

Wild type and *vte₄* mutant *Arabidopsis thaliana* responses to different water frequencies: genetic engineering towards stress tolerance

A. A. Khalatbari^{1*}, H. Z.E. Jaafar^{1*}, A. M. Khalatbari¹, M. Mahmood² and R. Othman³

¹Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, 43400. ²Department of Biochemistry, Faculty of Biotechnology and Biomolecular sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, 43400. ³Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, 43400. *Corresponding author: amkh_united2002@yahoo.com . drhawazej.postgrads@gmail.com.

Abstract

Growth and development of plants are severely affected once exposed to soil water deficit. In addition, plants experience different levels of water stress in which their adaptation and response will be different. In this paper, we sought to investigate the growth, development and water relations of both wild-type (Col-0) and *vte₄* mutant *Arabidopsis thaliana* under different water frequencies. These water treatments, including control condition, 4 days of water withholding and 8 days of water withholding were imposed on the plants. Each water treatment was replicated three times in a complete randomized design with factorial arrangement. Wild type and mutant *A.thaliana* plants were subjected to the abiotic stress (water stress) for up to 24 days. The results indicated that under water stress, the performance of wild type plants were stronger than *vte₄* mutants. Under control condition, specific leaf area, rosette dry weight and rosette dry weight at bolting of wild type *A.thaliana* scored the highest values of 47.66 mm²/mg, 13.67 mg and 201.5 mg, respectively in comparison with *vte₄* mutant. However, both wild type and *vte₄* mutant plants were negatively affected as the water treatments continued. The root mass fraction showed an increase in *vte₄* mutant and wild type *A.thaliana* as they scored 0.136g/g and 0.17 g/g, respectively. Under the same treatment, water potential indicated a reduction for both plan types where *vte₄* and wild type plants obtained the values of -1.4 and -1.3 MPa, respectively after 24 days of stress initiation. As a result, our findings suggested that different water treatments significantly differed in growth characters in which the absence of γ -tocopherol methyltransferase (γ -TMT) gene in *vte₄* had an impact on the plant's response towards the water deficit.

Keywords: *Arabidopsis thaliana*, water stress, wild type, mutant

1. Introduction

Plants encounter water stress either when the transpiration rate gets intense or when the root water supply becomes limiting. In first place, water stress is the result of the water deficit, drought or high salinity of soil (Wang *et al.*, 2014). As an instance, when plants experience high soil salinity or low soil temperature, water remains in soil solution but plants cannot uptake it. This situation is regarded as 'physiological drought'. Yearly drought happens in many parts of the world, regularly affecting field grown plants under arid and semi-arid climates. It is that regions with adequate but non-uniform precipitation also experience water limiting environments (Jenks and Hasegawa 2008). In recent years, many studies have indicated that the average yields from the major crop plants may decrease by more than 50% exposed to different stresses. However, plants also have demonstrated the developed adaptations to stresses with a series of physiological and biochemical interventions that involves the function of many stress-related genes (Vinocur and Altman 2005). Drought is addressed as a situation which results in reducing plant water potential and turgor to the extent that eventually plants encounter difficulties in performing normal physiological functions (Farooq *et al.*, 2009). Drought is as an abiotic stress which is found to be multidimensional in nature, and it has a huge effect on plants at various levels of their organization (Yordanov *et al.*, 2000). It is the fact that under prolonged drought, many plants dehydrate and die. Water stress in plants lowers the plant-cell's water potential and as a result their turgor reduces, which escalate the solutes' concentrations in the cytosol and extracellular matrices (Yordanov *et al.*, 2000). Consequently, cell division and enlargement decrease leading to growth inhibition and reproductive failure (Schuppler *et al.*, 1998). As a result, accumulation of abscisic acid (ABA) and compatible osmolytes like

proline will occur which cause wilting. At this point, formation of radical scavenging compounds such as a glutathione and ascorbate and over production of reactive oxygen species (ROS) exacerbate the adverse influence (Hamilton *et al.*, 2000; Pei *et al.*, 2000). Genetic engineering and plant biotechnology have provided the new pathways for the biosynthesis of various solutes into plants, causing the production of transgenic plants with improved stress tolerance and resistance. Over expression of stress-associated genes in transgenic plants resulted in improved stress tolerance as well (Wang *et al.*, 2003). Plant modification in terms of enhancing tolerance is majorly based on the manipulation of genes that maintain and protect the structure and function of cellular components. It has to be said that the genetically responses to abiotic stress conditions such as water and salt stresses are more complex to control and engineer. Recent engineering methods depend on the transfer of one or several stress-associated genes (Umezawa *et al.*, 2006).

Arabidopsis thaliana is a widely used model organism in different fields of study and it is indispensable to determine the optimal conditions of its maximum growth. Therefore, the physiological and morphological study of this plant under various oxidative stresses such as water stress can be made possible (Heath *et al.*, 2013). As drought conditions are experienced by plants, different ecotypes of *Arabidopsis* demonstrate various response strategies, some employing an escape strategy, while other apply a tolerance strategy (Meyre *et al.*, 2001). Considering different response strategies affecting growth and development of the plants, the type of the plant must be taken into consideration when analyzing results (Meyre *et al.*, 2001). Recent studies on (*vte*) *A.thaliana* mutants as a vitamin E-deficient plant have revealed that one of the most prominent functions tocopherols play is acting as antioxidants

in chloroplasts, thus protecting plants from photo-oxidative stress and photoinhibition (Havaux *et al.*, 2005). Hence, studying the response of mutant plants (*vte*) compared to wild type of *A.thaliana* under varied water stress seems crucial and will provide important information on mechanism of the plants against this abiotic stress. The objectives of this study were: 1) to determine the effect of different water frequency of 4 and 8 days interval and control (daily watering based on field capacity) on growth parameters of wild type and mutant (*vte₄*) plants 2) to investigate water relations of these *A.thaliana* types subjected to different water frequency 3) to evaluate the effect of mutant (*vte₄*) and wild type plants on morphological traits.

