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Enhanced Drought Tolerance of Arabica Coffee (*Coffea arabica* L.) by Grafting Method

(Peningkatan Toleransi Kemarau Kopi Arabica (*Coffea arabica* L.) melalui Kaedah Cantuman)

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ABSTRACT

The objective of this study was to evaluate grafting method to improve the drought tolerance of Coffea arabica*. Using* C. arabica *species as scions, and* C. robusta *as rootstock, the grafted plant was compared with the non-grafted plant* (C. arabica) *under water deficit condition. The result shown that growth parameters such as plant height, leaf length, and leaf width of the grafted coffee plants were higher than those of the non-grafted. The leaf area, fresh and dry weight of plants were highly reduced in non-grafted coffee plants. The leaf chlorophyll content (SPAD) and chlorophyll fluorescence (Fv/Fm) values of the grafted and non-grafted coffee plants decreased significantly with increasing duration under water deficit condition. The SPAD and Fv/Fm values of the two coffee types were also increased significantly with increasing duration after re-watering. Compared to the non-grafted plants, higher values of SPAD, Fv/Fm and relative water content in the leaf were observed in the grafted coffee plants. Moreover, lower values of relative ion leakage were observed in the grafted coffee plants after three days of water withholding and one month after re-watering. On the other hand, the grafted coffee plants showed enhanced drought tolerance by reducing the percentages of wilting plant under water deficit condition, and increasing the recovery percentages after re-watering.*

Keywords: Coffee; drought tolerance; grafting; growth; physiology

ABSTRAK

Objektif kajian ini adalah untuk menilai kaedah cantuman untuk meningkatkan toleransi kemarau Coffea arabica*. Dengan menggunakan spesies* C. arabica *sebagai skion dan* C. robusta *sebagai stok akar, tanaman yang dicantumkan dibandingkan dengan tanaman yang tidak dicantumkan* (C. arabica) *dalam keadaan kekurangan air. Hasil kajian menunjukkan bahawa parameter pertumbuhan seperti tinggi tanaman, panjang daun dan lebar daun tanaman kopi yang dicantumkan lebih tinggi berbanding tanaman kopi yang tidak dicantumkan. Luas daun, berat tanaman segar dan kering sangat berkurang pada tanaman kopi yang tidak dicantumkan. Nilai kandungan klorofil daun (SPAD) dan pendarfluor klorofil (Fv/Fm) tanaman kopi yang dicantum dan tidak dicantumkan menurun dengan ketara dengan peningkatan tempoh dalam keadaan kekurangan air. Nilai SPAD dan Fv/Fm daripada kedua-dua jenis kopi juga meningkat dengan ketara selari dengan peningkatan tempoh selepas penyiraman semula. Berbanding dengan tanaman yang tidak dicantumkan, nilai SPAD, Fv/Fm dan kandungan air relatif dalam daun dilihat lebih tinggi pada tanaman kopi yang dicantumkan. Tambahan pula, nilai kebocoran ion relatif yang lebih rendah diperhatikan pada tanaman kopi yang dicantumkan setelah tiga hari tidak disiram dan satu bulan setelah penyiraman semula. Sebaliknya, tanaman kopi yang dicantumkan menunjukkan peningkatan toleransi kekeringan dengan mengurangkan peratusan tanaman layu dalam keadaan kekurangan air dan meningkatkan peratusan pemulihan setelah penyiraman semula.*

Kata kunci: Cantuman; fisiologi; kunci; pertumbuhan; toleransi kemarau

Introduction

In almost all coffee growing areas, drought is considered as one of the major climatic limitations for coffee plant growth and yield (DaMatta & Ramalho 2006). Different species of coffee may also differ in morphological and physiological mechanisms that allow them to produce reasonably well under limited water supply (DaMatta 2004). Many reports have been published on the morphology, physiology, and biochemistry of both Arabica and Robusta coffee with respect to drought (Barros et al. 1997; Carr 2001; DaMatta 2004; DaMatta & Ramalho 2006; DaMatta et al. 1993; D'Souza et al. 2009; Cheserek & Gichimu 2012). However, not much progress has been achieved in breeding for the drought resistance of coffee worldwide because it is a long-term process.

