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Research article

Effect of water deficit and propagation methods on physiological responses of Robusta coffee (*Coffea canephora*) varieties

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Abstract

Importance of the work: Drought is a major issue for Robusta coffee production. However, data on the physiological response of Robusta coffee to water deficit are limited.

Objectives: To determine effect of water deficit and propagation methods on the physiological characteristics of Robusta coffee and to investigate relationships among the physiological characteristics of Robusta coffee.

Materials & Methods: Three varieties of Robusta coffee and different propagation methods were evaluated under control and water deficit conditions. Plant water status, leaf gas exchange and the soil-plant analyses development (SPAD) index were all measured.

Results: Water deficit reduced plant water status and leaf gas exchange characteristics—impaired photosynthesis (P_n) via reduced stomatal conductance (g_s) and transpiration (E), water use efficiency (WUE), light-adapted quantum efficiency of PSII (ΦPSII) and electron transport rate (ETR)—in Robusta coffee but did not affect the SPAD index. The physiological responses to Robusta coffee differed depending on the propagation method. Cutting-propagated Robusta coffee had a higher rate of leaf gas exchange than seeded and grafted Robusta coffee. Robusta coffee had physiological differences between varieties. For the SC05 variety, both the grafting and cutting methods had the highest potential for leaf gas exchange under water-stressed conditions. Furthermore, a relationship among leaf gas exchange characteristics was found (correlation coefficient, r = 0.74** to 1.00**). Leaf gas exchange and leaf water status were also related (r = 0.86** to 0.93**).

Main finding: There was a physiological response to water deficit of Robusta coffees propagated using different methods, with a relationship among the physiological characteristics of Robusta coffee under water deficit conditions.

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Introduction

Coffee is a high-value economic crop on the global market and one of the most important agricultural products in international trade (DaMatta et al., 2018). Coffee is also a significant source of income for many countries in Latin America, Africa and Asia (DaMatta et al., 2010, 2018). More than 100 coffee species are widely grown in tropical regions around the world, but Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) are two of the most popular or economically important coffee species, accounting for 99% of global coffee production (Silva et al., 2013). Thailand's coffee plantation area decreased from 29,681 ha to 20,757 ha between 2016 and 2020, with the average total yield decreasing from 21,190 t/yr to 10,212 t/yr, while Thailand's demand for coffee beans was 81,417 t/yr. Thus, coffee beans must be imported from abroad due to the increasing demand for coffee beans from processing plants, while there is insufficient production to meet consumer demand, (Office of Agricultural Economics, 2020). Robusta coffee constitutes the majority of Thailand's coffee production where it is grown mainly in the south and accounts for 75% of total coffee production (Kiattisin et al., 2016). However, the majority of coffee-growing areas are not irrigated (Roonprapant et al., 2021) so farmers rely on seasonal rainwater for cultivation.

Drought conditions that were more frequent and intense, as well as climate variability, had an impact on the growth and yield of Robusta coffee (DaMatta et al., 2010, 2018). Water deficit causes the stomata of the coffee plant to close, reducing the rate of transpiration and photosynthesis (DaMatta and Ramalho, 2006), resulting in decreased growth of the plant and crop yield (Dias et al., 2007). Other research found that water deficit altered plant morphology and reduced biomass accumulation (Farooq et al., 2009). Furthermore, physiological changes might occur, such as decreased plant water status, which leads to impaired photosynthesis (P_n) via reduced stomatal conductance to water vapor (g_s) and transpiration (E), according to Wang et al. (2018). Drought-tolerant varieties have been grown as one strategy to mitigate the effects of drought (Pinheiro et al., 2005). Understanding Robusta's drought tolerance requires studying its physiological responses, which could then be used to select coffee varieties for drought tolerance.

The drought tolerance of coffee might be influenced by its propagation methods. Coffee is propagated using several techniques, including seedling, cutting and grafting, according to Júnior et al. (2013) and Myers et al. (2020). These authors summarized some of the important factors associated with the different techniques. Seedling propagation produces a plant with a strong root system due to its taproot. However, the coffee plant takes a long time to grow and is easily genetically altered through cross-pollination. Cuttings are a popular and simple method; however, the seedlings have no taproots. The grafting method entails inserting the scion of a good coffee variety into a rootstock with a strong root system. As a result, a good coffee variety has a more tolerant root system to different environments. Coffee propagated through grafting did better than coffee propagated through cuttings (Júnior et al., 2013). Robusta coffee plants propagated using different methods could produce different morphological characteristics as well as different physiological mechanisms. Plant drought tolerance could be influenced by propagation methods (Kumar et al., 2017; Karunakaran and Ilango, 2019). Thus, suitable propagation methods might aid in the drought tolerance of Robusta coffee.

The physiological responses of different Robusta coffee varieties and their various propagation methods under water deficit conditions could be useful in the development of Robusta coffee varieties for drought tolerance. Furthermore, suitable propagation methods could be chosen to lead to the expansion of high-yielding coffee varieties to drought-prone areas, thereby increasing the production potential of Robusta coffee. This research aimed to determine the effect of water deficit and propagation methods on the physiological characteristics of Robusta coffee, as well as to investigate the relationship among the physiological characteristics of Robusta coffee.

Materials and Methods

Experimental design and treatments

From February 2017 to February 2018, the experiment was carried out at the Tropical Vegetable Research Development Center (TVRDC) at Kasetsart University's Kamphaeng Saen Campus in Nakhon Pathom, Thailand, under greenhouse conditions and a natural photoperiod. The experiment was conducted as a split-plot in a randomized complete block design (RCBD) with four replications. The main plot had two water regimes: the control conditions (CT) and the water deficit conditions (WD). The subplot was six types of Robusta coffee variety grown using different propagation methods.

Three varieties of Robusta coffee were used in this experiment, including FRT141 from Nestlé Agricultural Services in Chumphon province, Thailand. It has been proven to have a strong root system and to be a drought-tolerant variety (Roonprapant et al., 2021). In this experiment, FRT141 coffee seedlings grown from seeds were also used as rootstocks for grafting the SC05 and PP01 varieties, which are high-yielding and high-quality varieties widely cultivated in Thailand that were also used in the evaluation. Therefore, the six types of coffee plants consisted of: 1) FRT141-seedling; 2) FRT141-cutting; 3) SC05-grafting; 4) SC05-cutting; 5) PP01-grafting; and 6) PP01-cutting.

