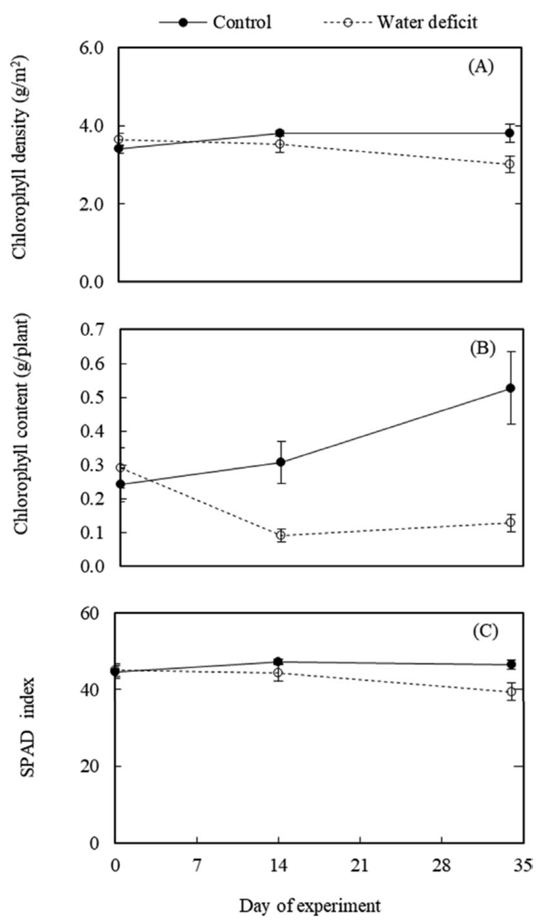

Fig. 3 Effect of water deficit stress on: (A) chlorophyll density; (B); total chlorophyll content per plant; (C) SPAD index of Robusta coffee grown under control and water deficit conditions, where error bars represent ± SE

Drought tolerance of Robusta coffee genotypes

Water deficit had a significant effect on chlorophyll stability and the total dry weight of the Robusta coffee at 34 d (Table 1). Compared to the Robusta coffee in the control condition, Robusta coffee in the water deficit condition had a 24.13% decrease in chlorophyll density, a 75.79% decrease in chlorophyll content, a 14.40% decrease in SPAD index and a 46.06% decrease in total dry weight. In addition, in both control and water deficit conditions, there were significant differences in the values for chlorophyll stability and the total dry weight among the Robusta coffee genotypes. The FRT141 genotypes had the highest chlorophyll density, chlorophyll content and total dry weight under control conditions, with values of 5.95 g/m^2, 1.03 g/plant and 17.50 g/plant, respectively. However, under control conditions, there was no significant difference between the genotypes in the SPAD index, which was in the range 43.47–56.18. In water deficit conditions, with 3.70 g/m^2, 3.42 g/m^{2}and 3.04 g/m^2, the SC05, PP01 and FRT141 genotypes, respectively had the highest chlorophyll densities. The highest total chlorophyll concentration was in the FRT141, PP01 and SC05 genotypes, with 0.23 g/plant, 0.22 g/plant and 0.16 g/plant, respectively. The highest SPAD index values were 48.57, 43.98 and 41.80 for the SC05, PP01 and FRT141 genotypes, respectively. The FRT141, PP01 and SC05 genotypes, with total dry weights of 10.07 g/plant, 8.45 g/plant and 8.12 g/plant, respectively, had the highest total dry weights. The ability of coffee to produce dry weight under limited water exposure was used to determine its drought tolerance (Silva et al., 2013; Chemura et al., 2014). The biomass weight of Arabica coffee under water deficit conditions was used in another study to identify drought-tolerant genotypes (Cheserek et al., 2015; Mohammed et al., 2021). According to the current findings, the FRT141, PP01 and SC05 genotypes showed drought tolerance. Furthermore, in water deficit conditions, these Robusta coffee genotypes had high chlorophyll stability.

Relationship between chlorophyll stability and drought tolerance of Robusta coffee

Correlation analysis at the three ages (0 d, 14 d and 34 days; n = 18) revealed a strong positive correlation between chlorophyll density and the SPAD index under both control and water deficit conditions, with correlation coefficients (r) of 0.85** and 0.85** (Figs. 4A and 4B), respectively. A similar correlation was obtained when both water levels were included in the calculation of the correlation (n = 36), r = 0.83**; data not shown). Additionally, a high positive correlation was established between the chlorophyll content and chlorophyll density in both the control and water deficit circumstances, including when calculating the pooled correlation of both water levels, (r = 0.85**, 0.55* and 0.83**, respectively), as shown in Table 2. A significant positive correlation was discovered between the chlorophyll content and the SPAD index in both the control and water deficit conditions, including when calculating the pooled correlation of both water levels (r = 0.60**, 0.50* and 0.57**, respectively). Furthermore, in water deficit conditions, r = 0.60**, 0.58* and 0.49* were significant positive correlations between the chlorophyll content and F_v/F_m, P_n and E, respectively.

