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# EFFECTS OF SALT STRESS ON WILD TYPE AND *VTE*₄ MUTANT *ARABIDOPSIS THALIANA*: MODEL PLANT TO ENGINEER TOLERANCE TOWARDS SALINITY

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One of the major environmental constraints impairing plant distribution and yield is believed to be salt stress. Additionally, engineered abiotic stress resistance or/and tolerance is considered as an indispensable target in order to enhance plant productivity. In this study, the effects of salinity on physiological and morphological of wild type (Columbia-0) and vte4 mutant Arabidopsis thaliana were investigated under different NaCl concentrations. These salt treatments, including control condition, 50mM and 100mM NaCl were imposed on the plants. Each salt 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 (salinity) for up to 11 days to evaluate the parameters of growth, development and water relations. As a result, the performance of wild type plants was stronger than vte4 mutant under different salt treatments. Under control condition, rosette dry weight, maximum quantum efficiency (PSII) and specific leaf area obtained the highest values of 13.85 mg, 0.81 Fv/Fm and 49.84 mm<sup>2</sup>/mg, respectively. As salt treatments continued both wild type and  $vte_4$  mutant plants were affected negatively. When plant types was

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considered, wild type *A.thaliana* recorded higher value of 0.82 gW/gFW for relative water content (RWC) under 50mM NaCl whereas mutant plants gained the value of 0.78 gW/gFW under the same condition. However, root mass fraction indicated an increase for both wild type and  $vte_4$  mutant plants after 11 days of salt stress onset. The reduction of water potential was observed for wild type and mutant *A.thaliana* where it scored -1.3 MPa and -1.4, respectively. As a conclusion, these findings implied that under different salt treatments morphological and physiological responses of wild type and  $vte_4$  mutant were affected in which wild type plants showed more tolerance. Lack of  $\gamma$ -tocopherol methyltransferase ( $\gamma$  -TMT) gene in  $vte_4$  seemed to impair defence mechanism of this mutant against salinity.

Key words: Arabidopsis thaliana; mutant, salt stress, wild type

#### INTRODUCTION

Salt stress is one of the major factors regarding as a huge constraint which ultimately results in limiting agricultural productivity around the world (SHAFIEIZARGAR et al. 2013). Salinity is caused by whether saline soils or irrigation (MUNNS and TESTER, 2008). There are number of reasons that can enhance the rate of salinity including application of marginal crop land, over irrigation and uprising need for food production. Therefore producing plants with salinity stress resistance or tolerance seems to play a crucial role for crop improvement. In plants, salt stress is an outcome of osmotic-related stress or dehydration effects leading to water potential reduction. Furthermore, high level of solute concentration and damage of toxic effects of excess sodium ions are considered to result in salinity stress (YAMAGUCHI and BLUMWALD 2005; MUNNS and TESTER, 2008). Many studies showed that the accumulation of compatible solutes in plants results in resistance to different abiotic stresses like high salinity, high temperature and drought. Typically, Salt stress is regarded as one of the most important oxidative stresses (MILLER et al., 2010). Provided the plant is resistant to this specific stress, the plant will apply some certain routes to encounter these negative effects by sequestration or exclusion of sodium ions or by accumulating osmoprotectants or compatible solutes. It has been proposed that osmoprotectants are able to balance stress-related decrease in water content which causes stabilization of macromolecular structures. Free radicals increase in response to stress and compatible solutes can scavenge these free radicals accumulated by salinity stress (CHEN and MURATA, 2002). Salinity has been addressed as an oxidative stress that increases the production of ROS (Reactive oxygen species) and results in ROS-associated injury (CHINNUSAMY et al., 2005). In addition, ROS-scavenging mechanisms has been found to play an indispensable role in protecting plants against abiotic stresses like temperature stresses and high light (ABBASI et al., 2007; MILLER et al., 2008). It should be included that beside the fact that ROS have indicated the potentiality of causing oxidative damage to cells under environmental stresses, it also can play an important role in plants as signal transduction molecules conducting plant responses to environmental stresses, different developmental stimuli and cell death (TORRES et al., 2006).