All coffee seedlings were grown in polyethylene bags containing 2 kg of soil mixed with coir, rice husk and manure (2:1:1:1). The seedlings were planted in the experimental greenhouse when they were aged 6 mth and were transplanted into plastic pots (43 cm in diameter) with a soil mixture of 3:1:1:1 soil, sand, rice husk and manure, respectively. The coffee plants were grown for another 10 mth to allow adaptation to the climate inside the greenhouse. The planting pots were placed so that the distances between the rows a d the plants in the rows were 1 m and 1 m, respectively. Coffee plants that were intact and consistent were chosen for the study when they were aged 16 mth. Drip irrigation was provided to control the amount of water supplied. Before beginning the experiment, the water use of the coffee plant was determined by weighing the water used by the plant each day. Under the control conditions, full water was provided throughout the experiment, representing 100% of the water used by the plants. When the experiment began in week one, the water deficit conditions were defined by limiting water intake to 75%, 50% and 25% for 2 wk each. Then, for another week, all plants received no water (0%). The age after water deficit included the time after the water level had been reduced until the eighth week of the experiment. Then, the water level was returned to normal (100%) and two weeks later, recovery crop data were recorded.

Meteorological conditions and soil moisture

The maximum photosynthetic photon flux density was in the range 120–522 μmol/m^2/s. during the experiment. The average air temperature was 25.9 °C (the maximum and minimum average temperatures were 31.1 °C and 24.9 °C, respectively) and the average relative humidity was 74.1% (the maximum and minimum average relative humidity were 96.9% and 52.2%, respectively, data not shown). A watermark soil moisture sensor (Spectrum Technologies Inc.; USA) was installed at a depth of 30 cm in the soil of the planting pot, in the center of the canopy between the plant and the edge of the pot. Every 15 min, all sensors connected to the data logger (Watchdog 1000 Series, Spectrum Technologies Inc.; USA) were recorded. Under the control conditions, the average soil water potential throughout the experiment was -3.83 ± 0.97 kPa. For the water deficit conditions, the average soil water potential decreased from -5.57± 0.41 kPa in week 1 to -1.34 ± 0.58 kPa, -5.96 ± 1.80 kPa, -9.50 ± 2.48 kPa, -11.24 ± 2.97 kPa, -34.32 ± 2.86 kPa, -48.81 ± 2.11 kPa and -123.49. ± 17.49 kPa in weeks 2–8, respectively. Following that, the irrigation was returned to the normal amount of water to observe recovery. At weeks 9 and 10, the soil water potential increased to -2.36 ± 1.13 kPa and -0.05 ± 0.02 kPa, respectively.

Measurement of plant water status

The plant water status was determined at two different times: predawn (pd) (0400−0600 hours) and midday (md) (1100−1300 hours). One coffee plant was selected at random from each treatment for each replicate to measure the change in leaf water status from the same plant each week throughout the experiment. Sampling selected the fully expanded leaves at the second or third leaf position from the apex branch in the middle of the plant's canopy. The method of Boyer (1995) was used to determine leaf water potential (Ψ) using a pressure chamber (model 3005; Soil Moisture Equipment Corp.; USA). Then, 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: RWC = (FW - DW) / (TW - DW).

Measurement of leaf gas exchange and SPAD index

For each replicate, one coffee plant was chosen at random from each treatment to measure the change in leaf gas exchange and soil-plant analyses development (SPAD) index (leaf greenness) from the same plant each week throughout the experiment. The fully expanded leaves were sampled at the second or third leaf from the apex branch in the middle of the canopy. 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) was used to measure the change in leaf gas exchange between 0830 hours and 1030 hours. The net photosynthetic rate (P_n) was determined under specific conditions as follows: photosynthetic photon flux density (PPFD) at 1,000 µmol/m^2/s (with 10% blue light), with the reference CO_2 concentration controlled at 400 µmolCO_2/m^2/s; leaf chamber temperature maintained between 28 and 33 °C; and relative humidity controlled to 75–85%. In addition to P_n data, the instrument provided stomatal conductance (g_s) and transpiration rate (E) data. The water use efficiency (WUE = P_n/E) of the leaves in each treatment was calculated. The simultaneous fluorescence measurement was used to quantify the light-adapted quantum efficiency of PSII (PSII) as follows: $\Phi PSII = (F_m\text{'}-F_s)/F_m\text{'}$, where F_m' is the maximum fluorescence obtained with a light-saturating point pulse and F_s is the steady-state fluorescence in the light (at 1,000 mol/m^2/s). ΦPSII denotes the number of electrons transferred per photon absorbed by PSII and the electron transport rate (ETR) was calculated as follows: ETR = ΦPSII × PPFD × 0.5 × 0.84. For C_3 plants, the constant 0.5 represents the fraction of excitation energy distributed to PSII, while 0.84 represents the fraction of incoming light absorbed by the leaves (Schreiber et al., 1998). The SPAD index was determined using a chlorophyll meter (model SPAD-502; Minolta Co. Ltd.; Japan) from the same sample that was used to evaluate the leaf gas exchange and was calculated by measuring three points on the leaf sheet and averaging the results.

Statistical analysis

Analysis of variance on physiological data from Robusta coffee was calculated using a split-plot in a RCBD design to study the effect of water deficit stress and genotype variability. The mean ± SE values were calculated for each Robusta coffee variety to compare the differences between propagation methods. Following that, analysis of variance was performed separately for the control and water deficit conditions to assess the response of the Robusta coffee variety to water deficit conditions. Mean comparisons were carried out using the least significant difference. Simple correlations were calculated among the physiological characteristics of Robusta coffee. The correlation of Robusta coffee under water deficit conditions was evaluated after stress and recovery at ages 8 wk and 10 wk, respectively, (n = 12).

Results

Effect of water deficit conditions on plant water status

The water potential of Robusta coffee leaves was investigated at two different times. The results showed that the total leaf water potential in the predawn (Ψ_{pd}) and midday (Ψ_{md}) had similar responses, but Ψ_{pd} was higher than Ψ_{md}. The Ψ_{pd} in the control and water deficit conditions were not significantly different at weeks 1–4 (Fig. 1A); however, when Robusta was exposed to water deficit at weeks 5–8, the Ψ_{pd} gradually decreased with values of -759, -1,056, -2,087 and -3,776 kPa, respectively. Robusta coffee for the water deficit conditions had a significantly lower Ψ_{pd} than for the control conditions. Robusta coffee was irrigated at a normal level again after the water restriction ended to assess the recovery. At week 10, Robusta coffee in the water deficit conditions increased Ψ_{pd} but this was not significantly different from the control conditions. Similarly, Ψ_{md} values were significantly different between the control and water deficit conditions from weeks 5–8 (Fig. 1B). For the water deficit conditions, the Ψ_{md} values of weeks 5–8 were -1,518, -2154, -2,790 and -4,250 kPa, respectively. Robusta coffee had significantly lower Ψ_{md} for the water deficit conditions than for the control conditions, even when the Robusta coffee was irrigated again at a normal level. Even at weeks 9 and 10, the $_{md}$ of Robusta coffee for the water deficit conditions increased but remained lower than Ψ_{md} for the control conditions.

