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Effect of Abscisic Acid on Growth and Physiology of Arabica Coffee Seedlings under Water Deficit Condition

(Kesan Asid Absisik ke atas Pertumbuhan dan Fisiologi Anak Benih Kopi Arabica dalam Keadaan Kekurangan Air)

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ABSTRACT

In this study, the effect of abscisic acid (ABA) on growth and physiology of Arabica coffee seedlings under water deficit condition was investigated. To examine the effect of ABA concentration on growth and physiology, six ABA concentrations (0, 10, 50, 100, 150, and 200 mgL^{-1}) were applied by spraying once a day for three days. Additionally, the effect of ABA on physiology of Arabica coffee seedlings under water deficit condition was examined by using two concentrations (50 and 100 mgL^{-1}) compared to non-ABA (0 mgL^{-1}). Foliar application of ABA decreased the growth traits of coffee seedlings in all the ABA concentrations. However, no statically significant difference was observed among the 0, 10, 50, and 100 mgL^{-1} treatments with growth traits except for the leaf area. Foliar application of ABA decreased the quantum efficiency of photosystem II (Fv/Fm) of Arabica coffee seedlings in watering condition. However, there was no significant difference between 0 (control) and 10 mgL^{-1} of ABA or 50 and 100 mgL^{-1} or 150 and 200 mgL^{-1} of ABA treatment with the Fv/Fm. The application of ABA enhanced drought tolerance of coffee seedlings by increasing the leaf chlorophyll content, Fv/Fm and relative water content in the leaf and reducing the relative ion leakage in the Arabica coffee seedlings. The application of ABA increased the relative water content in the soil and delayed the starting time of wilting point under water deficit condition.

Keywords: Abscisic acid; coffee; growth; physiology; water stress

ABSTRAK

Dalam kajian ini, kesan asid absisik (ABA) terhadap pertumbuhan dan fisiologi anak benih kopi Arabica dalam keadaan kekurangan air telah dikaji. Bagi mengkaji kesan kepekatan ABA terhadap pertumbuhan dan fisiologi anak benih kopi ini, enam kepekatan ABA (0, 10, 50, 100, 150 dan 200 mgL^{-1}) telah digunakan dengan penyemburan sekali sehari selama tiga hari. Selain itu, kesan ABA terhadap fisiologi anak benih kopi dalam keadaan kekurangan air telah dikaji menggunakan dua kepekatan (50 dan 100 mgL^{-1}) dan dibandingkan dengan benih tiada-ABA (0 mgL^{-1}). Semburan daun ABA telah mengurangkan sifat pertumbuhan anak benih kopi pada semua kepekatan ABA. Walau bagaimanapun, tidak terdapat perbezaan yang signifikan secara statistik pada ciri pertumbuhan benih antara rawatan ABA 0, 10, 50 dan 100 mgL^{-1} kecuali pada kawasan daun. Semburan daun ABA menurunkan kecekapan kuantum fotosistem II (Fv / Fm) benih kopi Arabica yang diairkan. Walau bagaimanapun, tidak ada perbezaan yang signifikan antara kepekatan rawatan ABA 0 mgL^{-1} (kawalan) dan 10 mgL^{-1} atau 50 dan 100 mgL^{-1} atau 150 dan 200 mgL^{-1} dengan Fv/Fm. Penggunaan ABA telah meningkatkan ketahanan anak benih kopi terhadap kekeringan dengan meningkatkan kandungan klorofil, Fv/Fm dan kandungan air relatif di dalam daun, serta mengurangkan kebocoran ion relatif benih kopi Arabica. Penggunaan ABA juga telah meningkatkan kandungan air relatif di dalam tanah dan melambatkan waktu mula titik layu dalam keadaan kekurangan air.

Kata kunci: Asid absisik; fisiologi; ketegasan air; kopi; pertumbuhan

Introduction

Coffee is one of the most important agricultural products in the international market. It is cultivated in different latitudes around the world, but its distribution depends on many factors, such as climate, location, soil types, shading and management practices (Wintgens 2004). Among approximately 100 *Coffea* species, *Coffea arabica* and *Coffea canephora* var. *robusta* economically dominate the world coffee trade and being responsible for 98-99% of the world's coffee bean production. Minor species include *Coffea liberica* and *Coffea excels*, which are mainly restricted to West Africa and Asia, and account for only 1-2% of the global production (Wintgens 2004). Presently, Arabica coffee accounts for 62% of coffee consumed, and Robusta coffee for the rest.

