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Research article

# Morphological and physiological responses to water deficit stress conditions of robusta coffee (*Coffea canephora*) genotypes in Thailand

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**Abstract**

Drought causes crop yield losses and inhibits growth in coffee plants. The morphophysiological responses were investigated of Thai robusta coffee genotypes under water deficit. The seedlings of six robusta coffee genotypes, five local Thai genotypes (SC05, PP01, PP05, SKE06 and TPO17) and a water deficit-tolerant genotype (FRT141) were evaluated under a hydroponics system that simulated water deficit stress using polyethylene glycol (PEG). Morphophysiological parameters were collected 2 wk after stress. The water deficit significantly reduced the mean (± SD) relative water content from 95.3 ± 3.0% in the control to 67.2 ± 6.9% in the stress condition. The mean total leaf water potential was -1.4 ± 0.4 MPa and -0.2 ± 0.1 MPa for the stressed and control plants, respectively. The stressed plants showed significantly decreased stomatal conductance, along with a lower transpiration rate (70–90%) and photochemical efficiency of photosystem II (PSII; 50–80%), which directly affected the net rate of photosynthesis that was almost zero. The number of leaves, total leaf area and total biomass greatly declined after stress. There were significant differences among genotypes. FRT141 had the highest mean number of leaves (29.8 ± 9.0 leaves/plant), total leaf area (912 ± 90 $cm^2$/plant) and total biomass (10.7 ± 2.4 g/plant) under water deficit, indicating that FRT141 was the most tolerant genotype under stress, followed by PP01 and SC05, respectively, while SKE06 and TPO17 were the most sensitive genotypes, respectively, with mean values of 1.3 ± 1.0–2.0 ± 2.0 leaves/plant, 34 ± 28–50 ± 45 $cm^2$/plant and 4.4 ± 2.6–3.1±1.2 g/plant. Furthermore, the tolerant genotypes showed fewer physiological changes, specifically less reduction in the light-adapted quantum efficiency of PSII, electron transport rate and maximum quantum efficiency and less increase in electrolyte leakage in root and leaf cells.

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## Introduction

Robusta coffee (*Coffea canephora*) is a coffee species that is economically important worldwide, accounting for approximately 20% of the total coffee trade on the global market. Over 70% of coffee worldwide is produced by small-scale family farms and largely grown under rainfed conditions, where irrigation is an exception (Rossing et al., 2014; DaMatta et al., 2018). Therefore, such activity can be severely affected by alterations in annual rainfall patterns and quantity. Drought has caused serious decreases in robusta coffee yields of approximately 50% in coffee production areas in Brazil and in particular in Vietnam, the 2nd largest producer worldwide (Nguyen and Nguyen, 2018; Semedo et al., 2018). In addition, Southeast Asia has experienced drought that has impacted its coffee production. In the past five years, robusta coffee production in Thailand has been in continuous decline, with reductions in planted areas and yield of 50 and 30%, respectively (Office of Agricultural Economics, 2020). In contrast, demand for coffee beans in the industry has increased. One of the most effective ways to relieve drought stress and improve robusta coffee yield and quality under water deficit is to select and improve drought-tolerant coffee genotypes that are capable of producing acceptable yields under water scarcity (Silva et al., 2013). At present, there is limited information on the existing variability among the coffee genotypes in this area with regard to traits associated with drought tolerance and coping with water deficit, even though drought stress has become increasingly serious in most coffee-growing areas. Hence, information regarding water deficit responses of Thai robusta coffee genotypes is important for improving and selecting drought-tolerant robusta coffee genotypes for this area. Many studies have investigated whether water deficit induces changes in some plant morphological characteristics, such as limiting the plant height, leaf area and stem diameter and causing a low accumulation of biomass (Farooq et al., 2009). In addition, changes in physiological features can occur; for example, reduced plant water status can lead to impaired photosynthesis ($P_n$) through reduced stomatal conductance to water vapor ($g_s$) and transpiration (E) (Wang et al., 2018). Nonetheless, the reduction in $P_n$ during a period of water deficit is also caused by nonstomatal factors related to damage to the photosynthetic apparatus (Petridis et al., 2012). An important parameter of the physiological state of the photosynthetic apparatus is the maximum quantum efficiency ($F_v/F_m$), which is indicative of the potential or maximum quantum yield of Photosystem II (PSII; Ahmadizadeh, 2013). In addition, biochemical constraints may directly affect cell membrane injury caused by stress and these effects can be assessed using the electrolyte leakage technique (Yang et al., 2009). Generally, drought-tolerant coffee plants have been characterized by an adequate water status achieved via a combination of deep rooting and stomatal control of access of $CO_2$ to carboxylation sites in conjunction with transpiration and the leaf area, including carbon assimilation for biomass partitioning (DaMatta et al., 2003; Pinheiro et al., 2005; Silva et al., 2013). Previously, nutrient solution culture experimental systems with different concentrations of polyethylene glycol (PEG) have been used to induce plant water deficit with an exactly defined level of water availability to plants, which is one of the effective methods for studying some plant responses to water deficit, such as in eucalyptus (Utkhao and Yingjajaval, 2015), apple (Kautz et al., 2015), pistachio (Esmaeilpour et al., 2016) and citrus (Zaher-Ara et al., 2016). However, there are few studies about the use of PEG in coffee plants under water deficit conditions, with regard to selected well-established morphological and physiological parameters.

Thus, the objectives of this study were to study the effect of drought stress on morphological and physiological responses, to investigate the variation responses of Thai robusta coffee under different water stresses and to identify water-deficit tolerant genotypes of robusta coffee under simulated water deficit stress conditions using nutrient solution culture.