Table 1 Chlorophyll density, total chlorophyll content per plant, SPAD index and total dry weight of Robusta coffee genotypes under control and water deficit conditions at 34 d of experiment

Genotype	Chlorophyll density (g/m^2)		Chlorophyll content (g/plant)		SPAD index		Total dry weight (g/plant)	
	Control	Water deficit	Control	Water deficit	Control	Water deficit	Control	Water deficit
FRT141	5.95±2.01^a	3.04±0.42^{ab}	1.03±0.39^a	0.23±0.05^a	56.18±3.92	41.80±0.50^b	17.50±3.34^a	10.07±1.74^a
SC05	3.59±0.41^b	3.70±1.17^a	0.44±0.15^{bc}	0.16±0.09^{ab}	46.68±3.84	48.57±1.70^a	12.15±2.19^b	8.12±1.77^{ab}
PP01	3.65±0.90^b	3.42±0.21^{ab}	0.52±0.15^b	0.22±0.17^a	46.48±8.16	43.98±1.10^b	13.89±1.60^b	8.45±4.29^a
PP05	2.89±0.54^b	2.92±0.12^{abc}	0.18±0.07^c	0.06±0.03^{bc}	43.47±5.58	35.87±4.97^c	7.77±1.70^c	5.29±0.95^{bc}
SKE06	4.01±0.59^b	2.79±0.43^{bc}	0.58±0.21^b	0.07±0.03^{bc}	47.50±5.10	37.58±3.41^c	12.11±3.39^b	4.94±0.35^c
TPO17	3.69±0.98^b	2.16±0.12^c	0.41±0.10^{bc}	0.04±0.03^c	46.20±8.33	37.45±1.18^c	11.81±2.99^b	3.71±1.47^c
F test	*	*	**	**	ns	*	**	**
Mean	3.96±1.34^A	3.01±0.69^B	0.53±0.32^A	0.13±0.11^B	47.75±6.75^A	40.87±5.09^B	12.54±3.76^A	6.76±2.99^B
CV (%)	19.50	18.28	27.29	28.37	13.86	6.64	15.05	28.35

CV = coefficient of variation; ns = non-significant ($p \geq 0.05$); * = significant ($p < 0.05$); ** = highly significant ($p < 0.01$);
Mean (± SE) superscripted with different capital letters are significantly ($p < 0.05$) different within each parameter; means (± SE) in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different among coffee genotypes under same conditions.

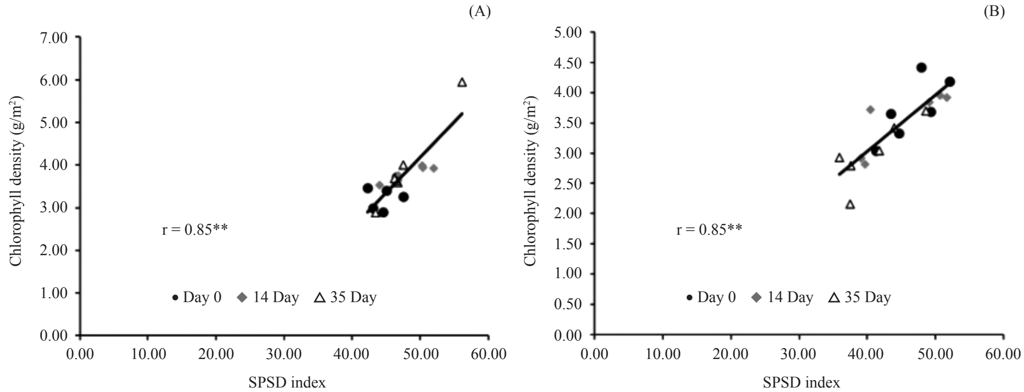

Fig. 4 Relationship between chlorophyll density and SPAD index of Robusta coffee genotypes pooled for three sampling dates ($n = 18$) under: (A) control conditions; (B) water deficit conditions, where r = correlation coefficient and ** = highly significant ($p < 0.01$)

Table 2 Correlation coefficients (r) between total chlorophyll content per plant with chlorophyll density, SPAD index, maximum quantum efficiency (Fv/Fm), net photosynthetic rate (Pn) and transpiration (E) of Robusta coffee genotypes pooled for three sampling dates under control and water deficit conditions