2. Material and Methods

2.1. Plant material and growth conditions

Seeds of *A.thaliana*, genotype Colombia-0 and *vte₄* mutant, were used in this experiment (The seeds were a gift from Dean DellaPenna, Department of Biochemistry, Michigan State University), and rinsed in running tap water. Seeds were sterilized in 70% (v/v) ethyl alcohol for 1 min, followed by rinsing twice with sterile deionized water. After thorough rinsing in sterilized water, the surface-sterilized seeds were sown in soil mix of commercial potting soil/vermiculate (2:1) kept under a 12 h photoperiod at 20 °C under standard cool white fluorescent bulbs at a photon flux density of 120-150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in growth chamber. Germination started within 2 to 4 days of sowing. The range of humidity was 50-75%.

2.2. Water treatment

Controlled watering was imposed to all plants until stage 1.04 (4th leaf is approximately 1 mm in size), after which watering continued for control plants (daily

watering with soil relative water content of 0.35-0.45 g water /g dry soil; corresponding to a predawn water potential of -0.35 Mpa), but then 4 days and 8 days interval was imposed to wild type and *vte₄* mutant plants with the same soil water content of control plants (Hummel *et al.*, 2010).

2.3. Growth, water relations and Fv/Fm analysis

In this experiment, the number of visible leaves was counted on 3 plants per treatment. The specific leaf area of plants was measured by LICOR 3100. The rosette dry weight and rosette dry weight at bolting were measured after drying the leaves in an oven at 80 °C for 24 h. Relative water content (RWC) and specific leaf area were determined on both the youngest fully expanded leaves and the whole rosette on five leaves or rosettes per treatment. Fresh weight (FW) was measured immediately after excision. RWC was determined as $100 \text{ (FW - DW) / (TW - DW)}$, where FW is the fresh weight, TW is the turgid weight after re-hydrating the leaves at 4 °C in darkness for 24 h, and DW is the dry weight after oven-drying the leaves at 80 °C to constant weight. Roots were collected and their dry weights measured separately (root mass fraction was calculated as root dry weight/plant dry weight) (Hummel *et al.*, 2010). Chlorophyll fluorescence parameters including initial fluorescence (F₀), maximum fluorescence (F_m), variable fluorescence (F_v), and maximum quantum efficiency of PSII (F_v/F_m) were observed from leaves. The F_v/F_m was determined by using a pulse-modulated fluorimeter mini-PAM (Walz) after 1 h of dark adaptation, as described (Gentry *et al.*, 1996 ; Khalatbari *et al.*, 2014).

2.4 . Experimental design and statistical analysis

Three watering regimes representing 100% of field capacity (control), 4 days and 8 days of water withholding

were imposed on the plants. Each watering treatment was replicated three times in a complete randomized design (CRD) with factorial arrangement. Data were analyzed based on simple ANOVA, using SAS computer package (SAS Institute Inc., 2007). Duncan New Multiple Range Test (DNMRT) was used for comparison of means of quantitative traits. Correlations between variables were established by SPSS16. Normality test was used to determine if a data set is well-modeled by a normal distribution and to compute how likely it is for a random variable underlying the data set to be normally distributed

3. Results

The application of different water treatments had significant effects on both wild type and *vte₄* mutant *Arabidopsis* plants. The experiment length was two months where there were apparent differences in terms of growth parameters in either plant type or water regimes. However, no interaction was observed between different water frequency and plant type in this study (Table 1).

Table 1. ANOVA factorial arrangement based on complete randomized designed (CRD) for growth parameters of *vte₄* mutant and wild type *Arabidopsis thaliana* after 24 days of water deficit onset (except for rosette dry weight at bolting).

SOV	d.f	specific leaf area	rosette dry weight	rosette dry weight at bolting	root mass fractions	relative water content	water potential	PDII (Fv/Fm)
A(Water Frequency)	2	954.05**	85.57**	34415.16**	0.0315**	0.0452**	1.77**	0.00190**
B(Varieties)	1	60.50**	2*	56.89**	0.005**	0.008**	0.067**	0.00027**
AB	2	0.5ns	0.46ns	0.722ns	0.00015ns	0.00010ns	0.00055ns	0.000005ns
C.V		4.46	5.33	1.52	5.32	3.37	10.73	0.67

** : Significant at ($P < 0.01$), * : Significant at ($P < 0.05$), ns: not significant

3.1. Effect of different water frequency on growth parameters of *arabidopsis* plants

As a conclusion, analysis of variance indicated that specific leaf area decreased for both wild type and mutant (*vte₄*) plants by imposing the water stress (Table 2). It should be noted that the highest value was observed for control condition after 24 days of water stress onset where it scored 47.66 mm²/mg. The lowest score was obtained from 8 days water withholding for specific leaf area after 24 days of water deficit initiation which recorded 22.5 mm²/mg. The specific leaf area of the whole rosette of stressed plants was reduced under severe deficit (8 days of

water withholding) by 47.2% (Table 2). In order to determine the effect of plant type on specific leaf area, *vte₄* mutant and wild type *Arabidopsis* plants were examined. After 24 days of water stress initiation, the wild type plants showed the higher score of 36.44 mm²/mg while the *vte₄* mutant value recorded was 32.77 mm²/mg (Figure 1A). It was the similar result for rosette dry weight with the decrease of the trait as the experiment continued. The highest value belonged to control condition where it gained the highest value of 13.67 mg on 24th day of water stress initiation. The value declined for rosette dry weight with 8.43 and 6.33 mg from 4 and 8 days water withholding respectively. Wild type plants indicated higher average of 9.81 mg

after 24 days of stress onset while the *vte₄* mutant one scored 9.14 mg (Figure 1B). Different water regimes indicated different results regarding root mass fraction (Table 2). The highest value was from 8 days of water withholding where the average was 0.225 g/g after 24 days of water deficit onset followed by 0.155 g/g from 4 days of water withholding. The lowest score of 0.08 g/g was recorded for control condition (Table 2). The root mass fraction enhanced by almost 31% from 4 days of water withholding and increased afterward. In order to investigate the weight of *Arabidopsis* rosette at flowering for both *vte₄* mutant and wild type plants, the rosette dry weight at bolting was measured. As the interaction of the different water regimes (frequency) and plant type for this trait was not significant, the rosette dry weight at bolting was considered separately for plant type and water regimes (Table 1). Based on different water treatments (frequency), the highest value was obtained under control condition in which the rosette dry weight at the time of bolting was