## Material and Methods

### *Experimental conditions and plant materials*

The experiment was conducted at the Tropical Vegetable Research Development Center (TVRDC) at Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand, under greenhouse conditions with a natural photoperiod, during September to October 2018. The experiment was arranged as a split plot in a randomized complete block design with four replications. The main plot consisted of two water regimes: the control condition (CT) and the water deficit condition (WD). The subplots consisted of six genotypes of robusta coffee, with five being SC05, PP01, PP05, SKE06 and TPO17 that are widely grown in Thailand and the sixth was FRT141, which is a drought-tolerant genotype provided by Nestlé Agricultural Services in Chumphon province, Thailand. All coffee seedlings from rooted stem cuttings were grown in polyethylene bags containing 2 kg of soil mix with coir, rice husk and cow manure (2:1:1:1). When the seedlings were

aged 6 mth, they were transplanted to a hydroponic system with half-strength Hoagland solution at pH 5.8 and an air pump. There were 16 plants in each genotype and treatment used in this experiment. The levels of the nutrient solution and pH were maintained daily by adding nutrient solution and pH adjustment by HCl. The plants were allowed to adjust to the hydroponic conditions for 2 wk before the water deficit treatments commenced. The control condition was half-strength Hoagland nutrient solution throughout the experiment (osmotic potential, $\Psi_{sol}$ = -0.035 MPa). Water deficit conditions were imposed using polyethylene glycol (PEG6000) (BASF Co. Ltd., Germany) to generate two levels of water deficit stress. During the first week, the solution was 5% PEG ($\Psi_{sol}$ = -0.07 MPa), and during the second week, the concentration was increased to 9% PEG ($\Psi_{sol}$ = -0.14 MPa), which was applied in stepwise succession. Similar PEG solutions have previously been used as a means for inducing drought stress in Eucalyptus, as expressed in several plant responses, including osmotic adjustment (Utkhao and Yingjajaval, 2015).

*Meteorological conditions and measurement of plant water status*

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

The plant water status was determined predawn (0400–0530 hours) in the control and stress treatments using the first fully expanded leaves of four different plants per genotype. The total leaf water potential ($\Psi_t$) was measured using a pressure chamber (model 3005; Soil Moisture Equipment Corp.; USA) following the recommendations of Turner (1981). The leaves were covered with a plastic sheath immediately prior to excision until the completion of the measurement to prevent transpiration. Afterward, the leaf samples were used to evaluate the relative water content (RWC). The leaf fresh weight (FW), turgid weight (TW) with full turgidity of the leaves and the dry weight (DW) were measured and calculated using Equation 1 (Barrs and Weatherley, 1962):

$$RWC = (FW - DW) / (TW - DW) \quad (1)$$

*Measurements of leaf gas exchange and chlorophyll fluorescence*

Fully expanded leaves (third to fourth leaves from the top of the plant) were selected for leaf gas exchange and chlorophyll fluorescence measurement. Four leaves, including one from each of four plants per treatment, were evaluated during 0830–1030 hours using a portable infrared gas analyzer (LI-6400; Licor Inc.; USA) equipped with a broad leaf 2.0 $cm^2$ fluorometer chamber (LI-6400-40, Licor Inc.; USA). The net photosynthetic rate ($P_n$) was determined under specific conditions as follows: PPFD at 1,000 µmol/$m^2$/s (with 10% blue light), with the reference $CO_2$ concentration controlled at 400 µmol $CO_2$/$m^2$/s, leaf chamber temperature maintained in the range 28–33°C and RH controlled to 75–85%. In addition to providing $P_n$ data, the instrument recorded the stomatal conductance ($g_s$), transpiration rate (E) and intercellular $CO_2$ concentration ($C_i$). The water use efficiency (WUE = $P_n$/E) of the leaves was calculated for each treatment. The light-adapted quantum efficiency of PSII (ΦPSII) was quantified using simultaneous fluorescence measurement defined by Genty et al. (1989) based on Equation 2:

$$\Phi PSII = (F_m' - F_s) / F_m' \quad (2)$$

where $F_s$ is the steady-state fluorescence in the light (at 1000 µmol/$m^2$/s), $F_m'$ is the maximum fluorescence obtained with a light-saturating point pulse, ΦPSII represents the number of electrons transferred per photon absorbed by PSII. The electron transport rate (ETR) was calculated based on Equation 3:

$$ETR = \Phi PSII \times PPFD \times 0.5 \times 0.84 \quad (3)$$

where the constant 0.5 is the fraction of excitation energy distributed to PSII for $C_3$ plants, and 0.84 is the fraction of incoming light absorbed by the leaves (Schreiber et al., 1998). The maximum quantum efficiency ($F_v/F_m$) was determined on the same leaf using a pulse amplitude-modulated fluorometer (PAM-2100; Heinz Walz; Germany). Measurements were conducted during 0400–0530 hours in the dark. $F_v/F_m$ was calculated based on Equation 4:

$$F_v/F_m = (F_m - F_o)/F_m \quad (4)$$

where $F_o$ is the minimum and $F_m$ is the maximum variable fluorescence.

*Electrolyte leakage*

Fully expanded leaves and roots were collected after the water deficit period. The leaves were immediately cut into 10 leaf discs of 6 mm diameter using a sharp cork borer; apical root segment samples were weighed (2.0 g), rinsed with distilled water to remove surface ions and put into a 10 mL tube with distilled water. The tubes were transferred to a water bath at 45°C for 2 hr and the initial electrical conductivity of the medium ($EC_1$) was measured using a conductivity meter (Horiba Laquatwin Conductivity Meter EC-22; HORIBA; Japan). The tubes were kept in a boiling water bath at 100°C for 20 min to release all the electrolytes and cooled at ambient temperature, before the final electrical conductivity ($EC_2$) of each tube was measured. The percentage of electrolyte leakage was evaluated based on Equation 5:

$$\%EL = (EC_1 / EC_2) \times 100 \quad (5)$$

*Morphological and biomass measurement*

At 2 wk after the initiation of water deficit treatments, the stem diameter, plant height and number of leaves were measured. Then, the leaves, stems and roots were separated. The total leaf area was measured using an area meter (LI-3100; Licor Inc.; USA). The dry masses of the leaves, stems and roots were obtained after oven drying the tissues at 75°C for 72 hr.