Drought is an environment factor that causes water deficit or stress in coffee plants (Pinheiro et al. 2005). Drought-stress and unfavorable temperatures are considered to be the major environmental factors limiting coffee plant growth and yield in most coffee growing areas (DaMatta & Ramalho 2006). These limitations are expected to become increasingly important in several coffee growing regions due to the recognized changes in the global climate. Moreover, coffee cultivation has spread towards marginal lands, where water shortage and unfavorable temperatures constitute the major constraints to coffee yield (DaMatta & Ramalho 2006).

Abscisic acid (ABA) is a very important agent in the mechanisms of resistance and adaptation in plants against various abiotic stress conditions (Bakhsh et al. 2011; Li et al. 2010; Vu et al. 2015). It plays an essential role in many physiological processes, including seed development, dormancy, germination, and reproduction (Finkelstein et al. 2002; Planes et al. 2015). In addition, it plays a pivotal role in abiotic stress tolerance (Leung & Giraudat 1998). ABA mediates responses to environmental stresses, such as heat, cold, salt, drought, and high irradiance (Cousson 2009; Larkindale & Knight 2002; Pospisilova et al. 2009; Taylor et al. 2000). Therefore, the objectives of this study were to evaluate the enhanced drought tolerance of Arabica coffee seedlings by application of ABA in order to improve the growth and physiology of coffee seedlings during the transplanting period from nursery to farm.

Materials and Methods

Plant Material and Soil Condition

The seeds of Arabica coffee were sown in a plastic bag that had been filled with mountain soil. One month after germination, the seedlings were fertilized at an overhead irrigation once a week with modified Hoagland solution (Hoagland & Arnon 1950). Three months after germination, the seedlings were transferred to pots (with top and bottom diameters of 15 cm and 12 cm, respectively; depth of 13 cm; and 8 bottom perforations), which were filled with 3 kg mountain soil. Two months after transplanting to plastic pots, the plants were used for treating. The soil for this experiment was obtained from the mountain at Son La province of Vietnam country. The chemical properties and particle sizes of soil used in this study are shown in Table 1.

ABA Application and Water Stress Treatment

Two experiments were carried out in a plastic house at the Vietnam National University of Agriculture. The effect of ABA concentrations on growth and physiology of coffee seedlings in the watering condition was examined. Two months after transplanting to plastic pots, five ABA concentrations (10, 50, 100, 150, and 200 mgL^{-1}) and control (non-ABA) were applied by spraying once a day for 3 days. This experiment was arranged in a completely randomized design with 60 plants for one treatment in three replications. In this experiment, the Fv/Fm parameter was measured after 10 days of treatment under plastic house condition. One month after treatment, ten seedlings per treatment were randomly selected for determination of growth traits, such as plant height (cm), leaf length (cm), leaf width (cm), leaf area (dm^2), leaf chlorophyll content (SPAD), fresh and dry weight of shoots and roots (g). The leaf area was measured by leaf area meter (Delta-T Device Ltd., Burwell, Cambridge, UK). The leaf chlorophyll content was measured using a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Osaka, Japan). The fresh shoots and roots 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 effect of ABA concentrations on the physiology of Arabica coffee seedlings in a water deficit condition was carried. In order to investigate the tolerance to drought stress, two months after transplanting to plastic pots, the coffee seedlings were applied by spraying once a day for 3 days with 0 (control), 50 and 100 mgL^{-1} of ABA. The coffee seedlings were fully irrigated with water after spraying, and then the irrigation was withheld until wilted seedlings were observed. For the individual physiology traits, the wilted seedlings were treated with and without re-watering. This experiment was arranged in a completely randomized design with 90 plants for one treatment in three replications. Physiology parameters, such as leaf chlorophyll content and Fv/Fm, were measured in treated plants from without watering to five days after re-watering. Six days after without watering and one month after re-watering, the coffee seedlings were harvested for analysis of relative water content in the leaf and relative ion leakage. A percentage of the wilted plants was measured in 30 seedlings per treatment until night day after without watering. For the relative water content in soil measurement, 24 h after treatment, eight pots of each treatment were covered with vinyl film to prevent water loss from the soil.

A portable fluorometer (model OS-30p; Opti-Sciences Chlorophyll Fluorometer, Hudson, USA) was used to measure the initial fluorescence (F_0), maximum fluorescence (Fm) and potential quantum efficiency of photosystem II (Fv/Fm). From these fluorescence data, the following parameters were calculated: Variable fluorescence ($Fv = Fm - F_0$) and effective absorbed energy conversion efficiency of photosystem II (Fv/F_0). Fluorescence determinations were performed between 08:00 h and 11:00 h on the same leaves used to evaluate the gas exchanges. They were then submitted to a 30 min dark adaptation period using leaf-clip holders, so that all the reaction centers in that foliar region acquired the 'open' configuration, indicating complete oxidation of the photosynthetic electron transport system.

