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Effect of water deficiency on growth and dry matter yield of selected in Robusta coffee (*Coffea canephora*) clones in Malaysia

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Abstract

Drought stress is one of the major bottle necks of growth and productivity of Robusta coffee (*Coffea canephora* pierre ex froehner) in many producing areas the crop. An experiment was carried out to determine the difference among known Robusta coffee clones for rate of vegetative growth and dry matter production and partitioning under water deficit stress condition and to identify drought tolerant materials. Twelve-month-old seedlings of six Robusta coffee clones (IC-2, IC-3, IC-4, IC-6, IC-8 and R-4) were subjected to two irrigation treatments: well-watered control and water-stressed by withholding irrigation for 3 weeks in a rain shelter. Growth response to soil drying and dry matter distribution among plant parts were measured to identify drought tolerant clones. Leaf growth of all the coffee clones was considerably affected by soil drying. There were significant differences noted between clones for the rate of reduction in leaf elongation, total leaf area, specific leaf area (SLA) and dry matter yield under water stress condition. Moreover, rate of survival of coffee plants during drought and recovery upon re-watering at the end of the soil drying period significantly varied with clone. Some of the clones, particularly IC-3 and IC-6, exhibited higher root: shoot ratio (0.493 – 0.613) and total leaf area (900 – 920cm^2) and lower SLA (82 – 83 cm^2/g) and rate of leaf fall (62 – 71%) than the other clones (with the respective values of 0.413 – 0.447, 160 – 440cm^2, 92 – 97 cm^2/g, 82 – 92%) under water-stressed condition. These clones (86 – 88%) survived stress and produced new leaves and flowers more rapidly after re-watering than R-4 (37%) and IC-8 (53%). Hence, clones IC-6 and IC-3 exhibited better performances and seemed to be less sensitive to water deficit stress. Drought tolerance attributes in these clones could be linked to some morphological modifications, mainly increased root: shoot ratio and reduced SLA.

Key words

Coffea canephora, Dry matter yield, Growth, Soil drying, Specific leaf area

Introduction

Robusta coffee (*C. canephora* Pierre ex Froehner) is one of the economically important species of the genus *Coffea*, accounting for about 20% of the total coffee trade in the world market. However, frequent water deficit stress appears to be the bottleneck for its production in areas with long dry spells. Since Robusta coffee is an obligate cross-pollinated species, it is likely to have wide population variation in morphological, physiological and biochemical traits associated with water stress tolerance. It has been reported that decrease in shoot growth and leaf area and increases in leaf thickness and root: shoot ratio with soil moisture depletion are believed to be among the important stress avoidance or tolerance mechanisms in plants (Mohammadi *et al*., 2007; Tesfaye *et al*., 2014).

On the other hand, Anim-Kwapong *et al*. (2011) showed that leaf scorching (damage to leaves due to water deficit stress) was inversely related to shoot growth parameters and can be used as an important attribute to screen Robusta coffee clones for drought tolerance. Similarly, it has been reported that photooxidative damage to photosynthetic apparatus due to severe drought stress may lead to death of cells, which could be reflected at the whole plant level by the appearance of chlorotic or necrotic lesions on damaged leaves and subsequent leaf shedding and loss of leaf area especially in the more sensitive genotypes. In line with this, Anim-Kwapong *et al*. (2011) observed that drought-tolerant clones of Robusta coffee exhibited increased ability to protect against oxidative stress, limited cellular damage and greater leaf retention capacity following periods of prolonged water stress, but the extent of photooxidative damage and, thus, leaf shedding was greater in drought-sensitive clone because of less effective antioxidant systems.

Some morphological and physiological traits which are important in selecting drought and heat tolerant coffee genotypes have been reviewed by Cheserek and Gichimu (2012). In this line, studies on Robusta coffee has shown deeper root system (Pinheiro *et al*., 2005) and larger root dry mass in drought tolerant clones than in drought sensitive ones (DaMatta and Ramalho, 2006). Similarly, morphological, physiological and biochemical variations among Arabica coffee genotypes for water deficit stress tolerance has been reported by D'Souza *et al*. (2009).