*Statistical Analysis*

Analysis of variance was performed based on the split-plot design to test the significance of the effects of the water stress condition and then analysis of variance was performed using a randomized complete block design to test the significance of the various responses of stress among the coffee genotypes grown under the same water conditions. The means were calculated using the least significant difference with the R software (R-language and environment for statistical computing and graphics; version 3.4.2; Vienna; Austria). Values were tested for significance at the $p < 0.05$ level. The percentage decrease or increase was evaluated based on Equation 6:

$$\%Change = [(CT_{value} - WD_{value}) / CT_{value}] \times 100\% \quad (6)$$

**Results**

*Meteorological conditions and changes in plant water status*

During the experimental period, the maximum photosynthetic photon flux density was 434–867 µmol/m$^2$/s, the average air temperature was 29.2°C (maximum and minimum average temperatures were 37.0°C and 24.9°C, respectively) and the average RH was 82.9% (maximum and minimum average values were 95.9% and 64.2%, respectively; data not shown). The plant water status parameters in the coffee leaves are presented in Fig. 1. Water stress was significantly reduced in both the RWC and total leaf water potential ($\Psi_t$) predawn measurements. The RWC in the leaves of the control plants was 95.3%, while in the water deficit-stressed plants it was 67.2% (Fig. 1A). Similarly, the $\Psi_t$ of water deficit stress was reduced to -1.4 MPa, while it was -0.2 MPa for the control plants (Fig. 1B). These results indicated that all coffee genotypes were affected by the water deficit treatment. However, there were no significant differences among the genotypes under both water conditions.

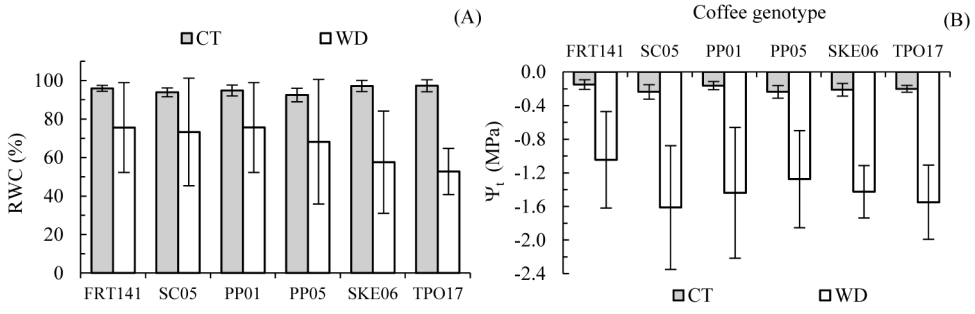


**Fig. 1** Effect of water deficit conditions on plant water status 2 wk after initiation of water deficit treatments: (A) relative water content (RWC); (B) leaf water potential ($\Psi_t$) of six robusta coffee genotypes grown under control (CT) and water deficit stress (WD) conditions, where error bars represent ± SD

*Effect of water deficit on leaf gas exchange*

The water deficit stress caused a significant decrease in all parameters of gas exchange compared to the control (Table 1). Under the control conditions, there were considerable differences between coffee genotypes in the $P_n$ values and there was a significant difference between the coffee genotypes. PP01, PP05 and FRT141 (5.15–8.08 μmol $CO_2/m^2/s$) had higher levels of $P_n$ than for SC05, SKE06 and TPO17 (2.6–4.1 μmol $CO_2/m^2/s$). The $P_n$ values across coffee genotypes greatly decreased under water deficit conditions. PP01, PP05 and FRT141 maintained higher $P_n$ levels than SC05, SKE06 and TPO17, but there were no significant differences among the stressed genotypes. Similarly, the E value of 1.75 mmol $H_2O/m^2/s$ in the control plants was approximately 0.26 mmol $H_2O/m^2/s$ under the water deficit conditions as a result of the decreases in $g_s$ (12 mmol $H_2O/m^2/s$) or the nearly closed stomata. This restricted transpiration and limited $CO_2$ flux into the leaves. PP01 and PP05 had high levels of E and $g_s$ under the control and water deficit conditions. Although FRT141 had lower values under the control conditions, this genotype maintained high levels under water stress conditions. In contrast, significant effects of the water deficit condition were observed as increases in $C_i$ (341 μmol $CO_2/m^2/s$) compared to the control (290 μmol $CO_2/m^2/s$). However, there were no significant differences among the genotypes for the control and water deficit conditions.

In addition, the effect of water deficit on photosystem II was in lowest in ΦPSII (0.03) and ETR (13.5 μmol $e^-/m^2/s$) while the control plants had the highest values for ΦPSII (0.10) and ETR (40.8 μmol $e^-/m^2/s$). Under the control conditions, PP01, FRT14 and PP05 (the high-$P_n$ group mentioned above) tended have better values for both these parameters than the other groups. PP01 and FRT141 maintained a high efficiency of photosystem II under drought conditions. The WUE had significantly different effects on conditions. The plants had 3.6 μmol $CO_2$/mmol $H_2O$ and 1.0 μmol $CO_2$/mmol $H_2O$ under the control and water deficit conditions, respectively. There were no significant differences among the genotypes under both conditions. Only PP05 increased WUE, while the other genotypes had a decrease under the water deficit conditions.

**Table 1** Net photosynthetic rate ($P_n$), transpiration rate (E), stomatal conductance ($g_s$), intercellular $CO_2$ concentration ($C_i$), light-adapted quantum efficiency of Photosystem II (ΦPSII), electron transpiration rate (ETR), water use efficiency (WUE) and maximum quantum efficiency of PSII ($F_v/F_m$) of six robusta coffee genotypes grown under control (CT) and water deficit stress (WD) conditions at 2 wk after initiation of water deficit treatment