201.5 mg whereas the lowest value was obtained from 8 days of water withholding where it scored 50.84 mg (Figure 2A). Following the plant type effect, the wild type plants obtained the higher average of 123.44 mg while the *vte₄* mutant gained the value of 119.89 mg at the bolting time (Figure 2B). Within two months of the experiment, the total leaf number of wild type and *vte₄* mutant was recorded. Under different water treatments (frequency), the total leaf number indicated the different response under each treatment (Figure 3, A and B). After 24 days of stress initiation, the highest value belonged to control condition where it scored 22 leaves. It should be taken into account that the lowest value was from 8 days of water withholding with 14 leaves. In addition, 4 days of water withholding obtained 18 leaves. (Figure 3A). The plant type also showed the alteration in total leaf number. After 24 days of water deficit onset, wild type plants obtained the highest score of 19 leaves while the *vte₄* mutant plants scored 17 leaves (Figure 3B).

Table 2. Impact of three water regimes (frequency) on the growth parameters of both wild type and *vte₄* mutant *Arabidopsis thaliana* after 24 days of water deficit onset.

Parameters	Water treatments (frequency)		
	(8 days)	(4 days)	(control)
Specific leaf area (mm ² /mg)	22.5 ^c	33.66 ^b	47.66 ^a
Rosette dry weight (mg)	6.33 ^c	8.43 ^b	13.67 ^a
Root mass fraction (g/g)	0.225 ^a	0.155 ^b	0.08 ^c
Relative water content (gW/gFW)	0.69 ^c	0.77 ^b	0.86 ^a
Water potential (MPa)	-1.27 ^c	-0.63 ^b	-0.183 ^a
PSII (Fv/Fm)	0.76 ^c	0.78 ^b	0.80 ^a

Means in the same row followed by different letters indicate a significant difference according to DMRT ($P \leq 0.05$).

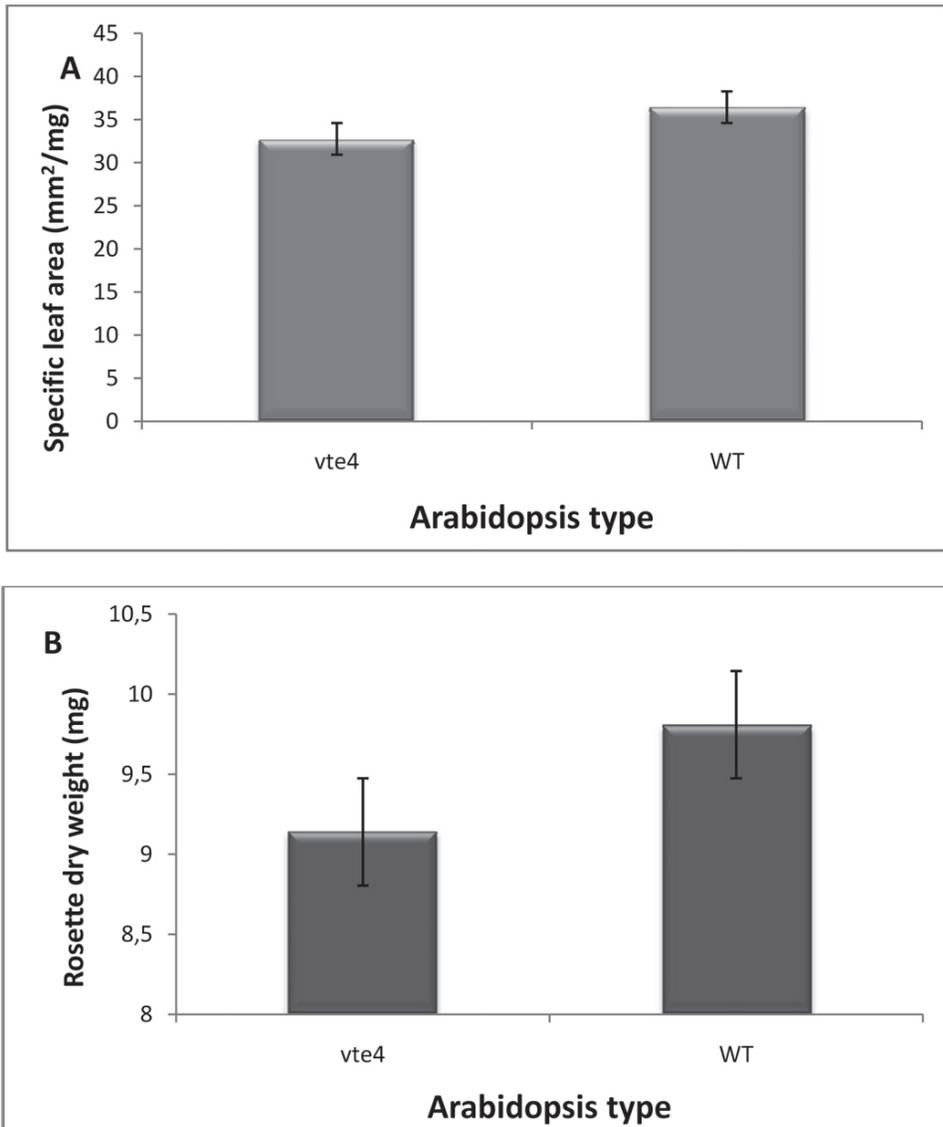


Figure 1. The effect of different plant types (wild type and *vte₄* mutant *Arabidopsis*) on (A) specific leaf area and (B) rosette dry weight after 24 days of water deficit onset.