Relative water content in the leaf (RWC) was assessed from the leaves of eight seedlings of similar sizes. The leaves were taken from the youngest fully expanded leaves. Ten leaf discs per plant were made up. Leaf discs were immediately weighed (fresh weight; FW). These samples were floated in distilled water inside the porous platform, in order to obtain turgid weight (TW) at a temperature range of 25-30 °C. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80 °C for 48 h in order to obtain dry weight (DW). Values of FW, TW and DW were used to calculate RWC, by using (1):

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100 \qquad (1)$$

Relative ion leakage was also assessed by the leakage of electrolytes from the leaves of eight seedlings of similar size. Leakage of electrolytes was determined by using 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, and then conduction (C_1) was measured. The vials were heated at 80 °C for 2 h and conduction (C_2) was measured again. The electrolyte leakage was expressed as a percentage of the relative ion leakage, which was calculated according to the equation (2) (Zhao et al. 2007):

$$\text{Relative electrolyte leakage (\%)} = \frac{C_1}{C_2} \times 100\% \qquad (2)$$

For the relative water content in the soil measurement, the potted seedlings covered with vinyl film were weighed daily. The decrease in pot weight was used as an estimate of relative water content. Percentage of wilted plant in drought stress were calculated when 75% of leaves per seedling withered.

DATA ANALYSIS

Data were analyzed by using the SAS v.9.3 software (SAS Institute Inc., Cary, NC, USA). The mean separations were calculated by using the Duncan's multiple range tests at $P \leq 0.05$.

RESULTS AND DISCUSSION

EFFECT OF ABA ON GROWTH AND PHYSIOLOGY OF SEEDLINGS IN WATERING CONDITION

Inhibition of plant growth by ABA has been reported in many papers (Alves & Setter 2000; Carrow 1996; Sharp et al. 1994; Vu et al. 2015). In this experiment, the plant height, leaf length and width of coffee seedlings decreased with increasing ABA concentration (Table 2). The highest values of the plant height, leaf length and width were observed in the control treatment (0 mgL^{-1}), but not statically different for seedlings treated with 10, 50, and 100 mgL^{-1} of ABA. There were not statistically different between the control and 10 mgL^{-1} of ABA with leaf area. However, the leaf area decreased significantly in seedlings treated with 50, 100, 150, and 200 mgL^{-1} of ABA (Table 2). On other hand, leaf chlorophyll content also decreased with increasing ABA concentration. However, no statistically difference was shown among 0, 10, 50, 100, 150, and 200 mgL^{-1} of ABA. This result agrees with those of Farooq and Bano (2006) in mung bean plants and Vu et al. (2015) in tomato plant. They also showed that the application of ABA causes a decrease in the chlorophyll content in plants.

After one month treating with ABA, the fresh and dry weight of shoots, leaves, and roots of the coffee seedlings decreased with increasing ABA concentration. This result agrees with previously reported data that ABA generally inhibits shoot and root growth (Munns & Cramer 1996; Vu et al. 2015). Although the fresh and dry weight of shoots, leaves, and roots of coffee seedlings decreased with increasing ABA concentration. However, there was no statistical difference among the 0, 10, 50, and 100 mgL^{-1} treatments for fresh and dry weight of the shoots, leaves and roots. The lowest values of fresh and dry weight of the shoots, leaves and roots were obtained in 200 mgL^{-1} treatment, but they were not statistical different from the 150 mgL^{-1} treatment. The highest values of the fresh and dry weight of the shoots, leaves and roots were obtained in the non-ABA treatment (0 mgL^{-1}) (Table 3).

In the watering condition, the quantum efficiency of photosystem II (Fv/Fm) decreased with increasing ABA concentration (Figure 1). This result agrees with that of Sewelam et al. (2017) in *Triticum aestivum* seedlings, which also showed that the application of ABA caused decreases in Fv/Fm. In this experiment, the highest value of Fv/Fm (0.81) was observed in the control treatment, but it was not statistically significant with 10 mgL^{-1} of ABA. The lowest value of Fv/Fm

(0.775) was observed in 200 mgL^{-1} of ABA. However, it was not statistically significant with 150 mgL^{-1} of ABA. There was also no significant difference between 50 and 100 mgL^{-1} of ABA treatment for Fv/Fm.