| Genotype | $P_n$ (μmol $CO_2/m^2/s$) | | E (mmol $H_2O/m^2/s$) | | $g_s$ (mmol $H_2O/m^2/s$) | | $C_i$ (μmol $CO_2/m^2/s$) | |
|---|---|---|---|---|---|---|---|---|
| | CT | WD | CT | WD | CT | WD | CT | WD |
| FRT141 | 5.15±1.3$^{ab}$ | 0.69±1.0 | 1.31±0.2$^{c}$ | 0.35±0.1$^{a}$ | 66±16.8$^{c}$ | 16±6.1$^{a}$ | 260±13.4 | 346±76.2 |
| SC05 | 3.33±2.8$^{b}$ | 0.20±0.4 | 1.78±1.2$^{bc}$ | 0.21±0.0$^{bc}$ | 102±71.5$^{bc}$ | 10±1.7$^{bc}$ | 334±20.7 | 358±69.5 |
| PP01 | 8.08±1.1$^{a}$ | 0.50±1.0 | 2.50±0.8$^{ab}$ | 0.44±0.1$^{a}$ | 160±73.3$^{ab}$ | 21±7.5$^{a}$ | 292±27.7 | 356±71.9 |
| PP05 | 7.52±1.3$^{a}$ | 1.00±0.2 | 3.01±0.4$^{a}$ | 0.31±0.0$^{ab}$ | 182±2.5$^{a}$ | 15±0.7$^{ab}$ | 319±12.8 | 278±26.5 |
| SKE06 | 2.66±1.5$^{b}$ | 0.00±0.3 | 1.09±0.9$^{c}$ | 0.12±0.1$^{c}$ | 53±43.3$^{c}$ | 6±4.3$^{c}$ | 261±91.4 | 345±121.0 |
| TPO17 | 4.16±2.3$^{b}$ | -0.06±0.8 | 0.80±0.2$^{c}$ | 0.14±0.0$^{c}$ | 42±7.2$^{c}$ | 7±1.6$^{c}$ | 276±74.5 | 363±107.6 |
| F-test | ** | ns | ** | ** | * | ** | ns | ns |
| Mean | 5.15±2.2$^{A}$ | 0.39±0.4$^{B}$ | 1.75±0.9$^{A}$ | 0.26±0.1$^{B}$ | 101±58.4$^{A}$ | 12±5.9$^{B}$ | 290±30.7$^{B}$ | 341±31.8$^{A}$ |
| | ΦPSII | | ETR (μmol $e^-/m^2/s$) | | WUE (μmol $CO_2$ /mmol$H_2O$) | | $F_v/F_m$ | |
| FRT141 | 0.12±0.0$^{ab}$ | 0.06±0.0$^{a}$ | 52.4±15.6$^{ab}$ | 26.2±5.3$^{a}$ | 3.9±0.3 | 1.2±2.2 | 0.795±0.0 | 0.779±0.0$^{a}$ |
| SC05 | 0.07±0.0$^{cd}$ | 0.03±0.0$^{bc}$ | 30.8±14.6$^{cd}$ | 13.6±8.0$^{bc}$ | 1.9±0.7 | 0.9±2.0 | 0.799±0.0 | 0.764±0.0$^{a}$ |
| PP01 | 0.13±0.0$^{a}$ | 0.04±0.0$^{ab}$ | 57.1±9.2$^{a}$ | 16.4±10.1$^{ab}$ | 3.4±0.6 | 0.9±2.1 | 0.799±0.0 | 0.744±0.0$^{a}$ |
| PP05 | 0.11±0.0$^{abc}$ | 0.02±0.0$^{bc}$ | 47.7±8.0$^{abc}$ | 9.2±2.0$^{bc}$ | 2.6±0.6 | 3.4±1.0 | 0.799±0.0 | 0.744±0.0$^{a}$ |
| SKE06 | 0.06±0.0$^{d}$ | 0.02±0.0$^{bc}$ | 24.6±8.1$^{d}$ | 9.0±3.5$^{bc}$ | 4.0±3.0 | 1.4±3.9 | 0.761±0.0 | 0.672±0.0$^{b}$ |
| TPO17 | 0.08±0.0$^{bcd}$ | 0.02±0.0$^{c}$ | 32.3±14.1$^{bcd}$ | 6.7±6.6$^{c}$ | 5.8±3.4 | -1.5±5.7 | 0.739±0.1 | 0.589±0.0$^{c}$ |
| F-test | * | * | * | * | ns | ns | ns | ** |
| Mean | 0.10±0.0$^{A}$ | 0.03±0.0$^{B}$ | 40.8±13.3$^{A}$ | 13.5±7.1$^{B}$ | 3.6±1.4$^{A}$ | 1.0±1.6$^{B}$ | 0.784±0.0$^{A}$ | 0.716±0.1$^{B}$ |

Means ± SD with different uppercase superscripts denote significant difference between the two water conditions; different lowercase superscripts denote significant difference among coffee genotypes under the same conditions; *, ** indicates significant difference among genotypes at $p < 0.05$ and $p < 0.01$, respectively and ns indicates not significant difference.

*Effect of water deficit on chlorophyll fluorescence*

The results showed that under the control conditions, the maximum quantum efficiency of PSII ($F_v/F_m$) of the control plants remained at 0.784, with no significant differences among the tested coffee genotypes, but the water deficit stress caused a significant reduction in $F_v/F_m$ (0.716), with significant differences among the genotypes (Table 1). The value of $F_v/F_m$ of TPO17 significantly decreased (0.589) under the control condition, followed by SKE06 (0.672), while SC05, FRT141, PP01 and PP05 maintained values greater than 0.744 but these values were not significantly different.

*Effect of water deficit on the membrane permeability*

Water deficit caused an increased EL in all genotypes compared with the control condition. The EL values of leaves were 16.9% and 26.0% for the control and water deficit conditions, respectively, but there were no significant differences among the genotypes for the control and water deficit conditions (Table 2). The EL values of the roots were similar to those for the leaves and it significantly increased by 72.0% in water deficit-stressed plants, though there were differences among the genotypes with those for FRT141 and PP01 (40.5–45.6%) being lower than those for PP05, SKE06, TPO17, and SC05 (86.4–90.2%). The EL values for the control plants were in the range 13.1–23.3% with no significant differences among the genotypes.