From weeks 1 to 7, there was no significant difference in the leaf water content in the predawn (RWC_{pd}) values between Robusta coffee grown under control and water deficit conditions (Fig. 1C). At week 8, RWC_{pd} was reduced to 38.5% for the water deficit conditions, which was significantly lower than for the control conditions (87.7%). During recovery, in weeks 9 and 10, Robusta coffee for the water deficit conditions increased RWC_{pd} to the same level as the control conditions. Furthermore, for weeks 1–6, there was no significant difference in RWC_{md} between the control and water deficit conditions for Robusta coffee (Fig. 1D). However, at weeks 7 and 8, the RWC_{md} values for the water deficit conditions reduced to 68.4% and 32.6%, respectively, which were significantly different from the control conditions. During the recovery period (weeks 9 and 10), the RWC_{md} values for the water deficit Robusta coffee increased but were not significantly different to those of the control.

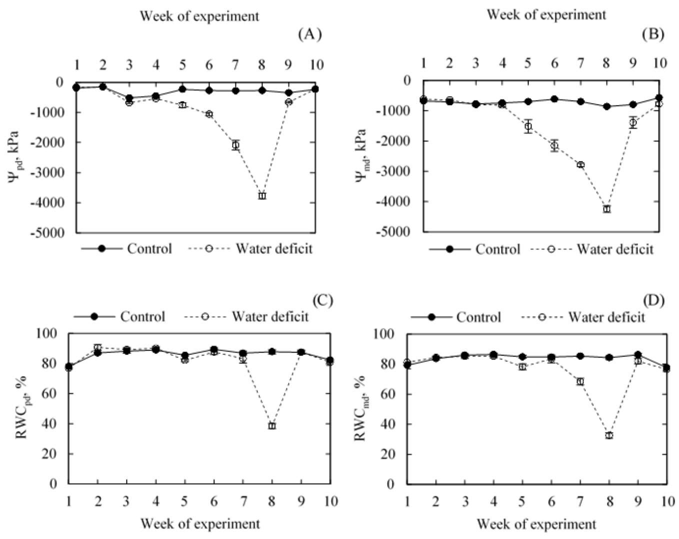

Fig. 1 Robusta coffee under control and water deficit conditions during weeks 1–10 of the experiment: (A) effect of water deficit conditions on leaf water potential at predawn (Ψ_{pd}); (B) effect of water deficit conditions on leaf water potential at midday (Ψ_{md}); (C) relative water content at predawn (RWC_{pd}); and (D) relative water content at midday (RWC_{md}), where error bars represent ± SE

Effect of water deficit on leaf gas exchange of Robusta coffee

The water deficit significantly reduced all gas exchange parameters compared to the control. During the 3 wk after the water deficit application, the P_n values of the Robusta coffee did not differ significantly between the control and water deficit conditions (Fig. 2A). When the water deficit in Robusta coffee was maintained until weeks 4–8, the P_n values gradually decreased to 2.24 µmol $CO_2/m^2/s$, 2.55 µmol $CO_2/m^2/s$, 3.28 µmol $CO_2/m^2/s$, 0.94 µmol $CO_2/m^2/s$ and -0.36 µmol $CO_2/m^2/s$, respectively, which differed from the control values. However, after the water deficit period ended, normal watering was resumed to observe recovery. At week 9, the P_n value of the stressed plants increased to 5.32 µmol $CO_2/m^2/s$, the same as the control. At week 10, the Robusta coffee with the water deficit conditions had a P_n value of 4.87 µmol $CO_2/m^2/s$, which was higher than for the control.

The g_s values of the Robusta coffee for the control and water deficit conditions were the same from week 1 to week 3 (Fig. 2B). However, from weeks 4–8, the g_s values of the stressed plants decreased to 32.54 mmol $H_2O/m^2/s$, 32.97 mmol $H_2O/m^2/s$, 20.97 mmol $H_2O/m^2/s$, 7.64 mmol $H_2O/m^2/s$ and 4.64 mmol $H_2O/m^2/s$, respectively. Furthermore, after resuming normal irrigation of the coffee plants the recovery, g_s values of the stressed plants increased significantly during weeks 9 and 10, with values of 96.00 mmol $H_2O/m^2/s$ and 54.91 mmol $H_2O/m^2/s$, respectively, which were higher than for the control conditions.

The transpiration rate (E) of the Robusta coffee did not differ between irrigation conditions from weeks 1 to 3 (Fig. 2C). However, from weeks 4 to 8, the Robusta coffee in the water deficit conditions gradually decreased with values of 0.50 mmol $H_2O/m^2/s$, 0.61 mmol $H_2O/m^2/s$, 0.32, 0.15 mmol $H_2O/m^2/s$ and 0.09 mmol $H_2O/m^2/s$, respectively, which differed from the control conditions. After the water limitation ended at week 9, the E value of stressed plant's (1.52 mmol $H_2O/m^2/s$) increased to be not significantly different from the control. At week 10, the Robusta coffee with the water deficit conditions had a higher E value than for the control conditions. This was consistent with a significant increase in g_s following recovery.

The WUE did not differ significantly between the control and water-stressed Robusta coffee plants from weeks 1 to 5 (Fig. 2D). The WUE increased by 12.18 µmolCO_2/mmolH_2O and 6.96 µmolCO_2/mmolH_2O, respectively, when irrigation was limited to weeks 6 and 7. At week 8, the WUE of the stressed plants was -4.21 µmol CO_2/mmol H_2O lower than for the control. However, after the water limitation was lifted, the WUE of the stressed plants increased to 4.13 µmolCO_2/mmolH_2O and 5.78 µmol CO_2/mmol H_2O, respectively, with no significant differences to the Robusta coffee under the control conditions.

There was no significant difference in the ΦPSII values between the control and water deficit conditions from week 1 to week 3 (Fig. 2E). However, when the Robusta coffee was water-stressed from weeks 4 to 8, the ΦPSII values of the stressed plants gradually decreased to 0.041, 0.064, 0.058, 0.053 and 0.022, respectively. After the water limitation ended, the stressed plant had a ΦPSII value of 0.093, which increased to no significant difference from the control, at week 9. The ΦPSII value increased to 0.107 at week 10, exceeding the control conditions.