Chlorophyll content	Chlorophyll density	SPAD index	F_v/F_m	P_n	E
Control (n = 18)	0.85**	0.60**	-0.41	-0.28	-0.47*
Water deficit (n = 18)	0.55*	0.50*	0.60**	0.58*	0.49*
Pooled (n = 36)	0.73**	0.57**	0.36*	0.32*	0.12

*, ** = significant ($p < 0.05$) and highly significant ($p < 0.01$), respectively

The chlorophyll content per leaf area (chlorophyll density) was related to the chlorophyll content per plant of Robusta coffee. Because Robusta coffee has a high chlorophyll density, it also has a high chlorophyll content per plant. Furthermore, both the chlorophyll content and the chlorophyll density are related to the SPAD index. Thus, the SPAD chlorophyll meter reading could be utilized as an indirect tool for assessing the chlorophyll content and chlorophyll density in Robusta coffee leaves as it allows for the easy, rapid and non-destructive measurement of plant samples. Several studies have demonstrated that the SPAD chlorophyll meter could be used to estimate the chlorophyll content in the leaves of a wide range of plants, including peanut, soybean, rice, corn, wheat, sorghum, potato, papaya, canola and jatropha (Arunyanark et al., 2008; Nyi et al., 2012; Parry et al., 2014; Dong et al., 2019). However, in the current investigation, the relationship between the SPAD index and chlorophyll density was greater than the relationship between the SPAD index and chlorophyll content per plant. It was demonstrated that using the SPAD index to estimate chlorophyll density was more accurate than using the SPAD index to estimate chlorophyll content per plant. This was similar to the findings of Netto et al. (2005), who discovered a relationship between the SPAD index and the chlorophyll and nitrogen densities per leaf area of Robusta coffee. In addition, Reis et al. (2009) found a relationship between the SPAD index and the chlorophyll and nitrogen densities per unit leaf area of Arabica coffee. However, the SPAD index might be applied to estimate the chlorophyll content per plant by multiplying the SPAD index value by the leaf area. In addition, the current study discovered that total chlorophyll content per plant was related to F_v/F_m, P_n and E in the water deficit condition, suggesting that the chlorophyll content in the leaves was important for maintaining the rate of photosynthesis in Robusta coffee under drought conditions.

The maximum quantum efficiency (F_v/F_m) of Robusta coffee was significantly positively correlated with the chlorophyll density and SPAD index in the water deficit condition, with r = 0.66** and 0.68** (Table 3), respectively. The corresponding

Table 3 Correlation coefficients (r) between maximum quantum efficiency (F_v/F_m) with chlorophyll density, SPAD index, net photosynthetic rate (P_n) and transpiration (E) of Robusta coffee genotypes pooled for three sampling dates under control and water deficit conditions

F_v/F_m	Chlorophyll density	SPAD index	P_n	E
Control (n = 18)	-0.30	-0.05	0.69**	0.77**
Water deficit (n = 18)	0.66**	0.68**	0.63**	0.60**
Pooled (n = 36)	0.37*	0.58**	0.71**	0.65**

*, ** = significant ($p < 0.05$) and highly significant ($p < 0.01$), respectively

r values were 0.37* and 0.58** when the correlation combining both water levels was calculated; however, no such relationship was observed under the control condition. A high positive correlation was also established between F_v/F_m and Pn in both the control and water deficit conditions, including when calculating the pooled correlation of both water levels (r = 0.69**, 0.63** and 0.71**, respectively). F_v/F_m and E had a high positive correlation in both control and water deficit conditions, including when the pooled correlation of both water levels was calculated (r = 0.77**, 0.60** and 0.75**, respectively). Furthermore, a very strong positive correlation was established between E and Pn in both the control and water deficit conditions (r = 0.82** and 0.95**, data not shown), respectively and when the pooled correlation of both water levels was calculated (r = 0.90**), as shown in Figure 5. The results showed a strong relationship between F_v/F_m, P_n and E under both the control and water deficit conditions, demonstrating that the ability of photosynthesis was dependent on the rate of water deficit in Robusta coffee. If the stomata were generally open to allow for a high rate of transpiration, this would also result in a high rate of photosynthesis. Transpiration is the evaporation of water from plants that occurs at the leaves while their stomata are open to allow CO_2 and O_2 to pass through during photosynthesis. However, although the number of stomata opening was reduced in the water deficit condition, the transpiration rate remained related to the photosynthesis rate of Robusta coffee. In the current study, the water deficit condition, Fv/Fm was only correlated with chlorophyll density and the SPAD index. It was demonstrated that in water deficit stress, plant photosynthesis was restricted by stomatal closure. Chlorophyll stability in the leaves is an important factor for the photosynthetic efficiency of Robusta coffee under water deficit stress.