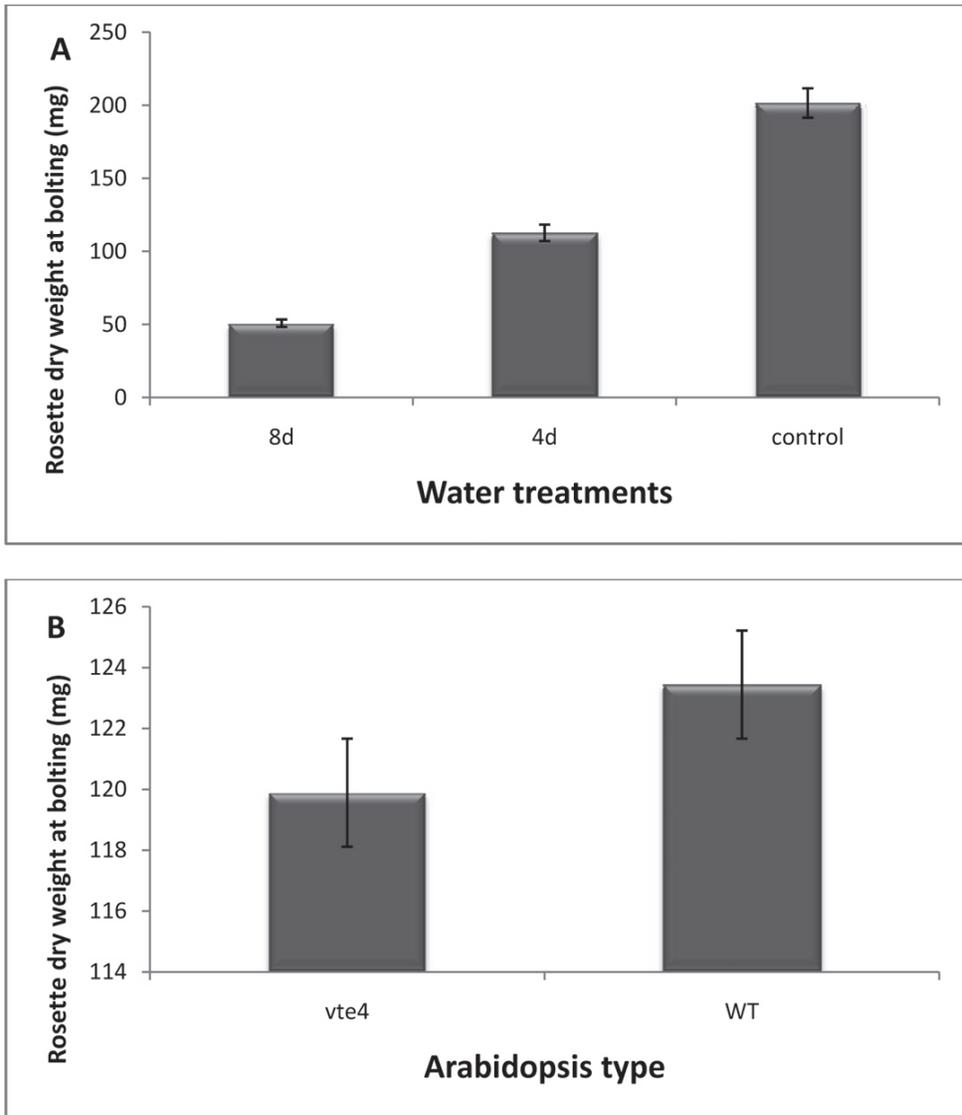


Figure 2. The effect of three water regimes (frequency) and different plant type on rosette dry weight at bolting. A: different water regimes (4 and 8 days interval and control), B: different plant type (wild type and *vte₄* mutant *Arabidopsis*).