Grafting has been used for a long time to increase the uniformity, vigor and resistance to biotic and abiotic stresses of vegetatively propagated plants (Lee & Oda 2003; Rivero et al. 2003). Genotypes of Arabica coffee have been found to differ in drought adaptation mechanisms, such as stomata control and soil water extraction efficiency (DaMatta & Ramalho 2006), plant water use, and biomass allocation to the stems and leaves (Dias et al. 2007). On the other hand, Robusta coffee showed a deeper root system (Pinheiro et al. 2005) and larger root dry mass in drought-tolerant clones, than in drought-sensitive ones (DaMatta & Ramalho 2006). Development of deep roots to increase the soil water catchment and biophysical control of water loss through reducing leaf area and closure of stoma can be used by the plant to maintain a positive plant water status (DaMatta et al. 2003).

Utilization of drought tolerant species as rootstocks with high water use efficiency and carbon exchange rates for commercially important Arabica cultivars seems to be a better approach in dealing with drought stress problems, and to improve the productivity of coffee. However, information on enhancing the drought tolerance of coffee plant by using grafting is limited. Therefore, the objectives of this study were to evaluate the morphological response of non-grafted (*C. arabica*) and grafted coffee plants (*C. arabica* species was used as scions, while *C. robusta* was used as rootstock) under water deficit condition, in order to determine the ability of the grafting method to improve the drought tolerance of coffee plants.

Materials and Methods

PLANT MATERIAL AND SOIL CONDITIONS

Six hundred seeds of two coffee species (*C. arabica* and *C. robusta*) were sown in plastic bags that had been filled with mountain soil. The soil was obtained from the Son La province (at the mountain in North Vietnam). Table 1 shows the chemical properties and particle sizes of soil used in this study. One month after germination, seedlings were fertilized by overhead irrigation once a week with modified Hoagland solution (Hoagland & Arnon 1950). Two months after germination, the same seedlings were used for grafting. One month after grafting, seedlings were transplanted to plastic pots.

TABLE 1. The chemical properties and particle size of soil in this study

Parameters	Particle size (mm)	Chemical properties and particle size
Particle size distribution in soil material (%)	2 - 0.02	28.60
	0.02 - 0.002	42.70
	< 0.002	28.70
OC (%)		0.97
Humic (%)		0.43
pH_{KCl}		5.10
N (mg/100 g)		0.97
P_2O_5 (mg/100 g)		0.43
K_2O (mg/100 g)		10.90
Ca^{2+} (mg/100 g)		0.70
Mg^{2+} (mg/100 g)		2.33
Fe^{3+} (mg/100 g)		34.60
Cl^- (mg/100 g)		8.16
Mg^{2+} (mg/100 g)		80.30
Zn^{2+} (mg/100 g)		3.20
Cu^{2+} (mg/100 g)		61.10

GRAFTING METHOD, HEALING, AND ACCLIMATIZATION PROCESS

Grafting was done by the splice grafting method according to Lee and Oda (2003), where *Coffea arabica* was used as scions, and *C. robusta* was used as rootstock. Firstly, plants were grafted by making approximate 45° cuts on both the rootstock and scion seedlings by using a sharp razor blade. After placing the scion on the rootstock, ordinary grafting clips were used to fix the grafted position, and hold the rootstock and scion tightly together. Rootstock and scion plants with similar stem diameters were chosen to increase the grafting success rate. After grafting, grafted plants were covered with plastic-film to maintain high relative humidity, before the grafted seedlings were placed at 25 °C in a growth room for 10 days. After 10 days, grafted seedlings were transferred to a plastic greenhouse in natural condition. The grafting clips were removed 10 days after plants were taken out of the growth rooms.

EXPERIMENTAL DESIGN

The pot experiment was carried out in a plastic greenhouse at Vietnam National University of Agriculture. Each of plastic pots (with 15 and 12 cm top and bottom diameters, respectively, 13 cm depth, and 8 bottom perforations) was filled with 2 kg mountain soil obtained from the Son La province, Vietnam. In order to investigate the drought stress tolerance of grafted coffee plant, the experiment was designed as a split-plot with 90 plants for one treatment in three replications. In this experiment, two soil water regimes (well-watered and water stress) were assigned as the main factor. The two coffee types (the non-grafted coffee plants and grafted coffee plants) were assigned as the sub factor.

WATER MANAGEMENT

Two months after transplanting to plastic pots, the non-grafted and grafted coffee plants were used for drought stress treatment. A modified method of Zhang et al. (2018) was used for drought stress treatment. For well-watered treatment, plants were irrigated until the end of the experimental period. For water stress treatment, plants were irrigated as well-watered treatment for two months after transplanting to plastic pots, and the irrigation was withheld for 6 days in 60 plants for plant growth and physiology measurements, and for 10 days in 30 plants for the determination of wilted and recovered plants. After 6 or 10 days of water stress, the plants were re-watered.