According to DaMatta and Ramalho (2006), cultivars more tolerant to drought generally differ morphologically and/or physiologically, with mechanisms allowing greater production under restricted water supply. Hence, understanding such mechanisms in genotypes naturally adapted to drought could help improve their agronomic performance. Some coffee genotypes are known to have few drought tolerance attributes (D'Souza *et al*., 2009). Such genotypes could be exploited in future drought tolerance breeding programmes (Anim-Kwapong *et al*., 2011). Hence, a complementary approach to improve Robusta coffee performance could involve identification and selection of agronomic/morphological traits associated with tolerance to drought stress that are relatively easy to measure (Anim-Kwapong and Adomako, 2010; Anim-Kwapong *et al*., 2011). In coffee, agronomic traits associated with yields have been reported for both *C. canephora* (Anim-Kwapong and Adomako, 2010). However, little information is available on the agronomic traits that are associated with genetic differences for drought tolerance and yield (Anim-Kwapong *et al*., 2011).

As drought episodes are more frequent than other stresses, drought-stress is considered to be major environmental factor limiting coffee yield in most coffee growing areas (DaMatta and Ramalho, 2006). In fact, Anim-Kwapong *et al*. (2011) reported existence of a large diversity among Robusta coffee genotypes around the world for drought tolerance, although these variabilities have not been well-studied and documented in relation to morphological traits. The objective of the present study was to determine differences among six widely grown Robusta coffee clones for morphological parameters under water deficit stress condition and to identify drought tolerant materials for stressful areas.

Materials and Methods

Six clones of *C. canephora* Pierre ex Froehner (clone IC-2; IC-3; IC-4; IC-6; IC-8 and R-4), which are widely grown by local coffee farmers in the Northern part of Malaysia, were used in this experiment. The plants were grown in pots (each with eight liter capacity) filled with a mixture of top soil (*Serdang* series), manure and sand (3:2:2 ratio by volume), irrigated daily and fertilized with 20g NPK per pot during establishment. When the plants were 12 months-old, water deficit stress was imposed by withholding irrigation until severe wilting symptoms were observed.

Treatments and plot arrangement*:* Seventy two uniformly grown seedlings were selected from each clone and arranged in plots of 12 plants, which were either well-watered (control) or water-stressed. The potted medium of well-watered plots was maintained at 100% field capacity (FC) (32% moisture content on dry weight basis) by applying irrigation at two-day-interval. On the other hand, water-stressed plots were re-watered when seedlings showed severe wilting (chlorosis and partial leaf drying or severe leaf scorching) symptoms at soil moisture content of 40%–45% of the FC (nearly 13 – 14% on dry weight basis) just 21 days after withholding irrigation. Experiment was laid down in a randomized complete block design (RCBD) with three replicates of twelve factorial treatment combination (two water regimes x six clones). Each plot consisted of 12 plants.

Experimental condition*:* Experiment was conducted in a rain shelter with average midday photosynthetic photon flux density (PPFD) of 672.3µmol m^{-2} s^{-1} and air temperature and relative humidity (RH) of 34.5^{0}C and 62.9%, respectively. Rain shelter was located at the Hydroponics Unit of Universiti Putra Malaysia 31m asl, Selangor, Malaysia. The mean maximum and minimum temperatures at UPM during the study period were 32.4 ºC and 22.8 ºC, respectively, with mean relative humidity of 65.5%.

Data collection : Dry matter yield and its partitioning among plant parts, changes in leaf elongation rate, extent of leaf fall, total and specific leaf area and rate of survival and recovery upon rewatering were measured. All plants in one of the two water-stressed plots were considered to determine degree of leaf fall and leaf area loss at the end of stress period and rate of recovery (initiation of new flushes) upon re-watering after 21 days of withholding irrigation. On the other hand, two plants were randomly sampled from each plot for determination of dry weights of leaves, stem and roots, total dry matter yield, root: shoot ratio and root volume.