*Effect of water deficit stress on morphological parameters and biomass*

There were no significant effects of water deficit on the stem diameter and plant height (Table 3). However, there were significant differences among the genotypes under both conditions, with PP05 having the highest stem diameter and FRT141 and PP01 having the greatest height for both conditions. Interestingly, significant effects of water deficit were observed in the leaf part. The mean number of leaves under the control condition was 27.7 leaves/plant and there were no significant differences among the genotypes. However, the total leaf area per plant was significantly different among the genotypes, with PP01 and FRT141 having 1,332 $cm^2$/plant and 1,085 $cm^2$/plant, respectively, while other genotypes had values in the range 491–760 $cm^2$/plant. The water stress conditions caused average decrease in leaf number and total leaf area of 8.9 leaves/plant and 231 $cm^2$/plant. However, only FRT141 had the significantly greatest values for both the number of leaves (29.7 leaves/plant) and the largest area of leaves (> 900 $cm^2$), while other genotypes retained less than 8.0 leaves/plant, so these plants also had the lowest total leaf area (< 200 $cm^2$), under water deficit conditions.

Furthermore, the effects of water deficit halved the total biomass (Table 4), with the greatest decrease in leaf dry mass being 1.5 g/plant for stressed plants while it was 4.9 g/plant for the control plants. In contrast, the stem and root mass slightly decreased, with no significant differences between the two conditions. FRT141 and PP01 had the highest total and leaf biomass under water deficit and control conditions. However, TPO17 also had the lowest stem dry weight, which was significantly different from those of the other genotypes under water deficit conditions. In addition, this genotype may have lost many shoots, resulting in it having the highest R:S ratio value.

**Table 2** Electrolyte leakage (EL) of leaves and roots of six robusta coffee genotypes grown under control (CT) and water deficit stress (WD) conditions at 2 wk after initiation of water deficit treatment

| Genotype | Leaf EL (%) | | Root EL (%) | |
|---|---|---|---|---|
| | CT | WD | CT | WD |
| FRT141 | 18.5±6.1 | 19.4±3.7 | 23.3±9.1 | 45.6±13.5$^{b}$ |
| SC05 | 16.2±1.6 | 26.4±1.1 | 22.9±4.5 | 90.2±16.9$^{a}$ |
| PP01 | 17.1±0.4 | 19.2±4.7 | 20.0±7.9 | 40.5±8.3$^{b}$ |
| PP05 | 18.0±0.5 | 30.9±10.6 | 15.9±0.6 | 87.0±9.5$^{a}$ |
| SKE06 | 15.6±1.0 | 30.6±19.1 | 13.1±0.8 | 86.4±8.9$^{a}$ |
| TPO17 | 16.2±1.4 | 29.2±4.3 | 14.5±2.9 | 87.1±7.9$^{a}$ |
| F-test | ns | ns | ns | ** |
| Mean | 16.9±1.1$^{A}$ | 26.0±5.4$^{A}$ | 18.3±4.4$^{X}$ | 72.8±23.2$^{Y}$ |

Mean ± SD with different uppercase superscripts denote significant difference between the two water conditions; different lowercase superscripts denote significant difference among the coffee genotypes under the same conditions.

**, ns denote significant difference at $p < 0.01$ and not significant different, respectively.

**Table 3** Growth parameters: stem diameter, plant height, number of leaves and total leaf area of six robusta coffee genotypes grown under control (CT) and water deficit stress (WD) conditions at 2 wk after initiation of water deficit treatment

| Genotype | Stem diameter (cm) | | Plant height (cm) | | Number of leaves (leaves/plant) | | Total leaf area ($cm^2$/plant) | |
|---|---|---|---|---|---|---|---|---|
| | CT | WD | CT | WD | CT | WD | CT | WD |
| FRT141 | 0.54±0.1$^{bc}$ | 0.55±0.1$^{bc}$ | 32.3±4.9$^{a}$ | 33.5±3.7$^{a}$ | 33.3±8.1 | 29.8±9.0$^{a}$ | 1,085±207$^{a}$ | 912±90$^{a}$ |
| SC05 | 0.44±0.0$^{c}$ | 0.51±0.1$^{bc}$ | 20.3±4.3$^{b}$ | 22.3±0.6$^{bc}$ | 22.8±5.9 | 8.0±5.9$^{b}$ | 562±77$^{b}$ | 161±158$^{b}$ |
| PP01 | 0.58±0.0$^{b}$ | 0.55±0.0$^{bc}$ | 34.0±2.6$^{a}$ | 32.5±4.8$^{a}$ | 33.7±0.5 | 6.5±1.5$^{b}$ | 1,332±74$^{a}$ | 118±40$^{b}$ |
| PP05 | 0.73±0.1$^{a}$ | 0.83±0.2$^{a}$ | 19.0±1.4$^{b}$ | 18.8±3.9$^{c}$ | 20.8±8.9 | 5.8±5.4$^{b}$ | 491±167$^{b}$ | 111±117$^{b}$ |
| SKE06 | 0.62±0.0$^{b}$ | 0.63±0.0$^{b}$ | 23.3±4.0$^{b}$ | 26.3±5.3$^{b}$ | 22.8±4.9 | 1.3±1.0$^{b}$ | 698±11$^{7b}$ | 34±28$^{b}$ |
| TPO17 | 0.62±0.1$^{b}$ | 0.41±0.1$^{c}$ | 29.8±3.6$^{a}$ | 20.0±2.0$^{c}$ | 33.0±11.7 | 2.0±2.0$^{b}$ | 760±225$^{b}$ | 50±45$^{b}$ |
| F-test | ** | ** | ** | ** | ns | ** | ** | ** |
| Mean | 0.59±0.1 | 0.58±0.1 | 26.4±6.7 | 25.6±6.9 | 27.7±6.2$^{A}$ | 8.9±10.6$^{B}$ | 821±324$^{A}$ | 231±337$^{B}$ |

Mean ± SD with different uppercase superscripts denote significant differences between the two water conditions; different superscript lowercase letters denote significant differences among the coffee genotypes under the same conditions;