DATA COLLECTION AND ANALYSIS

Growth traits such as plant height (cm), leaf length (cm), and leaf width (cm) were measured weekly. Six days after water withholding and one month after re-watering, plants were randomly selected to determine the leaf area. The leaf areas (cm^2) were measured by leaf area meter (Delta-T Device Ltd., Burwell, Cambridge, UK). One month after re-watering, plants were randomly selected for determination of fresh and dry weight (g/plant). The fresh plants were dried in an oven (MOV-212F, Sanyo Electric Co., Ltd., Osaka, Japan) at 80 °C for 72 h, before measuring the dry matter.

The leaf chlorophyll content and chlorophyll fluorescence were measured in treated plants from the second day after water stress begins until the fifth day of re-watering. The chlorophyll content was measured by a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Osaka, Japan). Three days after water withholding and one month after re-watering, the coffee plants were harvested for determination of leaf relative water content and relative ion leakage. After seven and ten days of water withholding, 30 plants per treatment were used to calculate the percentages of wilted plants. Three days and five days after re-watering, 30 plants per treatment were used to calculated percentages of recovery plants. Percentages of wilting or recovery plants were calculated when 75% of leaves per plant withered or recovered, respectively.

To determine the chlorophyll fluorescence (Fv/Fm), a portable fluorometer (model OS- 30p, Opti-Sciences Chlorophyll Fluorometer, Hudson, USA) was used to measure the initial fluorescence (F0), maximum fluorescence (Fm) and potential quantum efficiency of photosystem II (Fv/Fm). From these fluorescence data, the following parameters were calculated: variable fluorescence (Fv = Fm - F0), and the effective absorbed energy conversion efficiency of photosystem II (Fv/F0). Fluorescence determinations were performed between 08:00 h and 11:00 h on the same leaves. These leaves were submitted to a 30-minute dark adaptation period using leaf-clip holders, so that all the reaction centers in that foliar region acquired the 'open' configuration, indicating the complete oxidation of the photosynthetic electron transport system.

To measure the relative water content (RWC) of the leaf, 9 samples of 10 leaf discs, one per treatment were made up. Leaves were taken from the youngest fully expanded leaves. Leaf discs were immediately weighed (fresh weight; FW). These samples were floated in distilled water (temperature range: 25 - 30 °C) inside the porous platform in order to obtain turgid weight (TW). At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80 °C for 48 h to obtain the dry weight (DW). Values of FW, TW, and DW were used to calculate RWC using the equation below:

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Relative ion leakage was also assessed by the leakage of electrolytes from the leaves of ten plants of similar size. Leakage of electrolytes was determined by a conductivity meter (AG 8603, SevenEasy, Mettler Toledo, Switzerland). The leaf segments (disks of leaves with d = 1 cm^2) were washed, blotted dry, weighted, and put in stopped vials filled with the exact volume of deionized water. The vials were then incubated for 2 h in darkness with continuous shaking before the conduction (C_1) was measured. The vials were heated at 80 °C for 2 h, and the conduction (C_2) was measured again. The electrolyte leakage was expressed as a percentage of relative ion leakage, which was calculated according to this equation (Zhao et al. 2007):