EFFECT OF ABA ON PHYSIOLOGY OF SEEDLINGS IN WATER DEFICIT CONDITION

The effect of ABA concentrations on the chlorophyll content of coffee seedlings is shown in Figure 2. Significant difference was shown in the chlorophyll content of three treatments in water stress and after re-watering periods. The chlorophyll contents of all the treatments significantly decreased with increasing duration of treating water stress. However, the chlorophyll content of all the treatments significantly increased with increasing duration after re-watering. In the water deficit condition, the lowest value of chlorophyll content was observed in control. The highest value of chlorophyll content was observed in 100 mgL^{-1} of ABA, but it was similar to that of 50 mgL^{-1} of ABA. This result agrees with that of Anbarasi et al. (2015) in *Suaeda maritima* plant, which also showed that the application of ABA increased the chlorophyll a, chlorophyll b and total chlorophyll.

The quantum efficiency of photosystem II (Fv/Fm) of all treatments decreased significantly with increased water deficit duration (Figure 3). However, the lowest value of Fv/Fm was observed in the control. The highest value of Fv/Fm was observed in 100 mgL^{-1} of ABA, but it was similar to that of 50 mgL^{-1} of ABA. After re-watering, the Fv/Fm of all the treatments significantly increased with increasing duration time. However, the higher values of the Fv/Fm were also observed in the ABA treatments. This result agrees with that of Wang et al. (2010) in cucumber seedlings, which also showed that the application of ABA pre-treatment caused an increase of Fv/Fm in the water deficit condition. Therefore, in the water stress condition, the Fv/Fm values of coffee plants in ABA treatments were higher than that in the control in both water stress and after re-watering periods.

Relative water content in leaves is contemplated as a potential indicator of plant water status because it is involved in the metabolic activity in tissues. Decline in relative water content in leaves reflects a loss of turgor that results in limited cell expansion and thereby reduced growth in crop plants (Ashraf 2010; Lu et al. 2010). In this study, ABA application induced an increase in relative water content in the leaves of coffee seedlings under water stress condition (Figure 4). These results are in agreement with those of Agarwal et al. (2005) who reported that exogenous application of ABA increased the relative water content in the leaves in wheat under water stress. In addition, a higher value of relative water content in the leaves after pretreatment with ABA has been also recorded for other plants, such as tall fescue (Jiang & Huang 2002), tobacco (Pospısilova et al. 2005) and tomato (Vu et al. 2015). Therefore, in this study, ABA application induced an increase in relative water content in the leaf of coffee seedlings in drought stress. The highest value of relative water content in the leaf was observed in 100 mgL^{-1} of ABA, and it was similar than that of 50 mgL^{-1} ABA. Although the relative water content in the leaf of the control increased after one-month re-watering, but it was still lower than that of 50 and 100 mgL^{-1} of ABA treatments.

Electrolyte leakage is another component connected with different abiotic stresses. In this study, we found that ABA treatment led to a significant decrease in the relative ion leakage (Figure 5). These results are in agreement with those of Hala and Ghada (2009), who reported that foliar spraying of wheat plants with 50 and 100 μM ABA decreased the percentage of relative ion leakage in severe stressed plants. On the other hand, Vu et al. (2015) also showed that the application of ABA enhanced stress tolerance (cold and drought) of tomato seedlings by reducing the relative ion leakage. In this study, the highest value of relative ion leakage was observed in the control treatment. The lowest value of relative ion leakage was observed in 100 mgL^{-1} of ABA, and it was similar to that of 50 mgL^{-1} treatments. Although relative ion leakage in the control decreased after one-month re-watering, it was still higher than that of 50 and 100 mgL^{-1} of ABA.

The relative water content significantly decreased during the period without irrigation. However, relative water content increased with increasing ABA concentration (Figure 6). The ABA application enhanced drought tolerance of coffee seedlings by delaying the start time of wilting point. In the control, the start time of the wilting point was observed on the 6th day. However, in 50 and 100 mgL^{-1} of ABA, the start times of wilting point were observed on the 8th and 9th day without irrigation, respectively.