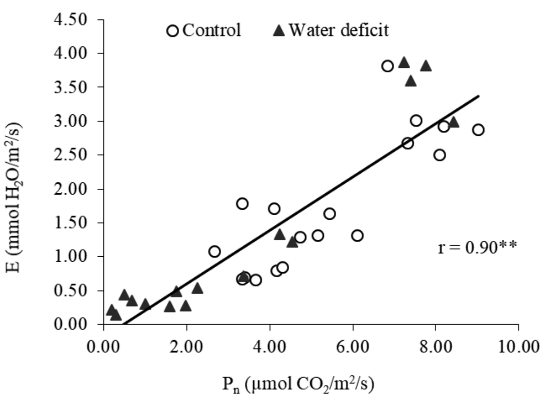

Fig. 5 Relationship between transpiration (E) and net photosynthetic rate (P_n) of Robusta coffee genotypes pooled for three sampling dates and two water levels (*n* = 36), where ** = highly significant ($p < 0.01$)

The total dry weight of Robusta coffee was significantly positively correlated with chlorophyll density in both the control and water deficit conditions, including when calculating the pooled correlation of both water levels (r = 0.68**, 0.52* and 0.62**, respectively), as shown in Table 4. A positive correlation was found between total dry weight and SPAD index in both the control and water deficit conditions, including when calculating the pooled correlation of both water levels (r = 0.51*, 0.54* and 0.56**, respectively). Furthermore, a significant strongly positive correlation was established between the dry weight and chlorophyll content in both the control and water deficit conditions, including when calculating the pooled correlation of both water levels (r = 0.90**, 0.76** and 0.89**, respectively). Additionally, a significant positive correlation was observed between total dry weight and F_v/F_m in the water deficit condition and when the pooled correlation was computed at both water levels (r = 0.51* and 0.38*, respectively), while no correlation was observed in the control condition. The chlorophyll content in the leaves is an important characteristic of photosynthesis and dry weight accumulation in plants (Banks, 2018). Previous studies have shown that the chlorophyll content in the leaves could be used to indicate drought tolerance in several crops, including peanut, soybean, wheat, barley, potato and oil palm (Arunyanark et al., 2008; Aryal et al., 2015; Monteoliva et al., 2021). The current study revealed that the dry weight capacity of Robusta coffee was related to the chlorophyll density and the total chlorophyll content per plant, as well as the SPAD index in both control and water deficit conditions. The finding that Robusta coffee's drought tolerance was based on its ability to build dry weight under water-stressed conditions suggested that chlorophyll

Table 4 Correlation coefficients (r) between total dry weight with chlorophyll density, SPAD index, total chlorophyll content per plant and maximum quantum efficiency (F_v/F_m) of Robusta coffee genotypes pooled for three sampling dates under control and water deficit conditions

Total dry weight	Chlorophyll density	SPAD index	Chlorophyll content	F_v/F_m
Control (*n* = 18)	0.68**	0.51*	0.90**	-0.32
Water deficit (*n* = 18)	0.52*	0.54*	0.76**	0.51*
Pooled (*n* = 36)	0.62**	0.56**	0.89**	0.38*

*, ** = significant ($p < 0.05$) and highly significant ($p < 0.01$), respectively

stability was an important mechanism that could be used to determine the drought tolerance of Robusta coffee genotypes and in the analysis of the relationship between the SPAD index and the chlorophyll stability and total dry weight of Robusta coffee under water deficit conditions. Consequently, it was proposed that the SPAD index might be used as an indirect trait in the effective selection of drought-tolerant genotypes of Robusta coffee.

In conclusion, water deficit stress affected the chlorophyll stability, rate of photosynthesis and the biomass of Robusta coffee. Water deficit stress reduced the values for the chlorophyll content, chlorophyll density, SPAD index, F_v/F_m, P_n, E and total dry weight of Robusta coffee. The chlorophyll stability and total dry weight of Robusta coffee differed between genotypes. The Robusta coffee genotypes with the highest chlorophyll content and chlorophyll density were also the genotypes with the highest total dry weight under water deficit conditions, such as FRT141, PP01 and SC05. Furthermore, the chlorophyll content and chlorophyll density were related to Robusta coffee photosynthesis under water deficit conditions. The chlorophyll content and chlorophyll density were also related to the total dry weight of Robusta coffee. Therefore, chlorophyll stability in leaves can be utilized to identify drought-tolerant genotypes in Robusta coffee. Furthermore, chlorophyll content and chlorophyll density were related to the SPAD index, as was the relationship between the SPAD index and the total dry weight of Robusta coffee under water deficit conditions. Thus, the SPAD chlorophyll meter can be used quickly to select for drought tolerance of Robusta coffee genotypes in a practical manner.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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