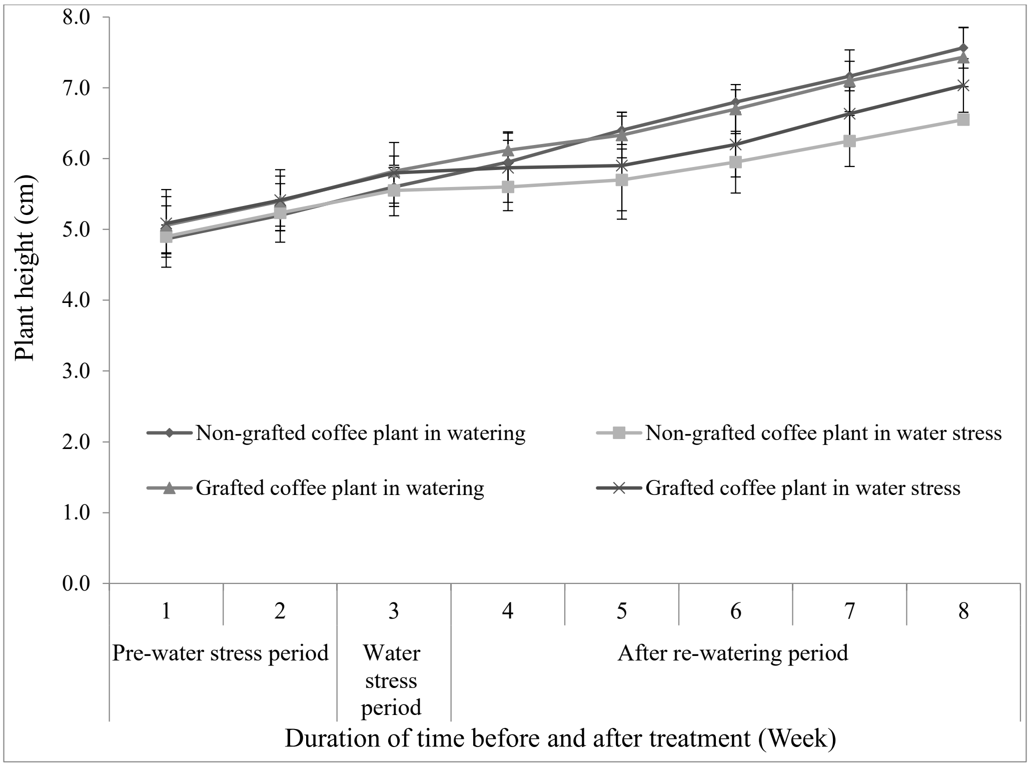
$$Relative\ electrolyte\ leakage\ (\%) = \frac{\mathrm{C1}}{\mathrm{C2}} \times 100$$

Data were analyzed using IRRISTAT 5.0. Mean separations were calculated using Duncan's multiple range tests at $p < 0.05$.

RESULTS

EFFECT OF WATER STRESS ON THE PLANT HEIGHT OF NON-GRAFTED AND GRAFTED COFFEE PLANTS

There was no significant difference between well-watered and water tress treatments of the two coffee types during the pre-water stress and water stress periods. But there was statistically significant difference between the well-watered and water stress treatments with plant height after the re-watering period. Comparing the two coffee types under well-watered condition, there was no



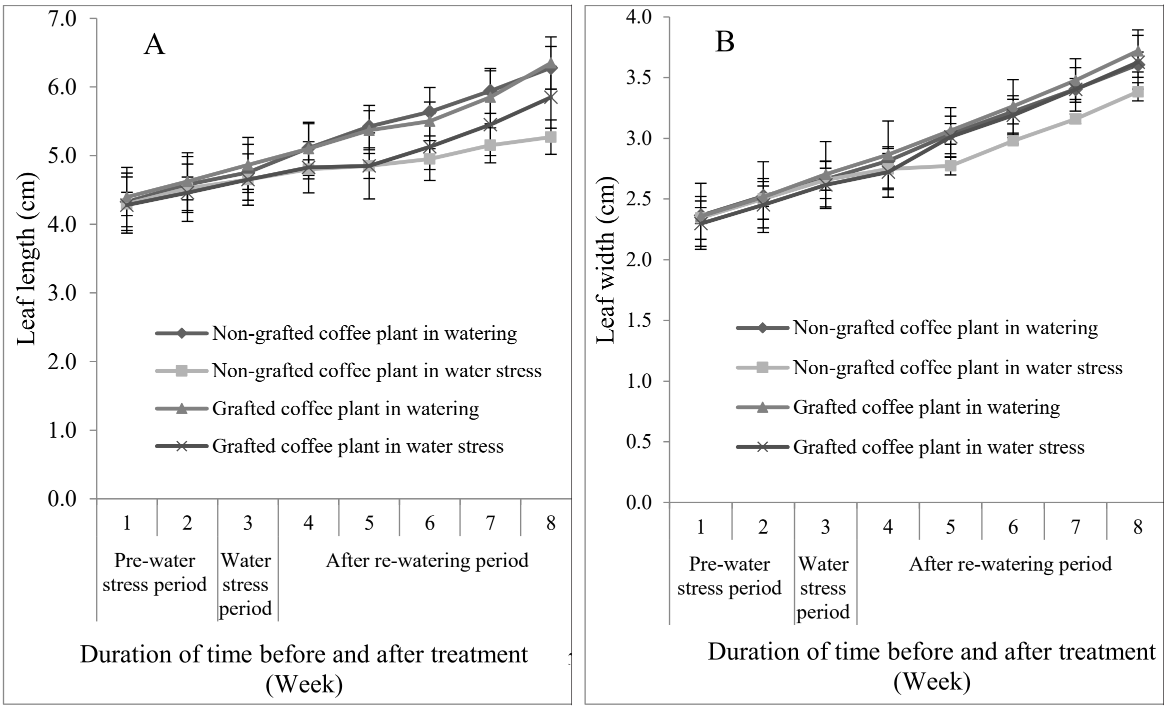
Vertical bars represent ± SD, n = 10

FIGURE 1. Effect of water stress on the plant height of non-grafted and grafted coffee plants