Leaf elongation rate was determined by measuring the length of leaves from petiole insertion point up to the tip. Newly emerging leaves were tagged to measure the increment in length at three-day-intervals. Similarly, total green leaf area of sampled plants was measured using an automatic leaf area meter (Delta T Device, Cambridge, UK). Specific leaf area (SLA) was calculated based on area (LA) and dry weight (LW) of fully expanded leaves on the third and fourth nodes from the apex of younger branches, where SLA = LA / LW.

The extent of leaf shedding or rate of leaf fall (RLF) was calculated as proportion of dried and fallen leaves (NFL) out of total number of leaves (TNL) produced by plant (where, RLF = NFL/TNL). Loss of total leaf area (LTLA) due to soil drying was estimated based on green leaf area of plants in water-stressed (LAWS) and well-watered (LAWW) plots just 21 days after withholding irrigation i.e., at the end of soil drying period (where, LTLA = (LAWW – LAWS)/LAWW).

Recovery rate of coffee plants upon rewatering was estimated using growth parameters. The proportion of plants that survived severe water stress (21 days without irrigation) and produced new vegetative parts (especially new flushes of buds and leaves) and flowers was calculated six days after rewatering. Plants sampled from each plot were separated into leaves, stem (including branches) and roots and dried in an oven at 80 °C to a constant weight. Then, each plant part was weighed to determine their respective dry weights, total dry matter yield and root: shoot ratio at the end of stress period. Root volume was measured before oven drying by displacement method (volume of water displaced by roots when the root system is submerged in a graduated cylinder filled with 600 ml of tap water).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and tested for significance using Least Significant Difference (LSD) at 0.05 P level by PC-SAS software (SAS Institute, Cary, NC, 2001).

Results and Discussion

Leaf growth of all the coffee clones was considerably affected by soil drying. There was a substantial difference between the clones for the rate of reduction in leaf elongation with increasing time of water stress. Leaf elongation rate (LER) in well-watered control plants also varied between the clones, but all the clones experienced a progressively increasing LER at early stages and a reduced rate at the later stages of leaf growth (Fig. 1). In general, LER in water-stressed plants was maintained at a similar level to that of well-watered controls until 6^{th} day after withholding irrigation for IC-6, but only for first three days of soil drying in IC-2 and IC-3. LER of water-stressed plants was consistently lower than those of controls for IC-4, IC-8 and R-4 throughout the soil drying period. Besides, LER in water-stressed plants increased at a diminishing rate after day 12 in IC-3 and IC-6 and after day nine in the rest of coffee clones. As a result, clone IC-6, followed by IC-2, IC-3 and R-4, exhibited considerably higher LER than IC-4 and IC-8, under water stress condition (Fig.1).

Clone IC-6, followed by IC-8, showed significant (P < 0.05) higher total leaf area (TLA) (2450 – 2700 cm^2) than IC-2, IC-3, IC-4 and R-4 (1650 – 1950 cm^2) under well-watered condition. Similarly, TLA of IC-6 and IC-3 (900 – 920 cm^2) was significantly higher (P < 0.05) than other clones (160 – 440 cm^2) under water-stressed conditions (Fig. 2).

Specific leaf area, which is an estimate of leaf thickness, significantly (P < 0.05) differed among the coffee clones, where highest value was recorded for R-4 (120 g cm^{-2}), followed by IC-6 and IC-8 (107 g cm^{-2}) under well-watered condition. Similarly, clones IC-4 (97 g cm^{-2}) and R-4 (95 g cm^{-2}) exhibited higher SLA than did the other clones under water-stressed condition. On the other hand, water-stressed plants of IC-6 and IC-3 had significantly (P < 0.05) the lowest SLA (82 – 83 g cm^{-2}), indicating the highest leaf thickness (Fig. 2).