ABA is known to protect plants from drought damage by inducing stomata closure to reduce water loss via transpiration (Borel et al. 1997; Li et al. 2000) and increasing hydraulic conductance for water movement from roots to leaves (Ludewig et al. 1988; Zhang et al. 1995). Likewise, in our study, the application of ABA enhanced the drought tolerance of coffee plants by delaying the starting time of the wilting point under water stress condition (Table 4). This result agrees with other studies to report that ABA improved drought tolerance *Pinus banksiana* (Rajasekaran & Blake 1999), *Tradescantia virginiana* (Frank & Farquhar 2001) and *Lycopersicon esculentum* (Vu et al. 2015). In addition, the study of both spray and drench applications of 500 mgL^{-1} ABA on a variety of popular bedding plants demonstrated a delayed time to wilting from 3 days in marigolds to 4 days in petunia (Waterland et al. 2010). Likewise, in the study of tomato, ABA has shown to enhance drought tolerance of seedlings by delaying the start time of wilting point from day 3 in the control to day 5 and 7 in the 50 and 100 mgL^{-1} ABA treatments (Vu et al. 2015).

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

Parameters		The 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

TABLE 2. Effect of ABA concentrations on growth characteristics of Arabica coffee seedlings in the watering condition

ABA concentration (mgL^{-1})	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (dm^2)	SPAD[z]
0 (Control)	20.03 a	9.65 a	6.24 a	3.67 a	45.5 a
10	19.97 a	9.55 a	6.10 a	3.33 ab	45.1 a
50	19.33 ab	9.34 ab	5.84 ab	3.18 b	45.6 a
100	19.02 b	9.23 ab	5.81 ab	3.11 b	45.7 a
150	18.45 b	9.12 b	5.31 b	2.96 c	43.8 a
200	18.15 b	9.08 b	5.21 b	2.72 c	43.5 a

[z]SPAD is the value of leaf chlorophyll content.

TABLE 3. Effect of ABA concentrations on fresh weight and dry weight of shoots, leaves and roots of Arabica coffee seedlings in the watering condition

ABA concentration (mg/L)	Fresh weight (g)			Dry weight (g)		
	Shoot	Leaf	Root	Shoot	Leaf	Root
0 (Control)	3.13 a	7.59 a	4.73 a	1.18 a	2.02 a	1.40 a
10	3.08 a	7.56 a	4.60 a	1.15 a	2.01 a	1.40 a
50	2.85 ab	7.16 ab	4.26 ab	1.05 ab	1.92 ab	1.25 ab
100	2.78 ab	7.02 ab	4.12 ab	1.02 ab	1.90 ab	1.24 ab
150	2.52 b	6.82 b	3.87 b	0.83 b	1.77 b	1.12 b
200	2.37 b	6.26 b	3.12 b	0.72 b	1.61 b	1.03 b

TABLE 4. Effect of ABA concentrations on percentage of wilted plant of Arabica coffee seedlings after without irrigation

ABA concentration (mgL^{-1})	Percentage of wilted plant after without irrigation (%)								
	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Eighth day	Ninth day
0 (control)	0.0	0.0	0.0	31.5	75.7	100.0	-	-	-
50	0.0	0.0	12.5	37.5	45.5	57.8	74.6	100.0	-
100	0.0	0.0	0.0	18.8	43.8	50.3	65.8	85.7	100.0

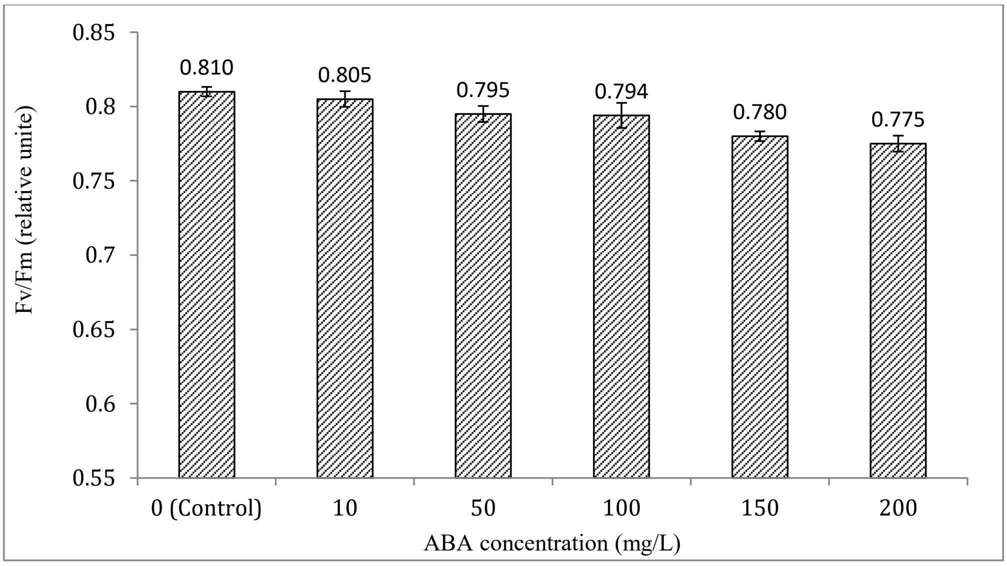

FIGURE 1. Effect of ABA concentrations on the quantum efficiency of photosystem II of Arabica coffee seedlings in the watering condition. Vertical bars represent ± SD, n = 10