In recent years, different approaches have been applied to generate stress tolerant or/and resistant plants using classical plant breeding techniques and genetic methods (KHALATBARI *et al.*, 2007; NAROUI RAD *et al.*, 2013). One way to improve plant resistance and enhancement of crop productivity under saline environments is to select genotypes that have improved yield in this condition. Currently, genetic engineering as an approach has been increasingly used in order

to produce crops for environmental stress resistance (CHAN *et al.*, 2011). Modification of plants by application of genetic engineering and plant biotechnology for increased tolerance towards salinity is largely rely on the manipulation of genes that protect and conserve the function and structure of cellular components. The responses of stress-related genes to abiotic stresses like salinity are more complicated to control and manipulate (CELA *et al.*, 2011). These innovating techniques depend on the transfer of one or several genes related with stress responsive pathways. It is noteworthy that the recent studies to enhance plant stress tolerance by genetic transformation have shown great achievements, however, the complexity of abiotic stress tolerance mechanisms has made this effort extensively difficult.

Arabidopsis thaliana as a model plant in plant biotechnology has the large number of mutants. *vte* mutants which represent vitamin E-deficient plant (lack the  $\alpha$ -tocopherol) have showed that one of the most important functions tocopherols play is acting as antioxidants in chloroplasts and protecting plants from oxidative stress like salinity (HAVAUX *et al.*, 2005). Therefore, studying the response of mutant plants (*vte*) compared to wild type of *A*-thaliana under saline environment can bring an insight into understanding of the mechanisms of the plants against this oxidative stress. The objectives of this study were: 1) to determine the effect of different NaCl concentration (50mM, 100mM and control condition) on growth parameters of wild type and mutant (*vte*<sub>4</sub>) plants 2) to study water relations of these *A*-thaliana types subjected to salinity 3) to investigate the effect of mutant (*vte*<sub>4</sub>) and wild type plants on physiological and morphological traits.

#### MATERIALS AND METHODS

## **Plant Material and Growth Conditions**

Seeds of *Arabidopsis thaliana*, genotype Colombia-0 and  $vte_4$  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 µmol m<sup>-2</sup> s<sup>-1</sup> in growth chamber. Germination started within 2 to 4 days of sowing. The range of humidity was 50-75%.

### Salt Stress 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 two NaCl concentration of 50mM and 100mM was added to wild type and  $vte_4$  mutant plants with the same controlled watering (HUMMEL *et al.*, 2010).

## Growth, Water Relations and Fv/Fm Analysis

In this experiment, the number of visible leaves was counted on 3plants 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 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 was scored

immediately after excision.RWC was determined as 100 (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). The Fv/Fm was determined by using a pulse-modulated fluorimeter mini-PAM (Walz) after 1 h of dark adaptation, as described (GENTRY *et al.*, 1996).

## **Experimental Design and Statistical Analysis**

Three different salt treatment including 50mM, 100Mm of NaCl concentration along with control condition were imposed to wild type and  $vte_4$  mutant *Arabidopsis thaliana*. Each 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.

## RESULTS

The application of different salt treatments showed significant effects on both wild type and  $vte_4$  mutant *Arabidopsis* plants. There were apparent differences in terms of morphological and physiological traits in either salt treatment or plant type within two months of the experiment. It should be noted that no interaction was observed between different salt treatment and plant type in this study (Table 1).

 

 Table 1. ANOVA factorial arrangement based on complete randomized designed 8CRD9 for growth parameters of vte mutant and wild type Arabidopsis thaliana after 11 days of salt stress onset

SOV	d.f	specific leaf area	rosette dry weight	PSII (Fv/Fm)	relative water content	water potential	Total leaf number
A	2	714.5**	74.25**	0.002**	0.047**	1.22**	0.0019**
В	1	64.22**	1.23ns	0.0003**	0.0086**	0.027ns	0.0027**
AB	2	0.39ns	0.003ns	0.00001ns	0.00015ns	0.0022ns	0.000005ns
C.V		3.87	5.30	0.66	3.44	11.25	0.66

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

#### Effect of Different Salt Treatments on Morphological Parameters of Arabidopsis Plants

The analysis of variance (ANOVA) showed that specific leaf area reduced for both wild type and mutant ( $vte_4$ ) plants by imposing the salt stress (Table 2). It noteworthy that the highest score was observed for control condition after 11 days of salt stress initiation where it recorded the value of 49.84 mm<sup>2</sup>/mg. The lowest value belonged to 100mM NaCl for specific leaf area after 11 days of stress onset which obtained 28.33 mm<sup>2</sup>/mg. When the specific leaf area of the whole rosette of stressed plants was determined under severe salt stress it indicated the reduction by 43% (Table 2).