Difference between coffee clones were significant (P < 0.05) for both rate of leaf fall and loss of total leaf area induced by water deficit stress. RLF was significantly (P < 0.05) higher (90 – 92%) for IC-8 and R-4, followed by IC-4 and IC-2 (82 -88%), than for IC-3 and IC-6 (62 – 71%). All the clones also showed same trend for LTLA, which was significantly higher (P < 0.05) (90 – 92%) for IC-8 and R-4, followed by IC-4 and IC-2 (78 – 80%), than for IC-3 and IC-6 (51 – 68%) (Fig. 3).

Rate of survival of coffee plants during drought and recovery upon re-watering at the end of the soil drying period significantly (P < 0.05) varied with clone. Some of the clones, particularly IC-3 and IC-6, survived the stress more and produced new leaves and flowers more rapidly after re-watering than did R-4 and IC-8. Survival rate and initiation of new flashes in plants, expressed as percent plants recovered (PPR) after the commencement of re-watering at the end of the drought period, was significantly higher (P < 0.05) for IC-3 (88%) and IC-6 (86%) than for IC-2 (60%), IC-4 (68%) and IC-8 (53%), whereas the lowest value of recovery was observed for R-4 (37%) just six days after re-watering (Fig. 3).

There were significant (P < 0.05) differences observed between the coffee clones for leaf and stem dry weight and root: shoot ratio, but variations due to total dry matter yield, root dry weight and root volume were not significant (P > 0.05). It was observed that well-watered plants had generally higher shoot and root dry weights, TDM yield and root volume than stressed plants, except for clone R-4. However, the root: shoot ratio tended to increase with water deficit stress, except for IC-4 and IC-8 (Table 1). Among the coffee clones, IC-4 had higher, while IC-3 produced lower TDM yield at the end of drought period. On the other hand, the root: shoot ratio of IC-3 was significantly (P < 0.05) higher under both well-watered and water-stressed conditions. Water deficit also resulted in relatively higher root: shoot ratio in IC-6 as compared to other clones (Table 1).

As observed in the present study with different Robusta coffee clones, the adverse effect of water stress on leaf elongation rate and total and specific leaf area has been reported for coffee (Tesfaye *et al*., 2013) and tomatoes (Kirda *et al*., 2004).