**, ns denote significant difference at $p < 0.01$ and not significant different, respectively.

**Table 4** Biomass of leaf, stem, root, total and root-to-shoot (R:S) ratio of six robusta coffee genotypes grown under control (CT) and water deficit stress (WD) conditions at 2 wk after initiation of the water deficit treatment

| Genotype | Biomass (g/plant) | | | | | | | | R:S ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | | Stem | | Root | | Total | | | |
| | CT | WD | CT | WD | CT | WD | CT | WD | CT | WD |
| FRT141 | 5.8±1.1$^{b}$ | 5.1±0.9$^{a}$ | 3.0±0.8$^{bc}$ | 3.4±0.8$^{a}$ | 2.6±0.7$^{a}$ | 2.2±0.6 | 11.3±2.5$^{b}$ | 10.7±2.4$^{a}$ | 0.30±0.0 | 0.26±0.0$^{b}$ |
| SC05 | 3.5±0.5$^{cd}$ | 1.1±0.8$^{b}$ | 2.2±0.2$^{c}$ | 2.4±0.8$^{ab}$ | 1.5±0.3$^{b}$ | 1.2±0.5 | 7.1±0.6$^{c}$ | 4.7±1.6$^{bc}$ | 0.28±0.1 | 0.37±0.1$^{b}$ |
| PP01 | 8.5±0.9$^{a}$ | 1.4±0.1$^{b}$ | 4.9±0.3$^{a}$ | 3.8±0.2$^{a}$ | 2.8±0.5$^{a}$ | 2.4±0.2 | 16.2±1.7$^{a}$ | 7.7±0.3$^{ab}$ | 0.20±0.0 | 0.46±0.0$^{b}$ |
| PP05 | 3.1±0.6$^{d}$ | 0.7±0.7$^{b}$ | 3.2±0.9$^{b}$ | 2.7±0.5$^{a}$ | 1.5±0.2$^{b}$ | 1.5±0.7 | 7.8±0.9$^{c}$ | 4.8±0.7$^{bc}$ | 0.24±0.0 | 0.51±0.3$^{b}$ |
| SKE06 | 4.5±0.9$^{c}$ | 0.2±0.1$^{b}$ | 3.1±0.9$^{bc}$ | 3.0±1.3$^{a}$ | 1.5±0.3$^{b}$ | 1.2±1.4 | 9.0±1.8$^{c}$ | 4.4±2.6$^{bc}$ | 0.20±0.0 | 0.33±0.2$^{b}$ |
| TPO17 | 4.3±0.8$^{cd}$ | 0.3±0.2$^{b}$ | 3.1±0.3$^{bc}$ | 1.1±0.3$^{b}$ | 1.9±0.6$^{b}$ | 1.7±0.9 | 9.4±0.6$^{bc}$ | 3.1±1.2$^{c}$ | 0.27±0.1 | 1.08±0.4$^{a}$ |
| F-test | ** | ** | ** | * | ** | ns | ** | ** | ns | * |
| Mean | 4.9±2.0$^{A}$ | 1.5±1.8$^{B}$ | 3.2±0.9 | 2.7±0.9 | 2.0±0.6 | 1.7±0.5 | 10.1±3.3$^{A}$ | 5.9±2.8$^{B}$ | 0.25±0.0$^{B}$ | 0.50±0.3$^{A}$ |

Mean ± SD with different uppercase superscripts denote significant differences between the two water conditions; different lowercase superscripts denote significant differences among the coffee genotypes under the same conditions;

*, ** denote significant difference at $p < 0.05$ and $p < 0.01$, respectively; ns denotes not significant difference.

*Changes in responses of morphological and physiological parameters of six coffee genotypes to water deficit stress*

There were significant differences among the coffee genotypes with reductions of ΦPSII and ETR, though FRT141 and SC05 had less reduction (by 50–55%), while SKE06, PP01, TPO17 and PP05 were reduced by 70–80% (Fig. 2A–B). The $F_v/F_m$ value had less of a reduction (only 4–10%) for almost all genotypes under the water stress condition, with TPO17 being significantly the highest with a decrease by 20% (Fig. 2C). The water deficit caused a decrease in other gas exchange measurements with about 80–100% for $P_n$ and 70–90% for E and $g_s$. In addition, the water stress caused a decrease in plant water status and the decreases were more than 600% for $\Psi_t$ and 20–40% for RWC. However, there were no significant differences in these measurements among the coffee genotypes (data not shown).

Among the morphological parameters, water stress caused the loss of leaves per plant and decreased the total biomass (Fig. 2D–F). Among the coffee genotypes, FRT141 had a significantly smaller reduction (only 10–16%) in the number of leaves and the leaf area that combined resulted in a decrease of 6% in the total biomass. Conversely, the other genotypes tended to have larger decreases (by 70–95%) in the number of leaves and leaf area by 30–67% for the total biomass. In addition, the stem dry mass was reduced in most of the coffee genotypes (lowest value was 64% in TPO17). The exceptions were two genotypes (FRT141 and SC05) for which the stem dry mass increased by about 14% relative to the controls. However, there were no significant decreases in the root dry mass (data not shown).