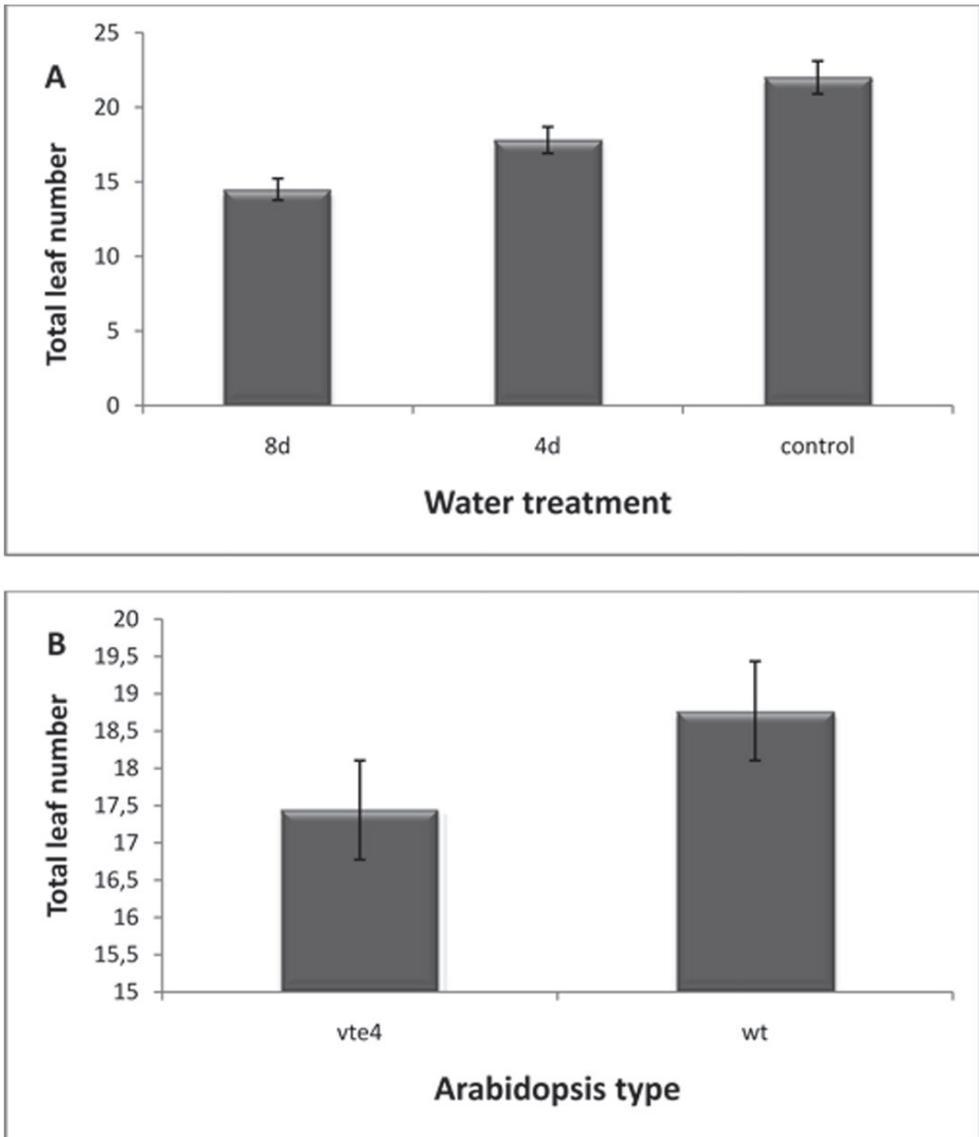


Figure 3. The effect of three water regimes (frequency) and different plant type on total leaf number. A: different water regimes (4 and 8 days interval and control), B: different plant type (wild type and *vte₄* mutant *Arabidopsis*).

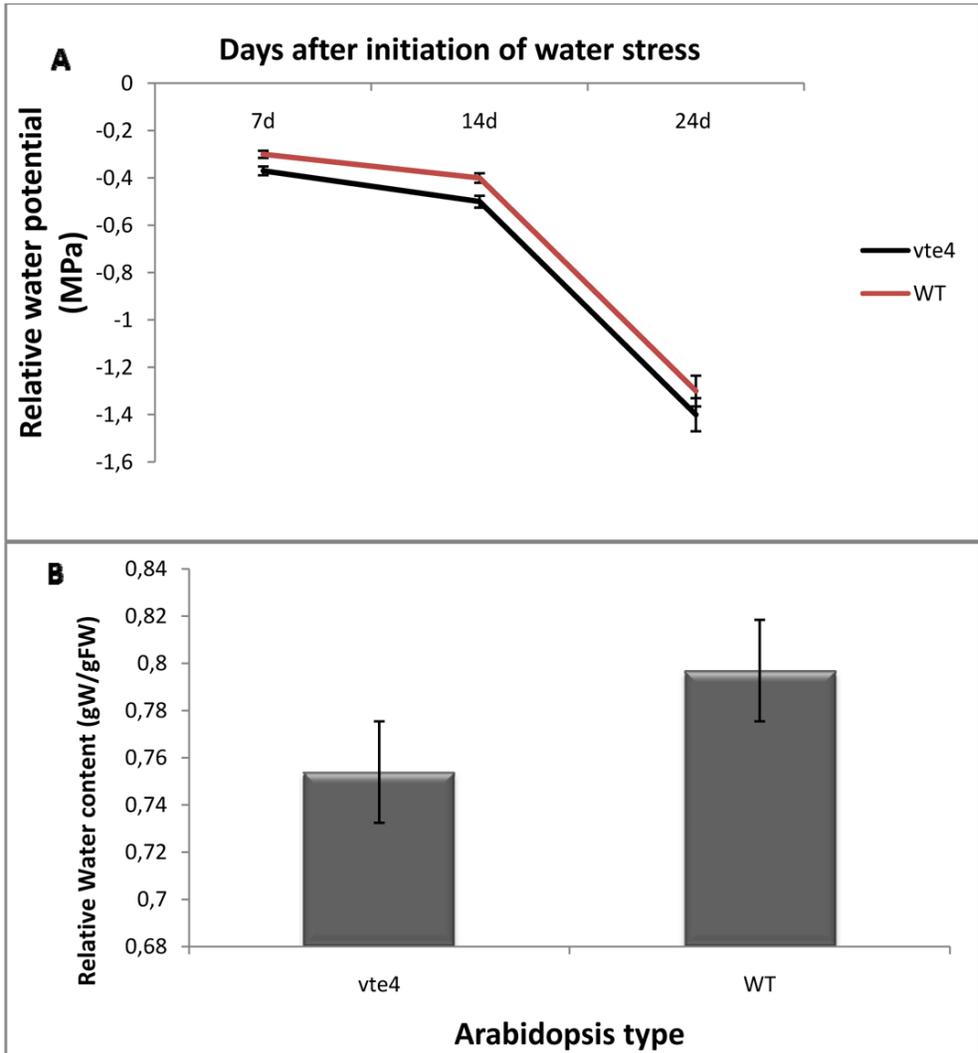


Figure 4. The effect of different plant types (wild type and *vte₄* mutant *Arabidopsis*) on relative water potential (A) and on relative water content (B) after 24 days water deficit onset.

3.2. Assessment of water relations in arabidopsis plants

To assess the water relations in *A.thaliana*, relative water potential was estimated for both *vte₄* mutant and wild type plants (Table 2). Control treatment indicated the highest value of -0.183 MPa after 24 days of stress initiation. The lowest average was gained from 8 days of water withholding with the value of -1.27 MPa in which the 4 days interval scored -0.63 MPa (Table 2). Additionally, the plant type effect was determined by measuring the water potential for both *vte₄* mutant and wild type plants. Wild type *Arabidopsis* plants obtained the average of -0.4 MPa after plants were stressed for 14 days in which the mutant recorded the average of -0.5 MPa (Figure 4A). In order to estimate relative water potential after 24 days of water withholding, wild type and *vte₄* mutant plants were examined. The wild type plant showed the higher value of -1.3 MPa whereas the *vte₄* mutant scored -1.4 MPa (Figure 4A). The relative water content was measured under three different water treatments (frequency) (Table 2). The highest value was recorded under control condition where it scored 0.86 gW/gFW (g water g⁻¹ fresh weight) on 24th days of stress initiation. 8 days interval obtained the lowest average of 0.69 gW/gFW. When 4 days interval was considered, the average showed the value of 0.77 gW/gFW (Table 2). Considering the effect of plant type on the relative water content, wild type *Arabidopsis* and *vte₄* mutant plants were determined (Figure 4B). The *vte₄* mutant plants showed the lower average of 0.75gW/gFW while wild type plants scored 0.80gW/gFW after 24 days of stress initiation (Figure 4B). With enhanced level of water availability, the relative water content and relative water potential