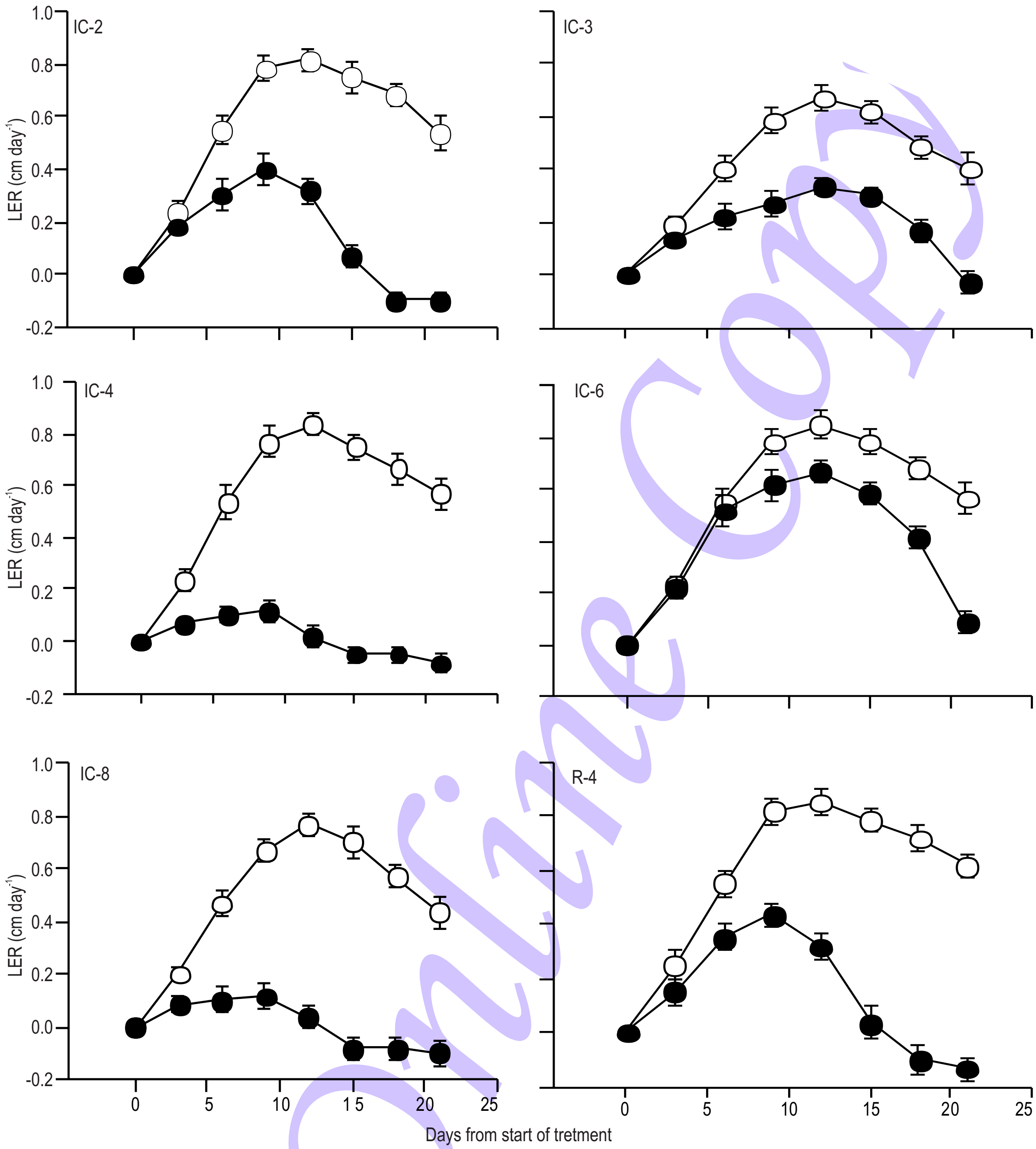

Fig. 1 : Rate of leaf elongation (LER) in six Robusta coffee clones as affected by soil drying (open symbols represent well-watered and closed ones water stressed treatments)

Hence, changes in LER and SLA are considered as sensitive indicators of the intensity of water stress in genotype evaluation programme (Liu *et al*., 2003) and, thus, good estimates the ability of a genotype to exploit its environment and survive under stress conditions (Tesfaye *et al*., 2008).

In the present study, LER in some of the coffee clones declined with reduction in plant water status. Similar results have been reported for grapevines (dos Santos *et al*., 2003) and common bean plants (Wakrim *et al*., 2005), where reductions in leaf growth and shoot vigor were associated with decrease in leaf

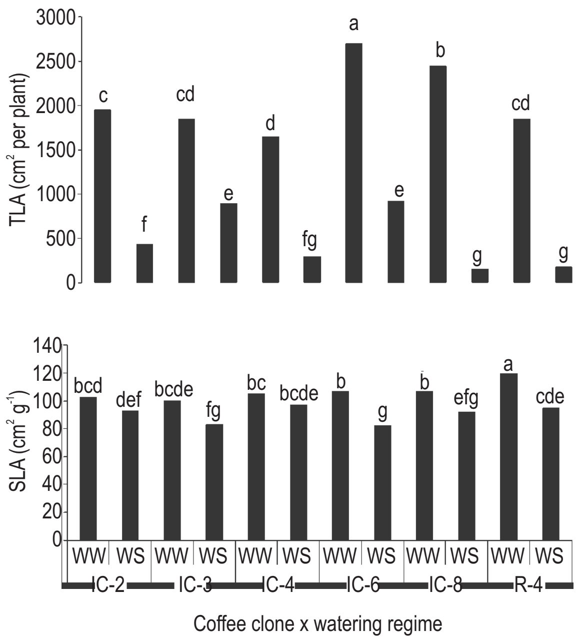

Fig. 2 : Total leaf area (TLA) and specific leaf area (SLA) of coffee clones as affected by soil drying for 21 days

water potential as a result of soil drying. Besides maintenance of higher total leaf area and LER, SLA of some of the coffee clones, such as IC-6 and IC-3, was reduced to the lowest level (leaf thickness increased) as compared to its values in other clones under water stress condition (Fig. 2).