In weeks 1 to 3, the ETR values of the Robusta coffee were the same in control and water deficit conditions (Fig. 2F). However, from weeks 4 to 8, the ETR values of the stressed coffee plants gradually declined to 17.2 µmol$e^-/m^2/s$, 27.2 µmol $e^-/m^2/s$, 24.7 µmol $e^-/m^2/s$, 22.4 µmol $e^-/m^2/s$ and 9.1 µmol $e^-/m^2/s$, respectively, which were lower than for the control conditions. The ETR value of the stressed plant was 39.4 µmol $e^-/m^2/s$ after the water limitation ended at week 9, increasing to no significant difference from the control.

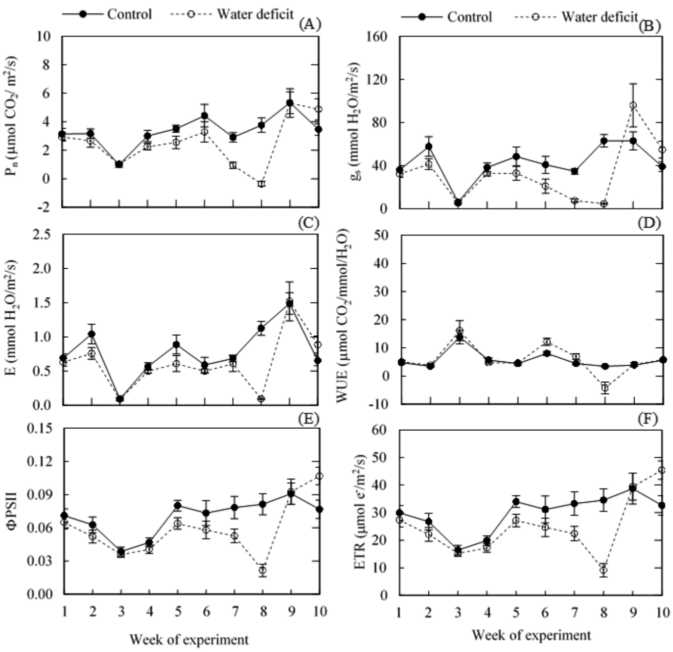

Fig. 2 Responses of Robusta coffee under control and water deficit conditions during weeks 1–10 of the experiment: (A) net photosynthetic rate (P_n); (B) stomatal conductance (g_s); (C) transpiration (E); (D) water use efficiency (WUE); (E) light-adapted quantum efficiency of PSII (ΦPSII); (F) electron transpiration rate (ETR), where error bars represent ± SE

Effect of propagation methods on leaf gas exchange of Robusta coffee

The leaf gas exchange of Robusta coffee was compared among the different propagation methods. Plants grown from FRT141 cuttings had higher E values than plants grown from seed under the control conditions (Fig. 3A). However, there were no significant differences in the E values between seedlings and cuttings every week after stress for the water-stressed conditions (Fig. 3B). However, after recovery, plants from cuttings had higher E values than plants from seedlings at week 9. Most E values for the SC05 variety under the control conditions did not differ significantly between grafting and cutting plants (Fig. 3C), except for weeks 6 and 8, plants from cuttings had higher E values than plants from grafting. The SC05 plants grown from cuttings had higher E values than those grown from grafts under the water-stressed conditions at weeks 5 and 6 (Fig. 3D). However, after recovery, plants from the cuttings had higher E values than plants from grafting for the PP01 variety under the control conditions at weeks 5 and 9 (Fig. 3E). Plants from grafting had the similar E values as cuttings almost every week under the water-stressed conditions (Fig. 3F). Furthermore, similar changes to the E values were found for the g_s values (data not shown). The results showed that plants propagated by cutting had higher rates of stomatal opening and transpiration than those grown from seedling of FRT141. Furthermore, in both the SC05 and PP01 varieties, plants propagated from cuttings had higher E and g_s values than the grafted plants.

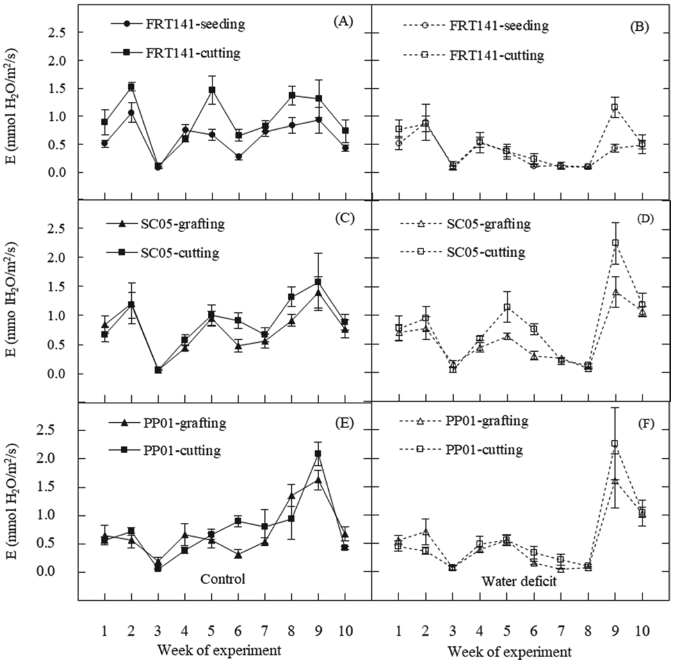

Fig. 3 Comparisons conditions during weeks 1–10 of experiment of: (A) FRT141 variety between seedling and cutting propagations under control conditions on transpiration; (B) and under water deficit conditions; (C) SC05 variety between grafting and cutting propagation under control conditions: (D) and under water deficit conditions; (E) PP01 variety between grafting and cutting propagation under control conditions; (F) and under water deficit conditions, where error bars represent ± SE

With the control conditions, there were no significant differences for most ΦPSII values between the seedlings and cuttings for the FRT141 variety (Fig. 4A), except for week 2, when the seedling plants had higher PSII values than the cuttings. Plants from seed had lower PSII values than from cuttings at weeks 6 and 8. During weeks 2 and 4 of mild water stress, the ΦPSII values of the seedling plant were higher than those of the cuttings for the water deficit conditions (Fig. 4B). However, when the water stress increased from weeks 5 to 8, there were no significant differences between the two propagation methods. In addition, during the recovery period in week 10, plants from the seedlings had higher ΦPSII values than for the cuttings. Under the control and water deficit conditions, most of the cutting plants for the SC05 variety had higher ΦPSII values than the grafted plants (Figs. 4C and 4D). Under the control conditions, most of the ΦPSII values for the PP01 variety did not differ significantly between plants from grafting and cutting (Fig. 4E), except for weeks 4 and 8, when plants from grafting had higher ΦPSII values than for cuttings. At 6 and 9 weeks, the grafted plants had lower ΦPSII values than the cuttings. There were no significant differences between plants from grafting and cuttings under the water deficit conditions at almost all ages (Fig. 4F), except for week 4, when the grafted plants had lower ΦPSII values than the cuttings. Similar changes in the ΦPSII values were observed for the ETR values (data not shown). The results revealed that seedling propagation of FRT141 and rootstock grafting in the SC05 and PP01 varieties did not significantly increase the ΦPSII and ETR of Robusta coffee compared to cuttings. In both the control and water deficit conditions, the values were lower than those from cuttings.