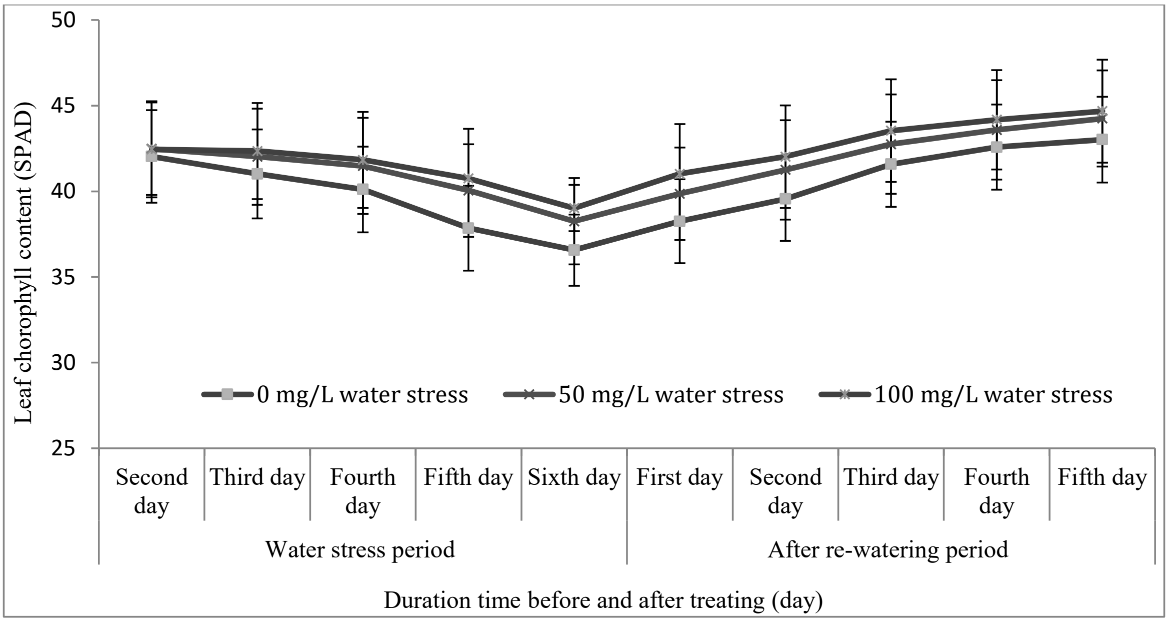

FIGURE 2. Effect of ABA concentrations on leaf chlorophyll content of Arabica coffee seedlings in water deficit condition. Vertical bars represent ± SD, n = 10