significant difference between non-grafted and grafted coffee plants with plant height when the duration time is increased ($p > 0.05$). However, there was significant difference between the non-grafted and grafted coffee plants with plant height under water stress condition. Comparing the two coffee types under water stress condition, the plant height of the grafted coffee plants was significantly higher than that of the non-grafted coffee plants ($p < 0.05$) (Figure 1).

EFFECT OF WATER STRESS ON LEAF LENGTH AND LEAF WIDTH OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

There was no significant difference between well-watered and water stress treatments with leaf length and leaf width of non-grafted and grafted coffee plants in the pre-water stress and water stress periods, but there was statistically significant difference between well-watered and water tress treatments with the leaf length and leaf width after the re-watering period. Comparing the two coffee types under watering condition, there was no significant difference between non-grafted and grafted coffee plants with leaf length and leaf width. However, there was significant difference between non-grafted and grafted coffee plants with leaf length and leaf width under water stress condition ($p < 0.05$). In the water stress condition, the leaf length and leaf width of the grafted coffee plants was higher than that in the non-grafted coffee plants (Figure 2).



Vertical bars represent ± SD, n=10

FIGURE 2. Effect of water stress on the leaf length (A) and leaf width (B) of non-grafted and grafted coffee plants

EFFECT OF WATER STRESS ON THE LEAF AREA OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

There was significant ($p < 0.05$) difference in leaf area of two coffee types between well-watered and water stress treatments after one-month re-watering. High percentage (11.12%) of leaf area reduction compared to well-watered treatment was observed in the non-grafted coffee plants. Lower percentage (5.62%) of leaf area reduction compared to well-watered treatment was observed in the grafted coffee plants. However, there was no significant difference in leaf area of two coffee types between well-watered and water stress treatments after 6 days without watering. At the sixth day after water stress, higher percentage (9.97%) of leaf area reduction compared to well-watered treatment was also observed in the non-grafted coffee plants, while the grafted coffee plants also indicated lower percentage (4.13%) of leaf area reduction compared to well-watered treatment (Table 2).

TABLE 2. Effect of water stress on the leaf area (cm^2) of non-grafted and grafted coffee plants

Coffee types	Treating conditions	Sixth day after treating water stress	% reduction compared to watering (%)	One month after watering	% reduction compared to watering (%)
Non-grafted plant	Well-watered	14.24[a]	9.97	18.79[ab]	11.12
	Water stress	12.82[b]		16.70[c]	
Grafted plant	Well-watered	15.62[a]	4.13	20.62[a]	5.62
	Water stress	14.97[a]		19.46[a]	
CV%		*5.1*		*3.7*	
$LSD_{0.05\ T\ x\ C}$		*1.53*		*1.75*	
Coffee types	Non-grafted plant	13.53[b]		17.75[b]	
	Grafted plant	15.30[a]		20.04[a]	
$LSD_{0.05\ C}$		*1.72*		*2.16*	
Treating conditions	Well-watered	14.93[a]		19.71[a]	
	Water stress	13.90[a]		18.08[b]	
$LSD_{0.05\ T}$		*1.25*		*1.05*	

Different letters indicate significant differences at $p < 0.05$; CV, Coefficient of variation; LSD, Least significant difference; T, Treating condition (watering condition and water stress condition); C, Coffee type

EFFECT OF WATER STRESS ON THE FRESH AND DRY WEIGHT OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

The fresh and dry weight of the non-grafted and grafted coffee plants significantly decreased ($p < 0.05$) under water stress treatment. One month after re-watering, higher value (14.02%) of the reducing percentage of fresh weight compared to well-watered treatment was observed in the non-grafted coffee plants. Lower value (4.00%) of the reducing percentage of fresh weight compared to well-watered treatment was observed in the grafted coffee plants. In addition, higher value (18.18%) of the reducing percentage of dry weight compared to well-watered treatment was also observed in the non-grafted coffee plants. Lower value (5.45%) of the reducing percentage of dry weight compared to well-watered treatment was also observed in the grafted coffee plants (Table 3).