The P_n values for the FRT141 variety did not differ significantly between the seedling plants and cuttings at almost all ages for the control conditions (Fig. 5A), except for week 4, when plants from seed had higher P_n values than those from cuttings. P_n values for the water-stressed conditions did not differ between seedling and cutting plants (Fig. 5B), except for weeks 2 and 4, when seedling plants had higher P_n values than cuttings. Most of the SC05 cuttings had higher P_n values than the grafted plants under the control conditions (Fig. 5C). Under the water deficit conditions, most P_n values for the grafted plants were not significantly different from those for cutting plants (Fig. 5D). Grafted plants had lower P_n values than cuttings, except for weeks 6 and 9. Under the control conditions, the P_n values of the grafted plants for the PP01 variety were not significantly different from those of the cuttings (Fig. 5E). Except for weeks 4 and 8, the grafted plants had higher P_n values than the cuttings. The grafted plants had lower P_n values than the cuttings at week 6. The P_n values of the grafted plants were not significantly different from those of the cuttings for the water-stressed conditions (Fig. 5F). Except for week 2, the grafted plants had a higher P_n value than the cutting plants, while in week 7, the grafted plants had a lower P_n value than the cutting plants. The results showed that cutting propagation had higher P_n values than seedling plants for FRT141. Cutting propagation had higher P_n values than grafting for the SC05 and PP01 varieties under both the control and water deficit conditions. Furthermore, for the water-stressed conditions, the seedling plants had higher WUE values than the FRT141 cuttings (data not shown). However, the WUE values of PP01 cuttings were higher than those for grafting under severe water deficit.

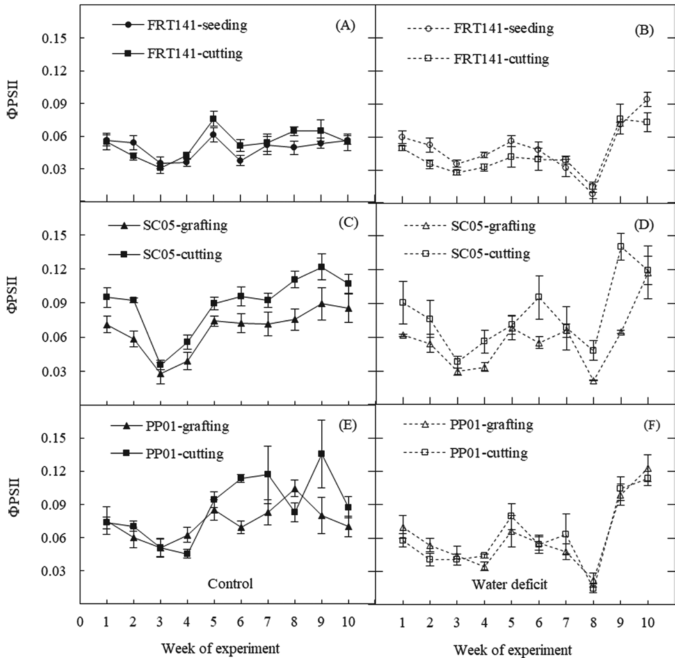

Fig. 4 Comparisons during weeks 1–10 of experiment of light-adapted quantum efficiency of PSII (ΦPSII) on: (A) FRT141 variety between seedling and cutting propagation under control conditions; (B) and water deficit conditions; (C) SC05 variety between grafting and cutting propagations under control conditions; (D) and water deficit conditions; (E) PP01 variety between grafting and cutting propagations under control conditions; (F) and water deficit conditions, where error bars represent ± SE

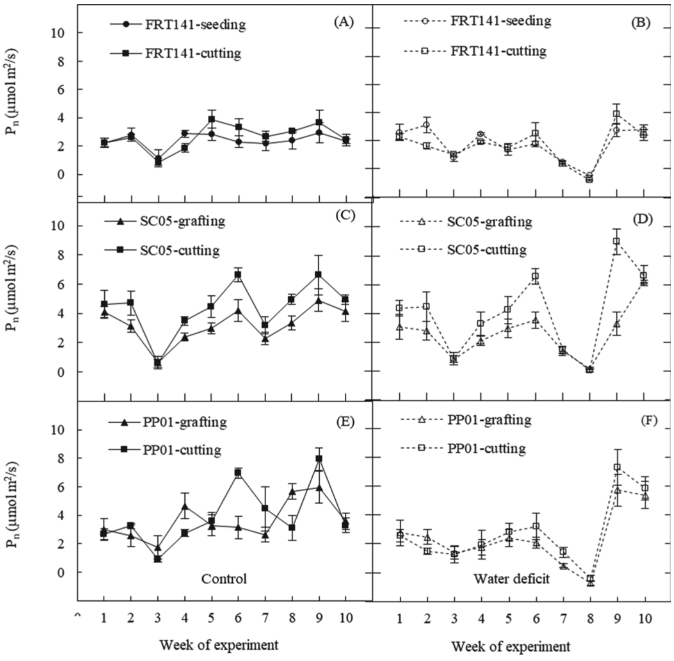

Fig. 5 Comparisons during weeks 1–10 of experiment of net photosynthetic rate (P_n) on: (A) FRT141 variety between seedling and cutting propagation under control conditions; (B) and under water deficit conditions; (C) SC05 variety between grafting and cutting propagation under control conditions; (D) and water deficit conditions; (E) PP01 variety between grafting and cutting propagations under control conditions; (F) and water deficit conditions where error bars represent ± SE

Comparison of physiological characteristics between Robusta coffee varieties

After 8 wk of water stress, the water deficit resulted in a significant reduction in all leaf gas exchange characteristics (Table 1). For both the control and water deficit conditions, there were significant differences in P_n, WUE and ETR between the Robusta coffee varieties; however, there were significant differences for ΦPSII among the varieties only under the control conditions. There were no significant differences in g_s and E between varieties for both the control and water deficit conditions. After recovery at week 10, the water-stressed Robusta coffee plants recovered well, with increases in E and WUE values that were not significantly different from those of the control, as well as significant increases in P_n, g_s, ΦPSII and ETR that were higher than for the control conditions. For both the control and water deficit conditions, there were significant differences in P_n, ΦPSII and ETR among the Robusta coffee varieties. Only for the control conditions did significant differences in g_s and E among the varieties emerge. However, there were no significant differences in WUE among the varieties for both the control and water deficit conditions.