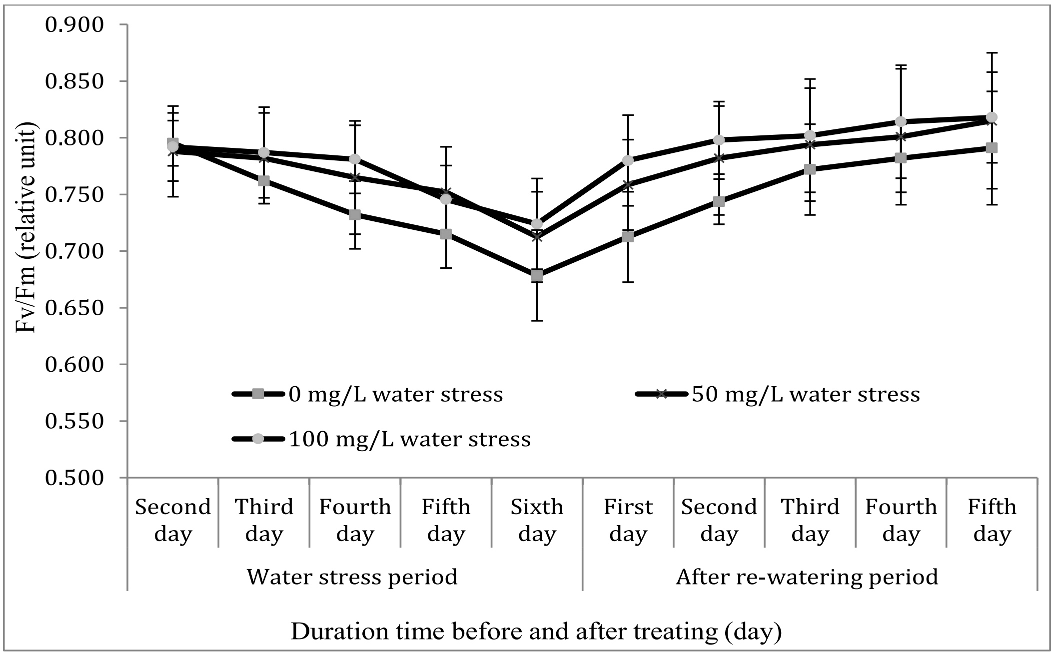

FIGURE 3. Effect of ABA concentrations on the quantum efficiency of photosystem II (Fv/Fm) of Arabica coffee seedlings in water deficit condition. Vertical bars represent ± SD. n = 10

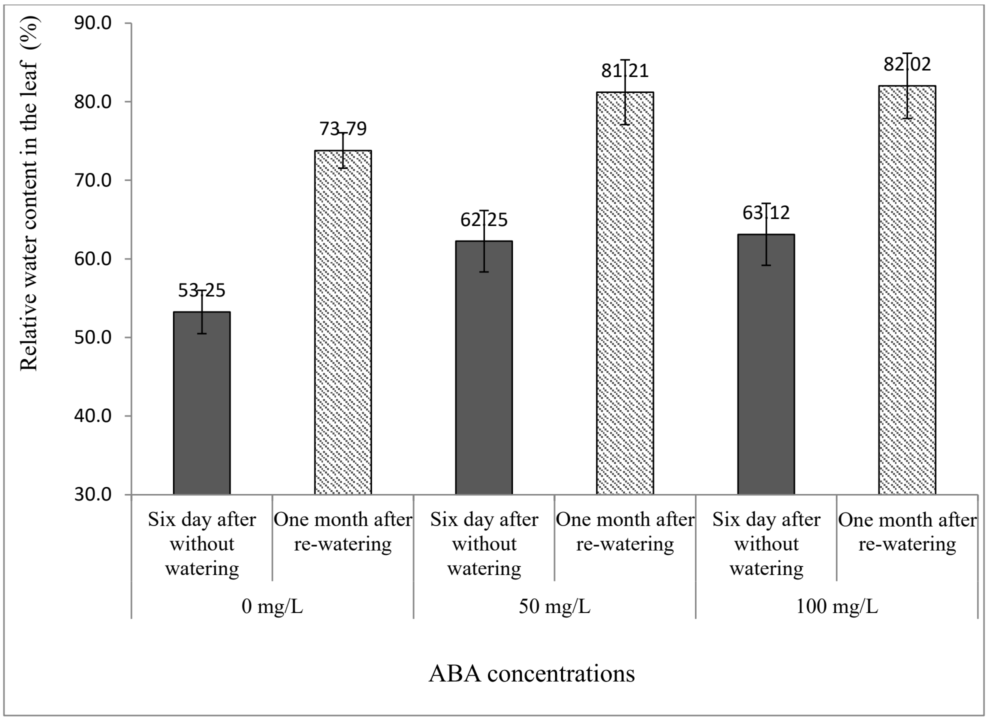

FIGURE 4. Effect of ABA concentrations on relative water content in the leaf of Arabica coffee seedlings in water deficit condition. Vertical bars represent ± SD, n = 8