Variation in the rate of leaf fall and loss of total leaf area among the coffee clones in the present study were quite in agreement with the findings of Anim-Kwapong *et al*. (2011), who reported that leaf shedding, frequently preceded by typical symptoms of oxidative stress, is a common response of some Robusta coffee clones to drought stress. Similarly, it has been reported that severe water stress significantly decreased leaf growth and leaf area duration in Arabica coffee (Tesfaye *et al*., 2008). Such reduction in leaf area of water stressed plants could be associated with both reduced growth (elongation or expansion) of individual leaves and increased rate of leaf senescence and abscission. In general, significantly lower rate of leaf shedding and loss of leaf area in IC-3 and IC-6 as compared to the other coffee clones in the present study might be associated with maintenance of turgor through enhanced rate of osmotic adjustment and increased root: shoot ratio. In line with this, it has been reported that maintenance of turgor by OA may reduce the rate of leaf senescence (help leaves stay green and delay wilting) by extending the life time of active tissues in drought-tolerant genotypes during water stress periods.

High rate of survival and recovery in IC-3 and IC-6 might be associated with lower SLA (increased leaf thickness) and higher root: shoot ratio. In line with this, it has been reported that increased root growth and higher root: shoot ratio have been observed in a number of cases to improve plant water status, delay the rate of stress development and increase survival rate under water stress conditions (Mingo *et al*., 2004). In contrast, lower rate of survival during water stress period and, thus, lower recovery rate after re-watering in coffee clones IC-8, R-4, IC-2 and IC-4 might be attributed to higher SLA and lower root: shoot ratio (Pinheiro *et al*., 2005; DaMatta and Ramalho, 2006).

As observed in the present study, the findings of Mingo *et al*. (2004) on tomato and Dorji *et al*. (2005) on hot pepper plants grown in split-root system have indicated that total biomass yield was not significantly affected by soil drying. In contrast, it has been reported that the rate of total dry matter accumulation significantly decreased with development of water deficit in coffee

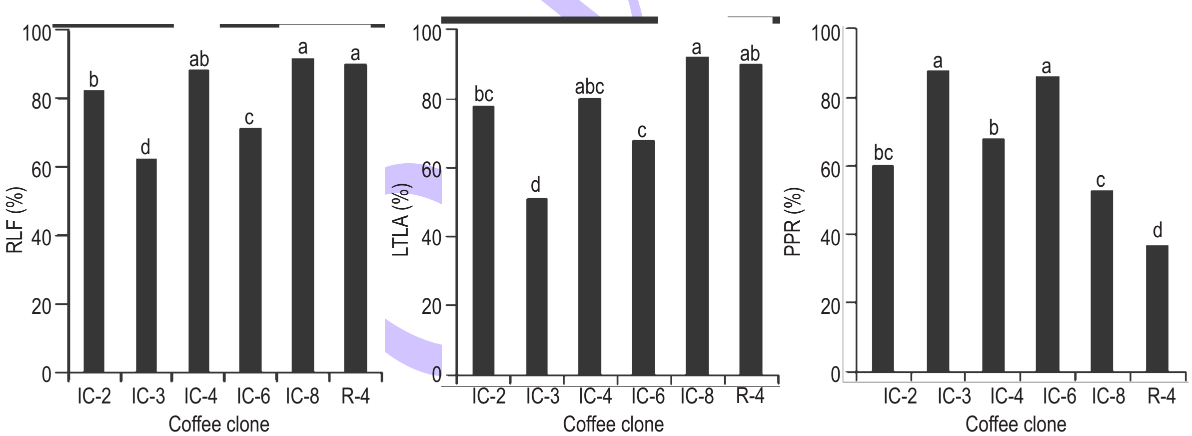

Fig. 3: Rate of leaf fall (RLF) and loss of total leaf area (LTLA) in Robusta coffee clones as a result of soil drying for 21 days and percent plants recovered (PPR) upon re-watering at the end of drought period