The leaf gas exchange in Robusta coffee varied depending on the variety. After stress, the PP01 variety from grafting and the SC05 variety from cuttings had the highest leaf gas exchange under the control conditions (Table 1). However, for the water-stressed conditions, the SC05 variety (from both cuttings and grafting) had the highest leaf gas exchange. After recovery, the SC05 variety from cuttings and grafting had the highest leaf gas exchange under the control conditions. The SC05 and PP01 varieties from cuttings and grafting had the highest leaf gas exchange levels for the water-stressed conditions. Furthermore, the comparison of leaf gas exchange between varieties revealed that the PP01 and SC05 varieties had higher leaf gas exchange than FRT141, which was drought tolerant under both the control and water deficit conditions, perhaps because of the FRT141 variety's ability to tolerate drought via leaf gas exchange not being expressed during the early growth stage.

Water deficit did not affect the SPAD index of Robusta coffee both after the stress and after the recovery stages (Table 2). However, there were significant differences in the SPAD index values among the Robusta coffee varieties for both the control and water deficit conditions, both after stress and after recovery. After stress, the SC05-cutting had the highest SPAD index for both the control and water deficit conditions, with values of 46.5 and 43.4, respectively. SC05-cutting and

Table 1 Net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE), light-adapted quantum efficiency of PSII (ΦPSII) and electron transpiration rate (ETR) of Robusta coffee varieties under control (CT) and water deficit (WD) conditions at weeks 8 (after stress) and 10 (after recovery) of experiment

Variety	After stress											
	P_n		g_s		E		WUE		ΦPSII		ETR	
	(µmol $CO_2/m^2/s$)		(mmol $H_2O/m^2/s$)		(mmol $H_2O/m^2/s$)		(µmolCO_2 /mmolH_2O)				(µmol $e^-/m^2/s$)	
	CT	WD	CT	WD	CT	WD	CT	WD	CT	WD	CT	WD
FRT141-seedling	2.43 ± 1.07^{c}	-0.48 ± 0.01^{b}	46.45±15.93	4.31±0.97	0.84±0.24	0.09±0.02	2.77 ± 0.55^{bc}	$6.52\pm1.91b^{c}$	0.050 ± 0.011^{d}	0.009±0.005	21.00 ± 4.61^{d}	3.70 ± 2.27^{b}
FRT141-cutting	3.07 ± 0.20^{c}	-0.81 ± 0.08^{b}	79.68±16.86	4.84±0.59	1.37±0.29	0.09±0.02	2.35 ± 0.53^{c}	-8.90 ± 0.68^{c}	0.065 ± 0.007^{cd}	0.015±0.005	27.50 ± 2.90^{cd}	6.20 ± 2.31^{b}
SC05-grafting	3.36 ± 0.84^{bc}	0.21 ± 0.12^{a}	51.68±8.69	3.50±0.57	0.92±0.18	0.07±0.01	3.63 ± 0.25^{ab}	2.48 ± 1.32^{a}	0.076 ± 0.016^{c}	0.022±0.000	32.00 ± 6.96^{c}	9.00 ± 0.08^{b}
SC05-cutting	4.97 ± 0.62^{ab}	0.05 ± 0.19^{a}	72.93±15.86	6.15±2.20	1.32±0.29	0.13±0.04	3.89 ± 0.65^{a}	0.92 ± 1.45^{a}	0.111 ± 0.013^{a}	0.048±0.015	47.10 ± 5.48^{a}	20.40 ± 6.51^{a}
PP01-grafting	5.67 ± 0.94^{a}	-0.73±0.25b	74.65±19.12	3.69±1.17	1.36±0.33	0.08±0.03	4.28 ± 0.46^{a}	-9.59 ± 2.02^{c}	0.105 ± 0.013^{ab}	0.022±0.008	44.50 ± 5.34^{ab}	9.30 ± 6.31^{b}
PP01-cutting	3.11 ± 1.55^{c}	-0.39 ± 0.37^{b}	53±34.91	5.37±1.48	0.94±0.62	0.11±0.03	3.98 ± 1.57^{a}	-3.63 ± 4.32^{b}	0.083 ± 0.015^{bc}	0.015±0.006	35.10 ± 6.30^{bc}	6.20 ± 2.65^{b}
F-test	**	**	ns	ns	ns	ns	*	*	**	ns	**	**
Mean	3.77 ± 1.50^{A}	-0.36 ± 0.43^{B}	63.06 ± 24.03^{A}	4.64 ± 1.60^{B}	1.12 ± 0.42^{A}	0.09 ± 0.03^{B}	3.48 ± 1.05^{A}	-4.21 ± 2.14^{B}	0.081 ± 0.025^{A}	0.022 ± 0.015^{B}	34.50 ± 10.59^{A}	9.10 ± 6.38^{B}
	After recovery											
FRT141-seedling	2.34 ± 0.26^{c}	2.78 ± 0.36^{b}	25.68±6.00	28.58 ± 7.09^{b}	0.43±0.09	0.49 ± 0.14^{b}	5.76±1.73	6.35±2.90	0.056 ± 0.007^{c}	0.094 ± 0.011^{ab}	23.90 ± 3.03^{c}	40.00 ± 4.94^{ab}
FRT141-cutting	2.45 ± 0.72^{c}	2.38 ± 0.68^{b}	45.13±22.57	31.88 ± 20.52^{b}	0.73±0.36	0.50 ± 0.30^{b}	4.12±1.70	5.86±1.33	0.055 ± 0.013^{c}	0.074 ± 0.015^{b}	23.20 ± 5.49^{c}	31.20 ± 6.28^{b}
SC05-grafting	4.15 ± 1.18^{ab}	6.23 ± 0.04^{a}	45.60±17.03	66.55 ± 1.43^{ab}	0.77±0.27	1.07 ± 0.06^{a}	5.56±0.91	5.73±0.38	0.086 ± 0.022^{ab}	0.118 ± 0.019^{a}	36.50 ± 9.18^{ab}	50.10 ± 6.98^{a}
SC05-cutting	4.94 ± 0.57^{a}	6.62 ± 1.23^{a}	50.95±11.38	72.88 ± 16.90^{a}	0.88±0.23	1.19 ± 0.34^{a}	5.83±0.99	5.71±0.54	0.107 ± 0.015^{a}	0.120 ± 0.022^{a}	45.40 ± 3.36^{a}	50.70 ± 9.18^{a}
PP01-grafting	3.57 ± 0.97^{abc}	5.39 ± 1.14^{a}	40.68±12.90	63.00 ± 15.98^{ab}	0.68±0.22	1.03 ± 0.28^{a}	5.40±0.69	5.53±1.65	0.070 ± 0.016^{bc}	0.123 ± 0.015^{a}	29.80 ± 6.91^{bc}	52.20 ± 6.24^{a}
PP01-cutting	$3.29\pm0.66b^{c}$	5.85 ± 1.37^{a}	25.73±4.36	66.58 ± 17.13^{a}	0.43±0.03	1.05 ± 0.16^{a}	7.61±1.34	5.53±0.54	0.087 ± 0.012^{ab}	0.114 ± 0.011^{a}	37.10 ± 4.97^{ab}	48.20 ± 4.48^{a}
F-test	*	**	ns	*	ns	*	ns	ns	**	*	**	*
Mean	3.46 ± 1.20^{B}	4.87 ± 1.93^{A}	38.96 ± 16.99^{B}	54.91 ± 23.43^{A}	0.65±0.28	0.89±0.37	5.71±1.64	5.78±1.53	0.077 ± 0.024^{B}	0.107 ± 0.024^{A}	32.60 ± 10.07^{B}	45.40 ± 9.93^{A}