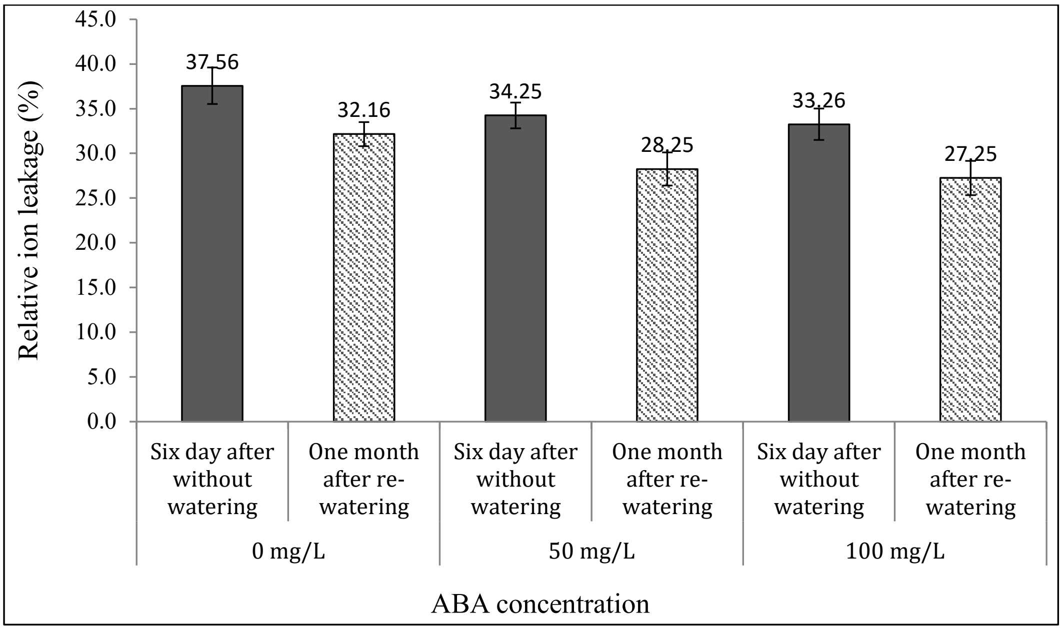

FIGURE 5. Effect of ABA concentrations on relative ion leakage of Arabica coffee seedlings in water deficit condition. Vertical bars represent ± SD, n = 8

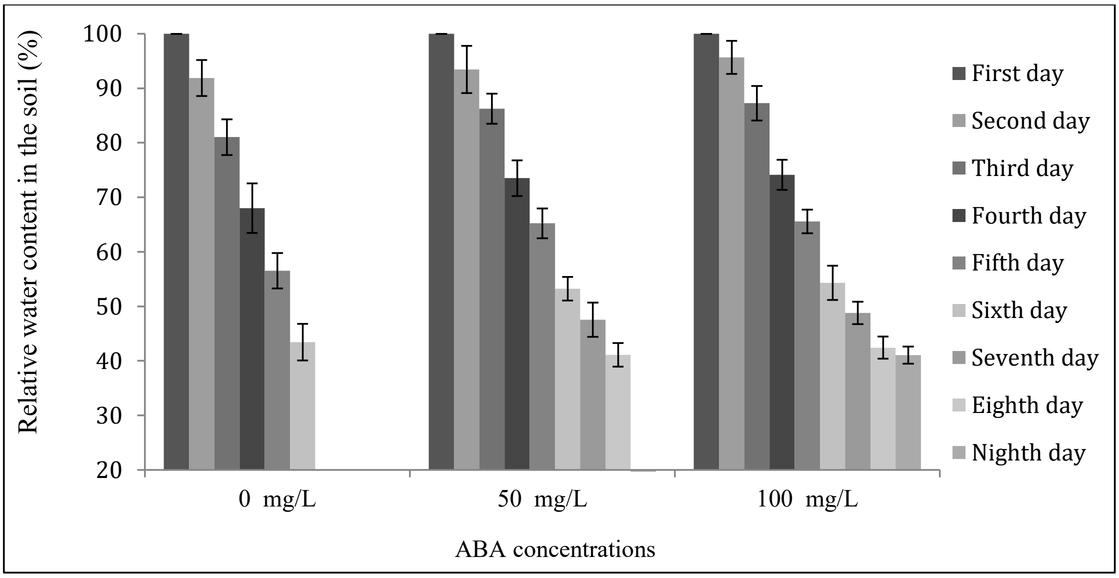

FIGURE 6. Effect of ABA concentrations on relative water content in the soil of Arabica coffee seedlings in water deficit condition. Vertical bars represent ± SD, n = 8

CONCLUSION

The foliar application of ABA reduced the growth and physiology traits of Arabica coffee seedlings in watering condition. However, the foliar application of ABA enhanced the drought tolerance in Arabica coffee seedlings by increasing the leaf chlorophyll value, Fv/Fm and relative water content in the leaves and decreasing the percentage of relative ion leakage. On the other hand, the application of ABA increased the relative water content in the soil and delayed the starting time of wilting point under water stress condition. From the results, it can be shown that 50 mgL^{-1} ABA is the optimal foliar concentration that can maintain of growth and physiology of Arabica coffee seedlings in water deficit condition.

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