TABLE 3. Effect of water stress on the fresh and dry weight of the non-grafted and grafted coffee plants

Coffee types	Treating conditions	Fresh weight (g/plant)	% reduction compared to watering (%)	Dry weight (g/plant)	% reduction compared to watering (%)
Non-grafted plant	Well-watered	1.07[b]	14.02	0.44[b]	18.18
	Water stress	0.92[c]		0.36[c]	
Grafted plant	Well-watered	1.50[a]	4.00	0.55[a]	5.45
	Water stress	1.44[a]		0.52[a]	
CV%		*3.4*		*4.3*	
$LSD_{0.05\ T \times C}$		*0.10*		*0.04*	
Average coffee types	Non-grafted plant	1.00[b]		0.40[b]	
	Grafted plant	1.47[a]		0.54[a]	
$LSD_{0.05\ C}$		*0.07*		*0.02*	
Average treating conditions	Well-watered	1.29[a]		0.50[a]	
	Water stress	1.18[b]		0.44[b]	
$LSD_{0.05\ T}$		*0.05*		*0.01*	

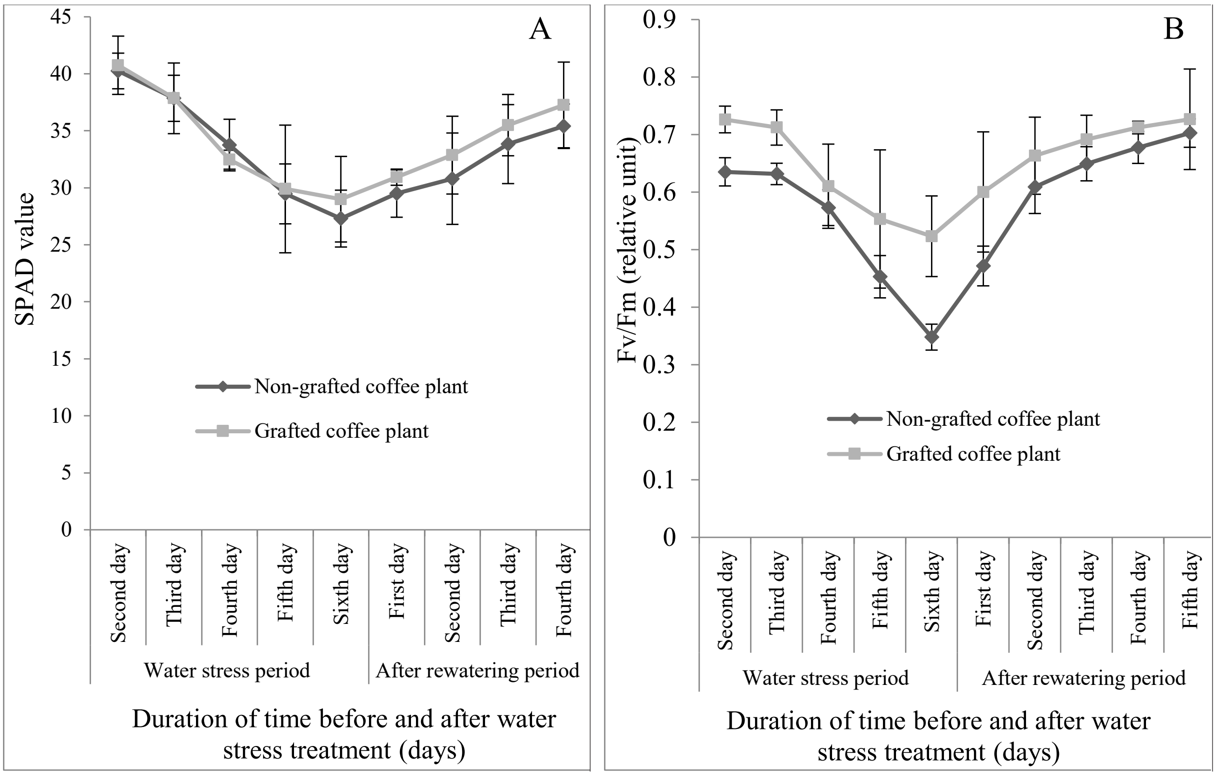
Different letters indicate significant differences at $p < 0.05$; CV, Coefficient of variation; LSD, Least significant difference; T, Treating condition (watering condition and water stress condition); C, Coffee type