To investigate the effect of plant type on specific leaf area,  $vte_4$  mutant and wild type *Arabidopsis* plants were studied. After 11days of salt stress initiation, the wild type plants demonstrated the higher value of 39.89 mm<sup>2</sup>/mg while the  $vte_4$  mutant obtained 36.11 mm<sup>2</sup>/mg (Figure 1 A). Rosette dry weight also indicated the reduction as the experiment was carried out. The highest value was recorded under control condition where it obtained 13.85 mg after 11 days of stress onset. The value decreased for rosette dry weight with 9.03 and 7 mg under 50mM and 100mM NaCl treatments, respectively. Interestingly, rosette dry weight was found non-significant when the plant type was determined (Table 1) (Figure 1 B).

## Table 2. Impact of salt stress treatments on the growth parameters of both wild type and yte mutant Aradopsis thaliana after 11 days of stress onset

Parameters	5	Salt treatment (NaC	1)
	(100mM)	(50mM)	(control)
Specific leaf area (mm²/mg)	28.33°	35.83 <sup>b</sup>	49.84 <sup>a</sup>
Rosette dry weight (mg)	7°	9.03 <sup>b</sup>	13.85ª
PSII (Fv/Fm)	0.77ª	0.79 <sup>b</sup>	0.81°
Relative water content (gW/gFW)	0.74°	0.79 <sup>b</sup>	0.88ª
Water potential (MPa)	-1.25°	-0.75 <sup>b</sup>	-0.35ª

Means in the same row followed by different letters indicate a significant difference according to DMRT (P=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 11 days of salt stress onset.

Different salt stress treatments showed different outcomes considering maximum quantum efficiency (Fv/Fm) (Table 2). The highest value belonged to control condition in which

the average was 0.81 Fv/Fm after 11 days of stress onset followed by 0.79 Fv/Fm under 50mM NaCl concentration. The lowest value of 0.77 Fv/Fm was under 100 mM NaCl concentration (Table 2). To evaluate whether plant type caused different maximum quantum efficiency (Fv/Fm) or not, the  $vte_4$  mutant and wild type *A.thaliana* were studied. Wild type *Arabidopsis* plants indicated the higher average of 0.8 Fv/Fm after 11 days of stress initiation compared to the mutant which obtained the average of 0.78 Fv/Fm (Figure 2).



Figure 2. The effect of different plant types (wild type and *vte*<sub>4</sub> mutant *Arabidopsis*) on maximum quantum efficiency (Fv/Fm) after 11 days of salt stress onset.

In this experiment which took 2 months, the total leaf number of wild type and  $vte_4$  mutant was determined. Under salt stress treatments, the total leaf number performed the different response under each treatment (Figure 3, A and B). After 11 days of stress onset, the highest value belonged to control condition where it scored 19 leaves. It should be noted that the lowest value was under 100 mM NaCl concentration with 13 leaves. Under 50mM NaCl concentration the total leaf number was 16 (Figure 3 A). The *Arabidopsis* plant type also indicated the change in total leaf number. After 11 days of salt stress initiation, wild type plants gained the highest value of 17 leaves whereas the  $vte_4$  mutant *A.thaliana* recorded 13 leaves (Figure 3 B).





Figure 3. The effect of three salt stress treatments and different plant type on total leaf number 4, 7 and 11 days after stress onset. A: different NaCl concentration (50mM, 100mM and control), B: different plant type (wild type and  $vte_4$  mutant *Arabidopsis*).

#### Assessment of Water Relations in Arabidopsis Plants under Salt Stress

In order to investigate the water relations in *Arabidopsis thaliana*, relative water potential was evaluated for both  $vte_4$  mutant and wild type plants (table 2).Under control condition the highest value of -0.35 MPa was obtained after 11 days of stress onset. The lowest score was recorded under 100mM NaCl concentration with the value of -1.25 MPa whereas the value of-0.75 MPa was observed under 50mM NaCl concentration (Table 2). Furthermore, the plant type effect was studied by measuring the water potential for both  $vte_4$  mutant and wild type plants. It is noteworthy that the water potential did not show any significant difference in terms of plant type (Table 2).Wild type *Arabidopsis* plants gained the average of -0.46 MPa after plants were exposed to salt stress for 7 days in which the  $vte_4$  mutant scored the average of -0.48 MPa (Figure 4). Evaluation of relative water potential for both wild type plant score the value of -1.3 MPa in which the  $vte_4$  mutant scored -1.4 MPa (Figure 4).