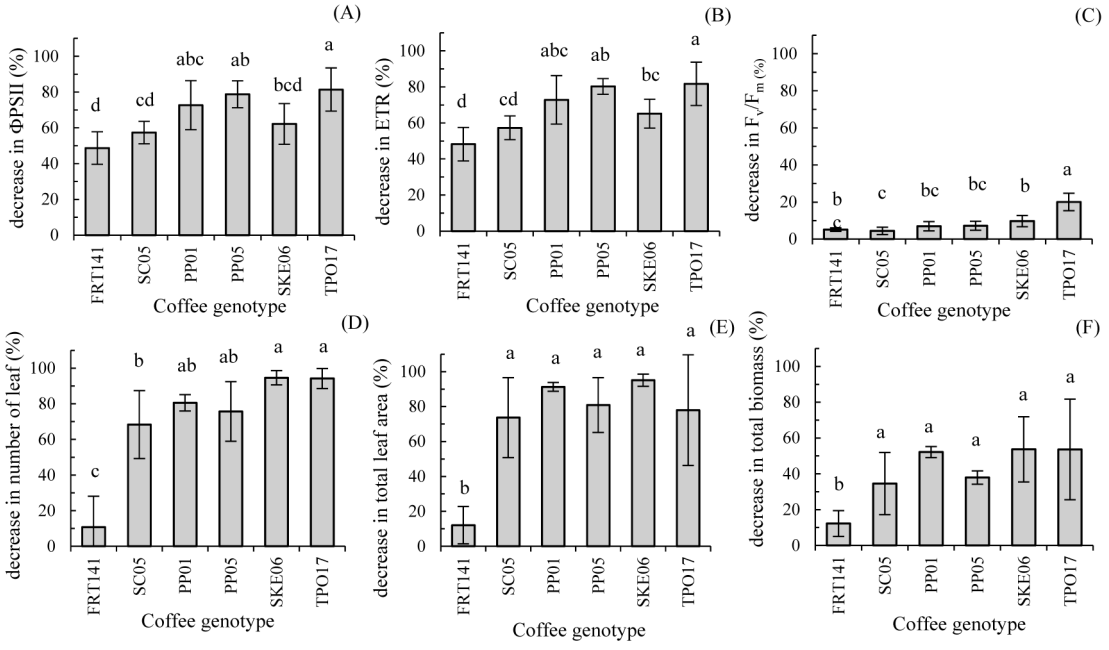


**Fig. 2** Parameters for six robusta coffee genotypes under water stress condition: (A) light-adapted quantum efficiency of PSII (ΦPSII); (B) electron transpiration rate (ETR); (C) maximum quantum efficiency of PSII ($F_v/F_m$); (D) number of leaves/plant; (E) leaf area/plant; (F) total biomass, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among the coffee genotypes

In contrast, water stress increased gas exchange in $C_i$ in almost all genotypes, except for PP05, where there was a decrease, although there were no significant differences among the genotypes (Fig. 3A). There were significantly different increases among the coffee genotypes for the morphological parameters of the R:S ratio and in particular for TPO17 which had significantly different increases in plant height and the R:S ratio compared to the others (Fig. 3B). The percentage increases in EL under the water deficit condition (Fig. 3C–D) for the leaf cells were the same as for the root cells among the coffee genotypes except that the levels of EL for FRT141 and PP01 slightly increased in the leaf and root cells by only 10% and 100%, respectively, while for SC05, the increases were approximately 60% and 300%, respectively. The greatest increases in leaf and root cells occurred with PP05, SKE06 and TPO17 by 70% and 500%, respectively. However, there were only significant differences among the genotypes for the root cells.

## Discussion

The data for the water deficit period showed that the plant water status parameters had the lowest values for both $\Psi_t$ and the RWC measurement compared to the control condition. Previously, many comparative studies in coffee plants under both pot and field conditions have shown that drought-tolerant coffee genotypes are better able to maintain a higher plant water potential or RWC or both compared to drought-sensitive genotypes under water deficit conditions (DaMatta et al., 2003; Pinheiro et al., 2005). However, although the current results did not show any significant differences in plant water status among the six coffee genotypes, other parameters showed various responses to the water stress condition. Generally, this has been directly associated with the maintenance of gas exchange under water stress conditions. The current gas exchange parameter results showed that the $P_n$ of coffee genotypes substantially decreased, reaching zero in some genotypes (SKE06 and TPO17), while other genotypes (FRT141, SC05, PP01 and PP05) had a positive carbon balance, although this decreased by more than 80% as a consequence of a considerable reduction in the plant water status, indicating stomatal regulation by hydraulic signals (Auge and Moore, 2002).

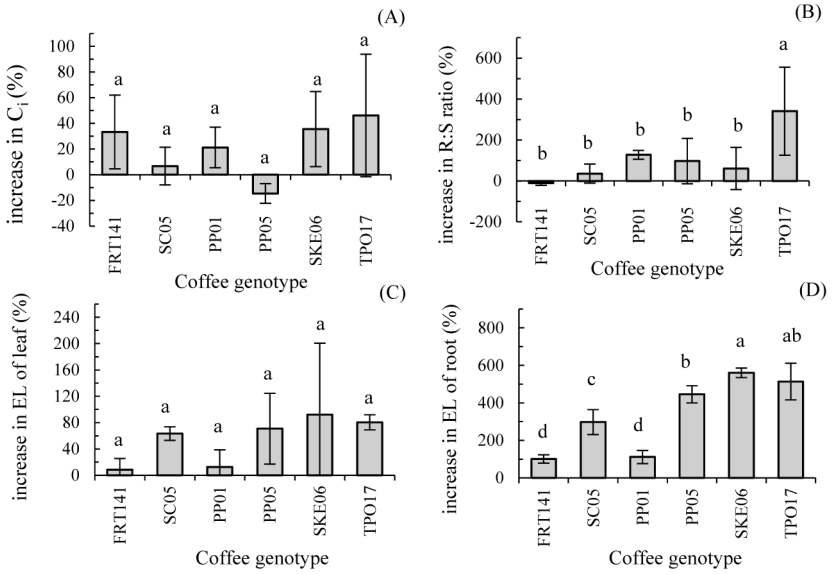


**Fig. 3** Parameters for six robusta coffee genotypes under water stress condition: (A) intercellular $CO_2$ concentration ($C_i$); (B) root to shoot ratio (R:S ratio); (C) electrolyte leakage (EL) of leaves; (D) electrolyte leakage (EL) of roots, where error bars represent ± SD and different lowercase letters denote significant ($p < 0.05$) differences among the coffee genotypes.