EFFECT OF WATER STRESS ON THE CHLOROPHYLL CONTENT (SPAD) AND QUANTUM EFFICIENCY OF PHOTOSYSTEM II (FV/FM) OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

The chlorophyll content (SPAD) of non-grafted and grafted coffee plants decreased significantly with increasing duration in water stress treatments. However, the SPAD value of the two coffee types increased significantly with increasing duration after re-watering. Higher value of SPAD was observed in the grafted coffee plants, but there was no statistically significant difference between the non-grafted and grafted coffee plants (Figure 3(A)). The Fv/Fm value of non-grafted and grafted coffee plants decreased significantly with increasing duration in water stress treatments. However, the Fv/Fm value of the two coffee types increased significantly with increasing duration after re-watering. Higher value of Fv/Fm was observed in the grafted coffee plants (Figure 3(B)).

EFFECT OF WATER STRESS ON THE RELATIVE WATER CONTENT AND RELATIVE ION LEAKAGE IN THE LEAF OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

In the water stress treatment, higher value of the relative water content in the leaf was observed in the grafted coffee plants at both checking times (third day after without watering, and one month after re-watering). Although the relative water content in the leaf of the non-grafted coffee plants increased after one-month re-watering, the values of relative water content in the leaf of the non-grafted coffee plants was also lower than that of the grafted coffee plants (Figure 4(A)).



Vertical bars represent ± SD, n = 10

FIGURE 3. Effect of water stress on the chlorophyll content (SPAD) (A) and chlorophyll fluorescence (Fv/Fm) (B) of the non-grafted and grafted coffee plants

There was statistically significant difference in the relative ion leakage between the two coffee types (non-grafted and grafted coffee plants). After the third day without watering, higher value (40.45%) of the relative ion leakage was observed in the non-grafted coffee plants. Lower value (35.65%) of the relative ion leakage was observed in the grafted coffee plants. Although the relative ion leakage of all coffee types decreased after one-month re-watering, higher value (37.13%) of the relative ion leakage was also observed in the non-grafted coffee plants (Figure 4(B)).

EFFECT OF WATER STRESS ON THE PERCENTAGE OF WILTING AND RECOVERING PLANT OF THE NON-GRAFTED AND GRAFTED COFFEE PLANTS

There was significant difference between the percentages of wilting and recovering plant of the non-grafted and grafted coffee plants. After seven days without watering, higher percentage value of wilting plant (66.67%) was observed in the non-grafted coffee plants. Lower value of wilting plant (33.33%) was observed in the grafted coffee plants. Ten days after without watering, 100% of the wilted plant was observed in the non-grafted coffee plants, while 86.66% of the wilted plant was observed in the grafted coffee plant. After re-watering, 88.33% of the grafted coffee plants were recovered on the third day; however, only 43.33% recovered plants were observed in the non-grafted coffee plants. On the fifth day after re-watering, 100% recovered plants were recovered in the grafted coffee plants; however, only 86.66% recovered plants were observed in the non-grafted coffee plants (Table 4).