showed an improvement in both *vte₄* mutant and wild type *Arabidopsis* plants (Arvidsson et al., 2011). The specific leaf area, rosette dry weight at bolting and total leaf number all showed strong positive correlation with relative water content. Specific leaf area and total leaf number gained (1.0) ($p<0.01$) in which rosette dry weight obtained (0.99) ($p<0.01$). In addition, there was a significant correlation between rosette dry weight at bolting with specific leaf area (1.0) ($p<0.01$) as well as total leaf number with specific leaf area (0.99) ($p<0.01$). Rosette dry weight and total leaf number also showed strong positive correlation with relative water potential (0.94) and rosette dry weight at bolting (0.99) respectively (Table 3). The linear regression was observed between rosette dry weight and water potential for both wild type and *vte₄* mutant. R² for wild type and *vte₄* mutant were 77.4% and 77.2% respectively (Figure 5A). The regression indicated that as the water potential decreased the value of rosette dry weight also showed reduction. Different levels of water treatments showed different results considering maximum quantum efficiency (Fv/Fm) (Table 2). The highest value was recorded for control condition where the average was 0.80 Fv/Fm after 24 days of stress onset followed by 0.78Fv/Fm from 4 days of water withholding. The lowest score of 0.76 Fv/Fm was from 8 days of water withholding (Table 2). To study whether plant type caused different maximum quantum efficiency (Fv/Fm) or not, the *vte₄* mutant and wild type *A.thaliana* were examined. Wild type *Arabidopsis* plants indicated the higher average of 0.79 Fv/Fm after 24 days of stress on set in comparison with the mutant which scored the average of 0.78 Fv/Fm (Figure 5B).

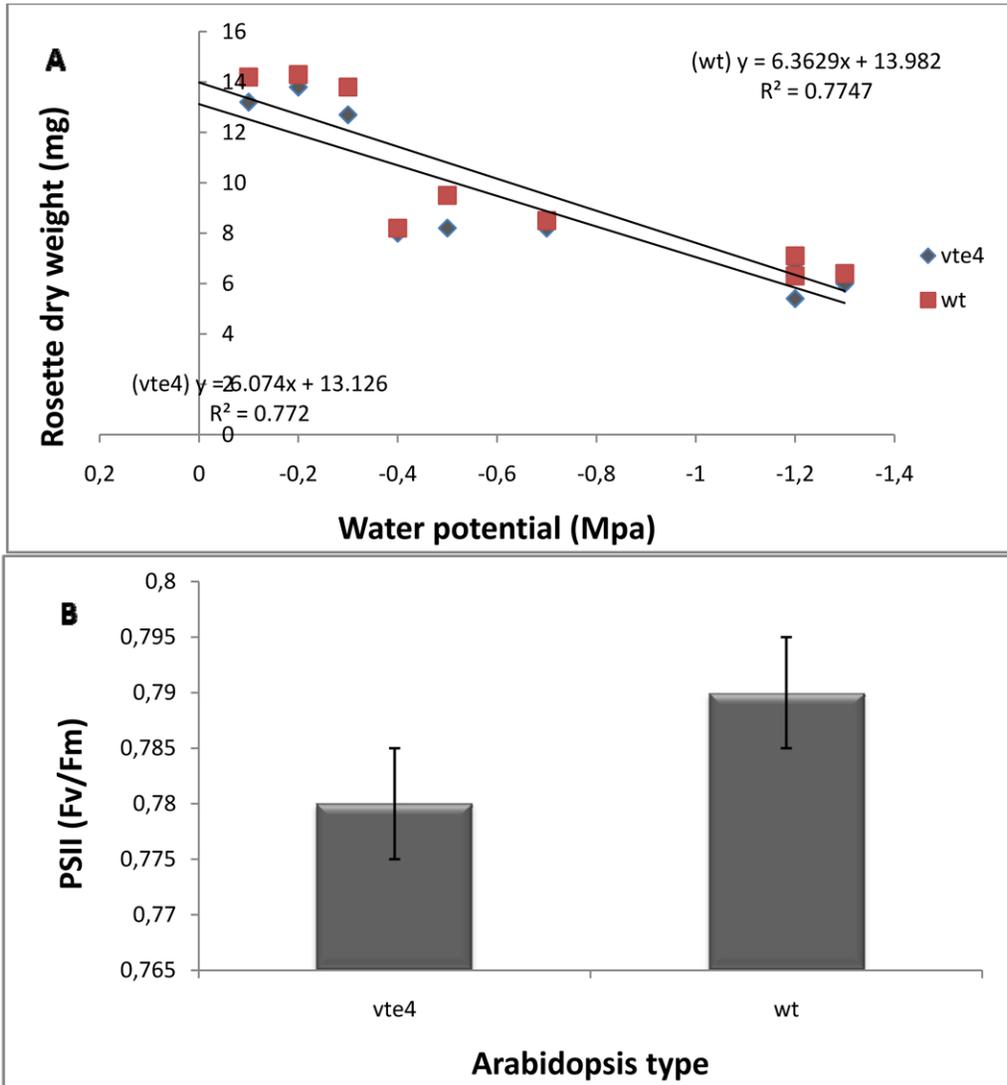


Figure 5. Simple regression between water potential and rosette dry weight of *Arabidopsis* plants (mutant *vte₄* and wild type) after 24 days of water treatment onset (A). The effect of different plant types (wild type and *vte₄* mutant *Arabidopsis*) on maximum quantum efficiency (Fv/Fm) after 24 days of water stress onset (B)

Table 3. Pearson's' correlation coefficients among the mean relative water content, specific leaf area, water potential, rosette dry weight, rosette dry weight at bolting, leaf number and root mass fraction of *vte₄* mutant and wild type *Arabidopsis thaliana* under three different water treatments (4 days, 8 days interval and control)

Parameters	1	2	3	4	5	6
1. relative water content	1					
2. specific leaf area	0.987**	1				
3. water potential	0.988	0.987	1			
4. rosette dry weight	0.983	0.984	0.942**	1		
5. rosette dry weight at bolting	0.989*	-0.998*	0.980	0.990	1	
6. leaf number	0.986*	0.988**	0.987	0.984	0.998*	1
7. root mass fraction	0.844	0.848	0.751	0.928	0.868	0.848