Mean ± SD superscripted with different capital letters denote significant ($p < 0.05$) differences between two water conditions;Mean± SD in the same column superscripted with different lowercase letters denote significant ($p < 0.05$) differences among the coffee varieties under same conditions; ns = non-significant at $p < 0.05$; * = significant at $p < 0.05$; ** = highly significant at $p < 0.01$

Table 2 Soil-plant analyses development index (SPAD) of Robusta coffee varieties under control (CT) and water deficit (WD) conditions at weeks 8 (after stress) and 10 (after recovery) of experiment

Variety	SPAD index			
	After stress		After recovery	
	CT	WD	CT	WD
FRT141-seedling	27.00 ± 3.12^{cd}	26.60 ± 3.98^{b}	30.30 ± 8.09^{b}	32.30 ± 3.31^{bc}
FRT141-cutting	23.40 ± 6.00^{d}	27.10 ± 8.77^{b}	30.20 ± 6.43^{b}	28.00 ± 3.24^{c}
SC05-grafting	34.60 ± 4.27^{bc}	30.70 ± 5.66^{b}	37.30 ± 0.91^{ab}	45.20 ± 4.84^{a}
SC05-cutting	46.50 ± 5.00^{a}	43.40 ± 5.33^{a}	46.00 ± 4.04^{a}	50.00 ± 8.33^{a}
PP01-grafting	35.20 ± 2.00^{b}	31.30 ± 4.30^{b}	43.70 ± 6.48^{a}	41.90 ± 6.55^{ab}
PP01-cutting	35.00 ± 4.56^{b}	32.20 ± 8.31^{b}	38.30 ± 7.21^{ab}	41.00 ± 3.99^{ab}
F-test	**	*	**	**
Mean	33.60±8.49	31.90±8.42	37.60±8.50	39.70±9.21

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different; * = significant at $p < 0.05$; ** = highly significant at $p < 0.01$

PP01-grafting had the highest SPAD index values after recovery for the control conditions, with 46.0 and 43.7, respectively. Furthermore, the SC05 variety from cuttings and grafting had the highest SPAD index values of 50.0 and 45.2, respectively, for water-stressed conditions. Although there was no significant effect of water deficit on the SPAD index, the results revealed that there was variability between the Robusta coffee varieties, with the SC05 variety having the highest SPAD index.

Relationship among the physiological characteristics of Robusta coffee

The physiological characteristics of Robusta coffee were correlated under water deficit conditions after stress at week 8 and recovery at week 10 ($n = 12$). A high positive correlation was found between P_n, g_s, E, WUE, ΦPSII and ETR (r = 0.74** to 1.00**) (Table 3). The findings revealed that under water stress conditions, photosynthesis of Robusta coffee was related to stomatal control and transpiration, including ΦPSII photochemical efficiency, as indicated by the ΦPSII and ETR. A strong positive correlation was also found between leaf water status characteristics such as Ψ_{pd}, $_{md}$, RWC_{pd} and RWC_{md} (r = 0.98** to 1.00**). Moreover, there was a strong positive correlation (r = 0.86** to 0.93**) between the leaf water status and the leaf gas exchange, indicating that P_n, g_s, E, WUE, ΦPSII and ETR were related to the leaf water status of Robusta coffee. Furthermore, a significant correlation was observed between the SPAD index and P_n, g_s, E, ΦPSII and ETR (r = 0.66* to 0.68*), indicating that the SPAD index could be used to evaluate these traits in Robusta coffee. However, there was no correlation between the SPAD index with WUE and the leaf water status of Robusta coffee.

Table 3 Correlation coefficients (r) among net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE), light-adapted quantum efficiency of PSII (ΦPSII) and electron transpiration rate (ETR), soil-plant analyses development index (SPAD), leaf water potential at predawn ($\Psi_{\mathbf{pd}}$), leaf water potential at midday ($\Psi_{\mathbf{md}}$), relative water content at predawn ($RWC_{\mathbf{pd}}$) and relative water content at midday (RWC_{md}) of Robusta coffee varieties under water deficit conditions across 8 (after stress) and 10 (after recovery) weeks of experiment ($n = 12$)

	P_n	g_s	E	WUE	ΦPSII	ETR	SPAD	Ψ_{pd}	Ψ_{md}	RWC_{pd}
g_s	0.99**									
E	0.99**	1.00**								
WUE	0.80**	0.74**	0.74**							
ΦPSII	0.97**	0.95**	0.96**	0.84**						
ETR	0.97**	0.95**	0.96**	0.84**	0.98**					
SPAD	0.68*	0.66*	0.67*	0.54^{ns}	0.68*	0.68*				
Ψ_{pd}	0.90**	0.89**	0.89**	0.83**	0.94**	0.94**	0.42			
Ψ_{md}	0.92**	0.91**	0.90**	0.84**	0.94**	0.94**	0.43	1.00**		
RWC_{pd}	0.90**	0.88**	0.88**	0.86**	0.92**	0.93**	0.46	0.98**	0.98**	
RWC_{md}	0.91**	0.88**	0.88**	0.89**	0.95**	0.95**	0.49	0.99**	0.98**	0.98**

* = significant at $p < 0.05$; ** = highly significant at $p < 0.01$; ns = non-significant ($p \geq 0.05$)

Discussion

Water deficit was observed in Robusta coffee plants during both the predawn and midday periods due to persistent water deficit. This study suggested that water deficit could reduce the leaf gas exchange of Robusta coffee. During water deficiency, the photosynthesis rate of Robusta coffee reduced due to the plant's stomata closing, which was the first mechanism in response to the water deficit. Stomatal conductance (g_s) could explain plant stomatal closure, with low values indicating that stomata close, limiting carbon dioxide and water flow. As a result, the net photosynthesis rate and transpiration of plants decrease (Cornic, 2000). In general, coffee has a low g_s (<100 mmol $H_2O/m^2/s$) for normal conditions (Batista et al., 2012; Chaves et al., 2012) and stomata close quickly when exposed to drought conditions (DaMatta et al., 2010). According to the current findings, the g_s of Robusta coffee began stomata closure within weeks of the onset of water deficit and decreased with the severity of the water deficit. This finding was shown to be highly active in the response of Robusta coffee stomata to water deficit stress, with g_s recovering rapidly after water stress. This was consistent with the findings of Pinheiro (2005), who reported a rapid recovery in the leaf water status of Robusta coffee after re-irrigation following drought conditions. Stomata play an important role in water regulation in plants (Wang et al., 2018) and when Robusta coffee was re-watered after stress conditions, the opening of stomata was greater than when the Robusta coffee was fully watered under the control.