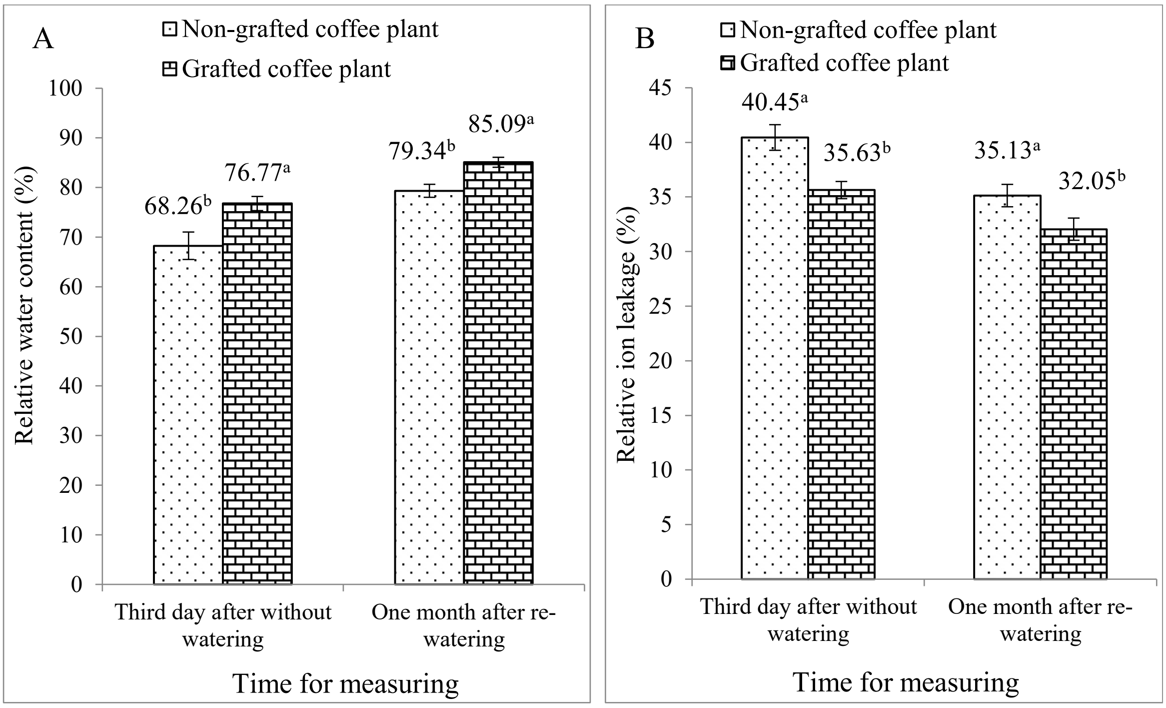


FIGURE 4. Effect of water stress on the relative water content in the leaf (A) and relative ion leakage (B) of the non-grafted and grafted coffee plants. Different letters above the columns indicate significant difference at $p < 0.05$

TABLE 4. Effect of water stress on the percentage of wilting and recovering plant of the non-grafted and grafted coffee plants

Coffee type	Wilting plant (%)		Recovering plant (%)	
	Seventh day without irrigation	Tenth day without irrigation	Third day after re-watering	Fifth day after re-watering
Non-grafted plant	66.67	100.00	43.33	86.66
Grafted plant	33.33	86.66	83.33	100.00

DISCUSSION

As a plant adjusts to water stress condition, the biomass allocation between the roots, stems, and leaves of the plant could change (Dias et al. 2007; Worku & Astatkie 2010). Decrease in growth parameters, such as shoots and leaf area under soil moisture deficit are believed to be among the important stress avoidance and tolerance mechanisms in plants (Tesfaye et al. 2014). Therefore, the plant height, leaf length, leaf width, leaf area, and fresh and dry weight of the non-grafted and grafted coffee plants under water deficit condition were lower than those under watering condition. However, the growth parameters of the grafted coffee plants were higher than those in the non-grafted coffee plants under water deficit condition. This result agreed with the results of Fahl et al. (2001), who reported that the grafted plants of *C. arabica* were taller, had more branches, and showed higher grain production than the non-grafted plants.

Several studies showed that rootstocks affected the resistance of the scion through regulating some morphological, physiological, and biochemical properties (Kumar et al. 2003; Nanda & Melnyk 2018; Patil et al. 2019). The rootstock with vigorous root system usually increased water and mineral uptake for grafted plants (Lee et al. 2010). Thus, features of the rootstocks could affect drought tolerance for grafted plants. For example, because *C. canephora* had the greater capacity of the root system than *C. arabica* in providing water to the shoot, using *C. canephora* as the rootstocks to graft *C. arabica* resulted in better performance of the grafted plants during the dry period (Fahl et al. 2001; Novaes et al. 2011). Greater gas exchange in the leaves and carbon gain were also expressed in grafted coffee plants on *C. canephora* rootstock (Fahl et al. 2001). Our study was also in agreement with those studies in which the grafted coffee plants showed better performance in water stress condition than the non-grafted coffee plants with higher values of SPAD, Fv/Fm, and relative water content, and lower value of relative ion leakage. In addition, the lower percentages of wilting plant under water deficit condition, and the higher percentages of recovering plant under re-watering were also observed in the grafted coffee plants.

Conclusion

We conclude that the grafted coffee plants had better performance in drought stress condition than the non-grafted coffee plants with higher values of the plant height, leaf length, leaf width, leaf area, the fresh and dry weight of plant, SPAD, Fv/Fm, and relative water content in the leaf, while lower value of percentage of relative ion leakage. On the other hand, the grafted coffee plants showed enhanced drought tolerance by reducing the percentages of wilting plant under drought stress condition, and increasing the percentages of recovering plant under re-watering. From these results shown that grafting of *C. arabica* on to *C. robusta* had highly favorable effects and could be used as a method to improve the drought tolerance of *C. arabica* plants.

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