The average gas exchange parameters were compared between the coffee genotypes under the control and water deficit conditions, which showed considerable differences among the coffee genotypes for the rate of $P_n$ under the control condition. PP01, PP05 and FRT141 had higher levels of leaf gas exchange compared to SC05, SKE06 and TPO17. There was a substantial drop in the gas exchange parameters as a result of stomatal closure (as indicated by the lowest $g_s$) under water deficit in all genotypes. Stomatal closure has been reported as the earliest response to water deficit stress, causing a reduced rate of loss of water from the plant system through transpiration (Flexas and Medrano, 2002). However, in the current results, WUE decreased in the water-stressed plants compared to the control plants, which could have been due to the lack of increased photosynthetic activity while leaf transpiration was still high, resulting in the reduction in WUE in the water-stressed plants. Another study indicated that a decrease in $P_n$ is usually caused by stomatal limitation under drought conditions when both $g_s$ and $C_i$ decline while nonstomatal limitation is the main reason for the decrease in $P_n$ when $C_i$ increases and $g_s$ reaches a minimum inflection point (Zhou et al., 2013). In the current study, water-stressed plants in each genotype had increased $C_i$, while $g_s$ reached a minimum. Therefore, a lowered $g_s$ was unlikely to be responsible for the reduction in $P_n$. The reduction in $P_n$ in most genotypes in the current study could have been affected by nonstomatal limitation which involved damage to PSII photochemical efficiency, as indicated by the lowest ΦPSII and ETR. The exception was PP05, which maintained high $P_n$ and reduced $C_i$ under water deficit, probably because of the $CO_2$ restriction caused by stomatal closure under water stress, which resulted in higher efficiency of transpiration; thus, it also had the highest WUE under water deficit.

The functionality of the photochemical apparatus was evaluated by measuring chlorophyll fluorescence parameters. The value of $F_v/F_m$ denotes the potential quantum efficiency of PSII and is used as a sensitive indicator of coffee plant photosynthetic performance, with optimal values for healthy plants generally being close to 0.8 (Burke, 2007). In contrast, under stress conditions, the above results indicated that the values of $F_v/F_m$ decreased with differential adaptation to drought among the six robusta coffee genotypes, especially in SKE06 and TPO17, which showed damage to the photochemical apparatus. Thus, the decline in the net photosynthetic rate of the stress may have been caused by both stomatal and nonstomatal mechanisms (DaMatta et al., 2003; Zhou et al., 2013). Interestingly, the changes in the light reaction parameters in response to water deficit were relatively small compared to the changes in net photosynthesis rates (especially in FRT141 and SC05). The current result showed that these genotypes had $P_n$ values that were greatly reduced by 80–90%,

with $g_s$ also reduced by 70–90%, but ETR and ΦPSII were only reduced by 50–55%. These genotypes also had less reduction in $F_v/F_m$. These changes confirmed other studies that reported high stability of the potential PSII photochemical efficiency to water deficit in robusta coffee (DaMatta et al., 1997; Lima et al., 2002), thus allowing for the maintenance of electron transport through photosystems at considerable rates. It is possible that photosynthetic reduction of $O_2$, via photorespiration and the Mehler-peroxidase pathway provide photoprotection by acting as a sink for excitation energy in the photosynthetic apparatus under water deficit conditions.

In addition, assessments using the electrolyte leakage technique showed that membrane injury increased by 80% in most of the coffee genotypes, except in FRT141 and PP01, which had less cell membrane damage, indicating that these two genotypes have higher cell membrane stability under drought stress. Therefore, this result indicated that these genotypes are more drought-tolerant genotypes compared to the others.

The largest alterations resulting from water deficit stress were observed in the leaf part of all coffee genotypes. Water stress caused decreases on average that were more than 70% in most genotypes. Prolonged drought stress reduced the plant water status, resulting in leaf abscission, which may be considered a whole-plant mechanism to limit transpiration. Leaf shedding in response to drought stress occurs sequentially from older to younger leaves, with the more drought-sensitive the genotype, the greater the extent of leaf shedding (DaMatta and Rena, 2001) as was seen in SKE06 and TPO17. These two genotypes are drought-sensitive genotypes that lost considerable amounts of their leaves. However, the strategy was not efficient enough, since the water status was severely impaired in their remaining leaves (DaMatta et al., 2003). In addition, under continuing water deficit events, the effects on biomass production could be related to stomatal closure and concomitantly lower $CO_2$ assimilation. These factors will reduce the energy available to achieve high productivity. Afterward, the increasing stress caused loss in the number of leaves, resulting in biomass loss (DaMatta et al., 2003). However, under both the studied conditions in the current work, there were no significant differences in the stem parameters, including the stem diameter, plant height and stem dry weight, as was the case for the root dry weight. Thus, the significant decrease in the shoot dry mass of robusta coffee depended on leaf damage and leaf fall. In general, changes in the total biomass and R:S ratio are considered sensitive parameters during genotype evaluation for drought tolerance, indicating the ability of a genotype to adapt to stress conditions (Tesfaye et al., 2008). In contrast, TPO17 had the highest R:S ratio because it had the highest decrease in the shoot and root dry masses. Thus, the physiological responses of this coffee genotype showed that it was drought-sensitive. The lower reduction in biomass and growth of FRT141 (the water deficit-tolerant genotype) was associated with some physiological traits, such as maintaining higher $CO_2$ assimilation and leaf area that influenced biomass (Kramer and Boyer, 1995). This was supported by Utkhao and Yingjajaval (2015) who studied eucalypts under drought stress over a 3 wk interval and reported that the $CO_2$ assimilation capacity of the leaf decreased to almost zero. Overall, under continuing and increasing drought stress, the leaves lost the photosynthesis integrity in a successive manner, starting with stomatal movement, carboxylation and light reaction, which more directly resulted in decreased growth and cumulative biomass.

In conclusion, the current results indicated that progressive water deficit was simulated by polyethylene glycol, which affected the morphological and physiological parameters among the six robusta coffee genotypes. Water deficit stress significantly reduced the plant water status, leaf gas exchange parameters, $F_v/F_m$, leaf area and total biomass, while the EL increased. However, the coffee genotypes were more tolerant to drought stress and maintained a high leaf area and total biomass and a low EL under water stress conditions. FRT141 maintained a higher leaf area and total biomass under water stress conditions (lowest decrease) compared to the other genotypes, followed by PP01 and SC05. These seemed to be less sensitive to drought stress compared to PP05, TPO17 and SKE06 under the water deficit condition. Therefore, it appeared that morphological and physiological parameters, as well as growth parameters, are important and need to be considered when screening crop genotypes for drought tolerance.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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