The stomatal response is a plant control mechanism to control plant water loss and maintain plant water balance. As a result, the rate of transpiration of Robusta coffee is reduced under water loss ocnditions (DaMatta and Ramalho, 2006). In addition, drought stress increased the WUE values of the Robusta with mild water deficits because early stomatal closure due to the water deficit had a greater inhibitory effect on transpiration than carbon dioxide diffusion into leaf tissues (Chaves et al., 2009; Sikuku et al., 2010). However, under severe drought stress, Robusta coffee was reported to have lower WUE values because mesophyll cells lose more water, inhibiting photosynthesis and resulting in a decrease in the WUE (Damayanthi et al., 2010). Severe drought stress reduces mesophyll cell efficiency in utilizing available carbon dioxide (Dias and Brüggemann, 2010). In addition to stomatal inhibition, which reduced photosynthesis in severe drought conditions, a significant decrease in ΦPSII was another cause of photosynthesis reduction by nonstomatal inhibition, particularly with severe drought stress (Farooq et al., 2009; Zhou et al., 2013). Furthermore, ETR was another characteristic that demonstrated the effect of water deficit on damage in the ΦPSII photochemical efficiency of plant leaves. The current study discovered that water deficit affected the ETR of Robusta coffee. This was consistent with the findings of Tounekti et al. (2018), who reported the effect of water deficit on the reduction of ETR in Arabica coffee. However, after recovery, the Robusta coffee plants restored ΦPSII and ETR to control values within a week of re-irrigation, which was in parallel with P_n. These results indicated that the photosynthetic apparatus of Robusta coffee plants had completely recovered. Therefore, water stress treatment for 8 wk did not cause permanent damage to leaf gas exchange in stressed plants.

In addition, the current research revealed that different propagation methods resulted in different physiological characteristics of Robusta coffee, even from the same variety. The rootstock used for grafting resulted in a different response by the Robusta coffee plants. Other research indicated a method for inserting the top of Arabica coffee onto the rootstock of Robusta coffee. It was discovered that this technique increased the photosynthetic efficiency of Arabica coffee (Novaes et al., 2011). In addition, grafting the top of Robusta or Arabica coffee onto the rootstock of Liberica coffee (*Coffea liberica*) was reported to increase nematode resistance and increase the growth and yield of coffee plants in nematode-infested areas (Myers et al., 2020; Pham et al., 2020). Under both the control and water deficit conditions, the current research found that Robusta coffee plants grown from cuttings had higher rates of stomatal opening and transpiration than those grown by grafting. To reduce water loss before the water deficit became severe stress, the stomata remained closed on the grafted plants. This response could be related to the control of stomatal closure caused by increased abscisic acid content in the leaves of the grafted rootstock being transported from the roots through the xylem when the coffee plant was stressed during water deficit (Silva et al., 2018). In addition, the current study discovered that grafted plants had higher leaf water potential than those obtained from cuttings during a severe water deficit (data not shown). Furthermore, Robusta coffee plants from cuttings had a high g_s value under control conditions, resulting in high P_n, ΦPSII and ETR values (data not shown). Consequently, for water deficit conditions, plants grown from cuttings had higher photosynthetic efficiency than plants grown from seedlings and grafting.

However, the current experiment investigated drought effects on coffee physiology on plants grown in containers. It is probable that the growth in the pots meant that the taproots from the seedling plants and the rootstock of the grafted plants could not take advantage of having a deeper root system such as the fibrous roots from cutting plants. Furthermore, the mechanism of drought tolerance depends not only on the root responses; thus, coffee plants may use a combination of mechanisms to delay dehydration and to minimize drought effects while growing in water deficit conditions (Mariga et al., 2016). Burkhardt et al. (2006) postulated various strategies for drought tolerance among wild coffee populations growing in Ethiopia. They observed that coffee plants with extensive root systems were still vulnerable to drought due to their hydraulic system and stomatal behavior.

The relationships between the physiological characteristics of Robusta coffee under water deficit conditions indicated that factors affecting photosynthesis rate under drought conditions were associated with stomatal inhibition in combination with nonstomatal inhibition, particularly with severe drought stress (Farooq et al., 2009; Zhou et al., 2013). The highly positive correlation between g_s and E demonstrated that stomata play a significant role in water regulation in plants (Silva et al., 2013). The ability of plants to maintain water was associated with the photosynthetic capacity of the Robusta coffee plants under drought conditions. Many perennial plants have a relationship between the rate of photosynthesis and leaf water status (Jones et al., 2010; Poorter et al., 2010). According to our research (data did not shown) the SPAD index could also indicate the photosynthetic potential and ΦPSII photochemical efficiency of Robusta coffee leaves. The current study discovered a relationship between the SPAD index and P_n, g_s, E, ΦPSII and ETR, indicating that the SPAD index could be used to evaluate these traits in Robusta coffee.

In summary, water deficit reduced the leaf water status and leaf gas exchange characteristics, such as P_n, g_s, E, WUE, ΦPSII and ETR in Robusta coffee, but did not affect the SPAD index. Despite being the same variety, the physiological responses of Robusta coffee plants differed due to the different propagation methods. Cutting-grown Robusta coffee plants had higher leaf gas exchange than seeded and grafted plants. The physiological characteristics of Robusta coffee varied depending on the variety. With water-stressed conditions, the SC05 variety had the highest leaf gas exchange, with both cuttings and grafted plants. The leaf gas exchange characteristics of Robusta coffee were related to the leaf water status. A relationship was also discovered between the SPAD index and P_n, g_s, E, ΦPSII and ETR in Robusta coffee. This finding suggested that the SPAD index could be used to evaluate the leaf gas exchange characteristics of Robusta coffee. However, for future research, it is recommended to undertake detailed physiological investigations under a variety of field conditions over a longer period of time.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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