Table 1 : Total dry matter (TDM) yield and its partitioning among plant parts in Robusta coffee clones grown under well-watered (WW) and water-stressed (WS) conditions

Clone	Watering regime	LDW (g)	SDW (g)	RDW (g)	TDM (g)	R:SRatio	RV (cm^3)
IC-2	WW	18.83 ab	17.17 abc	13.17 a	49.17 a	0.367 d	41.33 a
	WS	13.83 bc	16.83 abc	12.83 a	43.50 a	0.423 bcd	32.67 a
IC-3	WW	17.33 ab	15.00 abcd	18.50 a	50.83 a	0.577 ab	52.67 a
	WS	11.17 c	9.67 d	12.83 a	33.67 a	0.613 a	26.00 a
IC-4	WW	18.67 ab	19.33 a	16.33 a	54.33 a	0.427 bcd	49.67 a
	WS	17.17 abc	16.33 abc	14.00 a	47.50 a	0.413 cd	36.33 a
IC-6	WW	20.67 a	15.17 abcd	17.17 a	53.00 a	0.477 abcd	43.33 a
	WS	14.17 bc	11.50 bcd	12.83 a	38.50 a	0.493 abcd	32.00 a
IC-8	WW	20.33 a	17.83 ab	21.67 a	59.83 a	0.557 abc	53.00 a
	WS	18.17 ab	10.67 cd	12.33 a	41.17 a	0.427 bcd	35.00 a
R-4	WW	13.67 bc	12.67 bcd	9.67 a	36.00 a	0.350 d	21.67 a
	WS	13.83 bc	11.17 cd	11.50 a	36.50 a	0.447 bcd	31.33 a

Figures followed by same letter(s) within a column are not significantly different at P = 0.05; (LDW = Leaf dry weight, SDW = Shoot (stem and branch) dry weight; RDW = Root dry weight; R:S = Root to shoot ratio and RV = Root volume)

plants (Tesfaye *et al*., 2008). In the present study, it was observed that non-stressed plants had significantly higher leaf and stem dry weights than those subjected to soil drying. In agreement with these results, the findings of Mingo *et al*. (2004) and Zegbe *et al*. (2004) and Wakrim *et al*. (2005) on tomatoes and common bean plants respectively, have shown significant reduction of shoot dry mass as a result of soil drying in split-root system. Besides, decrease in shoot dry matter, reduction in root dry weight has also contributed to decline in total dry matter yield of water-stressed plants in most of the coffee clones in the present study. In contrast, it has been shown that soil drying considerably increased root biomass in tomato (Mingo *et al*., 2004) but did not affect biomass allocation to the roots of hot pepper (Dorji *et al*., 2005).

Nevertheless, the increase in root: shoot ratio of water-stressed coffee plants in the present work may indicate that dry matter accumulation in roots is less hampered by moisture stress than its partitioning in the shoot system. In line with this, it has been reported that biomass allocation to roots is usually increased even when total plant biomass is decreased by water stress (Poorter and Nagel, 2000). In general, changes in total dry matter yield and root: shoot ratio are considered among sensitive parameters during genotype evaluation for drought tolerance, indicating the ability of a genotype to exploit the environment in which it grows and adapts to stress conditions (Mingo *et al*., 2004; Tesfaye *et al*., 2008).

In conclusion, clone IC-6 and IC-3 exhibited quite a better performance in almost all the parameters considered in this study and seemed to be less sensitive to water stress than the other coffee clones. Drought tolerance attributes in these clones could be linked to some morphological modifications, mainly increased root: shoot ratio and reduced SLA (increased leaf thickness).

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