Figure 4. The effect of different plant types (wild type and  $vte_4$  mutant *Arabidopsis*) on relative water potential.

The relative water content was evaluated under three different salt stress treatments (Table 2). The highest score was obtained under control condition where it gained 0.88 gW/gFW (g water  $g^{-1}$  fresh weight) after 11 days of stress onset. 100 mM NaCl concentration obtained the lowest average of 0.74 gW/gFW. When 50mM NaCl concentration was studied, the average indicated the value of 0.79 gW/gFW (Table 2). To observe whether the plant type has effect on the relative water content or not, wild type *Arabidopsis* and *vte*<sub>4</sub> mutant plants were studied (Figure 5). The *vte*<sub>4</sub> mutant plants demonstrated the lower average of 0.78 gW/gFW whereas wild type *A.thaliana* gained 0.82 gW/gFW after 11 days of salt stress onset (Figure 5). As the level of NaCl concentration increased, the relative water content and relative water potential showed a reduction in both *vte*<sub>4</sub> mutant and wild type *Arabidopsis* plant.



Figure 5. The effect of different plant type ( $vte_4$  mutant and wild type Arabidopsis) on relative water content after 11 days of salt stress onset.

The specific leaf area showed strong positive correlation with relative water content in which it gained (0.998). In addition, there was a significant correlation between rosette dry weight with relative water content and specific leaf area where the first recorded (1.0) (p<0.01) and the latter one obtained (0.998). PSII (Fv/Fm) also indicated strong positive correlation with relative water potential (0.998) (Table 3).

 Table 3. Pearsons correlation coefficients among the mean relative water content, specific leaf area, water potential, rosette dry weight and maximum quantum efficiency (FvFm) of mutant

Parameters	1	2	3	4	5
1. relative water content	1				
2. specific leaf	0.998**	1			
3. water potential	0.953	0.972	1		
4. rosette dry weight	0.985**	0.988*	0.975	1	
5. PSII (Fv/Fm)	0.971	0.985	0.987*	0.973	1

\*\*. Correlation is significant at the 0.01 level

\*. Correlation is significant at the 0.05 level.

The linear regression was observed between relative water potential and maximum quantum efficiency (Fv/Fm) for both wild type and  $vte_4$  mutant *Arabidopsis thaliana* .R<sup>2</sup> for wild type and  $vte_4$  mutant were 70.39% and 73.11% respectively (Figure 6). The regression indicated that as the relative water potential decreased the value of maximum quantum efficiency (Fv/Fm) also showed reduction.



Figure 6. Simple regression between water potential and maximum quantum efficiency (Fv/Fm) of *Arabidopsis* plants (mutant *vte*<sub>4</sub> and wild type) after 11 days of salt stress onset.

### DISCUSSION

Tocopherols (vitamin E) are lipophilic antioxidants which are synthesized only in photosynthetic organisms.  $\alpha$ - and  $\gamma$ -tocopherol are predominant in higher plants Where  $\alpha$ -tocopherol is found accumulated in photosynthetic tissue specifically in leaves and  $\gamma$ -tocopherol is predominant in seeds. However, little information is available about the specific roles of  $\alpha$  - and  $\gamma$  -tocopherol in different plant tissues (ABBASI *et al.*, 2007). In addition, vitamin E (tocopherols) is regarded as one of the most important antioxidants that protect plants against oxidative stress like salinity. To investigate the effect of vitamin E on growth parameters of *Arabidopsis thaliana*, wild type and *vte*<sub>4</sub> mutant plants were studied.