** . Correlation is significant at the 0.01 level

* . Correlation is significant at the 0.05 level

4. Discussion

Plants are able to cope with oxidative stresses by various physiological, biochemical, molecular, and morphological changes as well as transitions in gene expression. The plants' response to water stress in terms of their physiology at the entire level is complicated and changes can be made via adaptive or destructive mechanism. There are numbers of factors which play crucial roles when a plant encounters drought such as plant species and variety, duration and intensity of water deficit, environmental conditions, alterations in water demand from the atmosphere

and plant growth as well (Chaves *et al.*, 2002). The result of this study showed that both *vte₄* mutant and wild type *A. thaliana* plants recorded the highest value under control condition consider in morphological traits such as rosette dry weight, specific leaf area, rosette dry weight at bolting and root mass fraction in comparison with other water regimes. This result was in accordance with previous study which indicated the fact that by providing the appropriate water availability to the plant, the growth parameters obtained the higher value compared to stress environment (Meyre *et al.*, 2001). In *A. thaliana* the biomass can be considered by weighing the plant rosette dry weight. Therefore,

the reduction of rosette dry weight and rosette dry weight at bolting under water deficit resulted in biomass decrease (Meyre *et al.*, 2001). In this study the specific leaf area, rosette dry weight and rosette dry weight at bolting were reduced as the frequency of water deficit escalated. Other study revealed that by imposition of water stress, the specific leaf area was permanently and significantly decreased from day 7 onward following the onset of water deficit in the severe water deficit treatment, whereas it was only reduced 10 d after the onset of the moderate water deficit (Hummel *et al.*, 2010). It has also been reported that the relative growth rate of the rosette recorded 14 days after the onset of stress was reduced from 0.182 (mg dry weight mg⁻¹ dry weight d⁻¹) in well-watered plants to 0.144 (mg dry weight mg⁻¹ dry weight d⁻¹) and 0.119 (mg dry weight mg⁻¹ dry weight d⁻¹) under moderate and severe stress, respectively. As a conclusion, rosette biomass was decreased by around 45% after 10 days of stress initiation and by around 75% at bolting time (after 20 days of water stress onset), respectively. The root mass fraction indicated the highest value from 8 days of water withholding in comparison with other treatments in both wild type and *vte₄* mutant *A.thaliana*. Skirycz *et al.*, (2011) also reported that the water deficit resulted in root mass fraction increase and it suggests that water deficit rapidly favored root growth. The root mass fraction increased by around 30% in stressed plants as early as 7 days after stress began and this difference was maintained afterwards (Skirycz *et al.*, 2011). In order to study the water relations in *A. thaliana*, both the water potential and relative water content (RWC) were determined. Different water treatment frequency demonstrated the different effect in which water potential obtained the lowest value after 8 days of water withholding. This outcome was in agreement with Meyre *et al.*, (2001) indicating the fact that by

withholding the water for a week in *A.thaliana*, the water potential and relative water content decreased. Currently, it has been studied that *A.thaliana* vitamin E-deficient (*vte*) mutants have lacked the protective function of tocopherol (α -tocopherol) which plays an indispensable role as antioxidants in chloroplasts, thus protecting plants from oxidative stresses such as water stress (Havaux *et al.*, 2005). Alongside with other antioxidants, tocopherols are able to scavenge lipid peroxyl radicals, avoiding the propagation of lipid peroxidation and regarded as excellent scavengers and quenchers of singlet oxygen which as a result can control its levels. It is noteworthy to know that *Arabidopsis vte₄* mutant lacks α -tocopherol, but the level of γ -tocopherol is accumulated in leaves. Oxidative stress, like salinity, high temperature, or drought stress results in a serious damage on cells as a secondary effect. It should be noted that oxidative stress causes the formation of ROSs (reactive oxygen species). ROS have negative effect on membranes by eradicating them which ultimately cause damages at cellular level under drought stress. Once the level of ROS is controlled in chloroplasts, tocopherols are able to control the amounts of lipid peroxidation products and affect gene expression towards stresses such as drought. As a result, the plants show more adaptation under stress condition. The outcome of this study is in accordance with the previous statement where the wild type *A.thaliana* performed stronger under water treatments compared to *vte₄* mutant (Heath *et al.*, 2013). The results of the present study is in agreement with other study providing the idea that vitamin E (α -tocopherol) play a significant role in order to protect the plants against oxidative stresses like drought, salinity and high temperature which supports the stronger performance of wild type *A.thaliana* in comparison with *vte₄* mutant under water deficit (different frequency) (Cela *et al.*, 2011). This study

indicated that wild type *Arabidopsis* plants obtained the higher value from 4 days and 8 days of water withholding considering morphological traits including rosette dry weight, rosette dry weight at bolting and Specific leaf area. Root mass fraction demonstrated the similar result where the wild type *A.thaliana* recorded the higher score (Cela et al., 2011). In terms of total leaf number, Wild type *A.thaliana* obtained the higher value with respect to different stage of plant growth. As a conclusion, rosette biomass was reduced by about 38% from 4 days of water withholding and by about 54% from 8 days of water withholding, respectively. The similar outcome was reported by Hummel et al., where the rosette biomass was decreased under water treatments. Regarding the water relations parameters, wild type *A.thaliana* also recorded stronger values under water treatments compared to *vt_{e4}* mutant. The wild type *Arabidopsis* gained the highest values when relative water content and water potential were determined. It was stated earlier that under water treatments the wild type *A.thaliana* showed the higher value in terms of water potential compared to mutant *Arabidopsis* (Bray 2002). When the 8 days of water withholding was considered, the wild type *A.thaliana* recorded -1.3 MPa while the *vt_{e4}* mutant plant obtained the lower value of -1.4 MPa. By increasing the frequency of water withholding (from 4 day to 8 days interval), water potential showed the reduction. The relative water content (RWC) gained the lowest value from 8 days of water with holding. This result is consistent with previous study in which the relative water content reduced under water treatments (Collakova and DellaPenna 2003). As a consequence, the changes in level of water availability had negative effect on rosette weight which resulted in reduction of relative water content. These out comes suggest that vitamin E (α -tocopherol) can act as a significant

antioxidant to protect the plants against oxidative stress like water stress which is regarded as one the most important environmental constraints limiting plant production. The *vt_{e4}* mutant *A.thaliana*, which lacks α -tocopherol due to absence of γ -tocopherol methyltransferase enzyme activity, showed more vulnerability towards different water treatments (frequency) in comparison with wild type, thus indicating that an altered tocopherol composition in chloroplast of *vt_{e4}* mutant plant changes the physiological response of mutant to water deficit.

5. Conclusion

In present study in terms of growth and development, both wild type and *vt_{e4}* mutant *Arabidopsis* plants were affected in a negative way when they were subjected to different water stress frequency. The rosette dry weight loss for wild type plant was approximately 25% in which the mutant scored 37% after 24 days of stress onset (8 days interval). To evaluate the water relations of plants, soil water potential and relative water content were measured. Mutant plants (*vt_{e4}*) scored lower value compared to wild type *A.thaliana*.

These outcomes suggest that vitamin E (α -tocopherol) can act as a significant antioxidant to protect the plants against oxidative stress like water stress which is regarded as one the most important environmental constraints limiting plant production. The *vt_{e4}* mutant *A.thaliana*, which lacks α -tocopherol due to absence of γ -tocopherol methyltransferase enzyme activity, showed more vulnerability towards different water treatments (frequency) in comparison with wild type, thus indicating that an altered tocopherol composition in chloroplast of *vt_{e4}* mutant plant changes the physiological response of mutant to water deficit.

Acknowledgment

The authors gratefully acknowledge Assoc. Prof. Dr Hawa Z.E. Jaafar for her kind consideration and contribution.

Acknowledgment

The authors gratefully acknowledge Assoc. Prof. Dr Hawa Z.E. Jaafar for her kind consideration and contribution.

References

- Arvidsson, S., Pérez-Rodríguez, P., Mueller Roeber, B. 2011. A growth phenotyping pipeline for *Arabidopsis thaliana* integrating image analysis and rosette area modeling for robust quantification of genotype effects. *New Phytologist*. 191(3): 895-907.
- Bray E.A. 2002. Classification of genes differentially expressed during waterdeficit stress in *Arabidopsis thaliana*: An analysis using microarray and differential expression data. *Annals of Botany*. 89, 803-811.
- Cela, J., Chang, C., Munné-Bosch, S. 2011. Accumulation of γ -rather than α -tocopherol alters ethylene signaling gene expression in the *vte4* mutant of *Arabidopsis thaliana*. *Plant and cell physiology*. 52, 1389-1400.
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, I., Faria, T., Pinheiro, C. 2002. How plants cope with water stress in the field?. *Photosynthesis and growth*. *Annals of Botany*. 89, 907-916.
- Collakova, E., Della Penna, D. 2003. The role of homogentisatephytyl transferase and other tocopherol pathway enzymes in the regulation of tocopherol synthesis during abiotic stress. *Plant Physiology*. 133(2): 930-940.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. 2009. Plant drought stress: effects, mechanisms and management. *Sustainable Agriculture*. Springer: 153-188.
- Gentry, T.W., Fredin, G.J., Riedl, D.A. 1996. Method for partitioning disk drives within a physical disk array and selectively assigning disk drive partitions into a logical disk array. Google Patents.
- Hamilton, D.W., Hills, A., Köhler, B., Blatt, M.R. 2000. Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. *Proceedings of the National Academy of Sciences*. 97, 4967-4972.
- Havaux, M., Eymery, F., Porfirova, S., Rey, P., Dörmann, P. 2005. Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. *The Plant Cell Online*. 17, 3451-3469.
- Heath, M., Kim, M., Marchand, M., Yang, S. 2013. Growth Response of *Arabidopsis thaliana* to Varied Water Stress. *The Expedition 2*.
- Hummel, I., Pantin, F., Sulpice, R., Piques, M., Roland, G., Dauzat, M., Christophe, A., Pervent, M., Bouteillé, M., Stitt, M. 2010. *Arabidopsis* plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiology*. 154, 357-372.
- Jenks, M.A., Hasegawa, P.M. 2008. Plant abiotic stress. Wiley-Blackwell.
- Khalatbari, A.M., Jaafar, H.Z.E., Ali Khalatbari, A.A. 2014. The impact of CO_2 enrichment on fiber dimension and lignocellulose properties of three varieties of kenaf (*Hibiscus cannabinus* L.). *Journal of soil science and plant nutrition*. 14, 676-687.
- Meyre, D., Leonardi, A., Brisson, G., Vartanian, N. 2001. Drought-adaptive mechanisms involved in the escape/tolerance strategies of *Arabidopsis Landsberg, erecta* and *Columbia* ecotypes and their F1 reciprocal progeny. *Journal of Plant Physiology*. 158, 1145-1152.

- Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G.J., Grill, E., Schroeder, J. I. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature*. 406, 731-734.
- Schuppler, U., He, P. H. John, P.C., Munns, R. 1998. Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. *Plant Physiology*. 117, 667-678.
- Skirycz, A., Vandenbroucke, K., Clauw, P., Maleux, K., De Meyer, B., Dhondt, S., Pucci, A., Gonzalez, N., Hoeberichts, F., Tognetti, V. B. 2011. Survival and growth of *Arabidopsis* plants given limited water are not equal. *Nature biotechnology*. 29(3): 212-214.
- Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., Shinozaki, K. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology*. 17, 113-122.
- Vinocur, B., Altman, A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology*. 16, 123-132.
- Wang, W., Vinocur, B., Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*. 218, 1-14.
- Wang, X., Mohamed, I., Xia, Y., Chen, F. 2014. Effects of water and potassium stresses on potassium utilization efficiency of two cotton genotypes. *Journal of Soil Science and Plant Nutrition*. 14, 833-844.
- Yordanov, I., Velikova, V., Tsonev, T. 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica*. 38, 171-186.