The present study showed that both  $vte_4$  mutant and wild type *A.thaliana* plants were negatively affected under NaCl treatment (50mM and 100mM) whereas the higher value was gained under control condition for both plant types regarding morphological and physiological parameters including specific leaf area, rosette dry weight and maximum quantum efficiency (Fv/Fm). Interestingly, even in the absence of salt stress, wild type *A.thaliana* performed stronger than  $vte_4$  mutant indicating the fact that lack of  $\alpha$ -tocopherol in mutants resulted in weaker phenotypic performance of the plants. This finding partially disagrees with another study in which the mutants performed almost equally with the wild type plants (CHAN et al., 2012). It is noteworthy that in Arabidopsis thaliana the biomass is measured by weighting rosette dry weight. Thus, the outcome of this study showed that under salinity the biomass of both wild type and vte4 mutant diminished by 50% (MEYRE et al., 2001) .Earlier ABBASI et al., (2007) reported that under salinity the mutant tobacco biomass decreased by 90%. The results demonstrated that specific leaf area, rosette dry weight and maximum quantum efficiency (Fv/Fm) decreased as the NaCl concentration increased where 100mM NaCl concentration obtained the lowest score. In addition, the salt stress led to dramatic phenotype change characterized by accelerated senescence A.thaliana mature leaves. This outcome is in agreement with previous study in which the growth parameters of mutant and wild type A.thaliana declined under saline condition (HUMMEL et al., 2010; CELA et al., 2011). The maximum quantum efficiency (Fv/Fm) recorded the lowest value under 100mM NaCl concentration compared to other treatments in both wild type and vte4 mutant Arabidopsis thaliana. CELA et al., (2012) reported the similar result in which the oxidative stress resulted in Fv/Fm decrease. It has been discussed that reduced leaf expansion can produce the unused photosynthate in growing tissues and as a result it generates feedback signals to downregulate photosynthesis. Hence, it implies the idea that under salinity the maximum quantum efficiency (Fv/Fm) will diminish (MUNNS and TESTER, 2008).

To investigate the effect of salinity on water relations in *A. thaliana*, both the water potential and relative water content (RWC) were studied. Different salt stress treatments showed different effect after 11 days of stress onset. Water potential gained the highest score under control condition whereas under 100 Mm NaCl concentrations the lowest value was obtained. This outcome was in accordance with other study pointing out the idea that additive NaCl in water uptake of *A.thaliana* results in reduction of water potential and relative water content (RWC) (CELA *et al.*, 2011).

Tocopherols are known to scavenge lipid peroxyl radicals, avoiding the propagation of lipid peroxidation and regarded as excellent scavengers and quenchers of singlet oxygen. In chloroplasts of plants alpha-tocopherol (vitamin E) is synthesized by  $\gamma$ -tocopherol methyltransferase ( $\gamma$  –TMT) or known as VTE4 gene. Leaves of *Arabidopsis thaliana* contain high levels of  $\alpha$ -tocopherol, however, they are very low in  $\gamma$ -tocopherol.  $\alpha$ -tocopherol in plants is known as the most active form of vitamin E protecting plants against oxidative stress (KAMAL-ELDIN and APPELQVIST, 1996). It has been shown that *Arabidopsis vte*<sub>4</sub> mutant lacks  $\alpha$ -tocopherol, but the level of  $\gamma$ -tocopherol is accumulated in leaves. Tocopherols are part of an intriguing signaling network controlled by ROS and antioxidants. Thus, they are considered as good candidates to affect plant cells signaling (CELA *et al.*, 2009).

ROS generate negative effect on membranes by damaging them which results in eradication at cellular level under oxidative stress such as salinity. The level of ROS is controlled in chloroplasts where tocopherols regulate the amounts of lipid peroxidation products. By doing so, they will be able to affect gene expression while plants encounter oxidative stress (CELA *et al.*, 2011). Our findings are in agreement with previous study in which the wild type *A.thaliana* demonstrated the better performance under salt stress compared to  $vte_4$  mutant.

Our result showed that wild type *Arabidopsis* plants gained the higher value under both 50mM and 100mM NaCl concentration with respect to morphological and physiological parameters including rosette dry weight, Specific leaf area and maximum quantum efficiency (Fv/Fm) in comparison with *vte4* mutant plants. It should be noted that regarding total leaf number wild type *A.thaliana* recorded the higher score whereas  $vte_4$  mutant number of leaf

diminished under salinity. As biomass in this particular plant is measured by weighting the rosette dry weight, the reduction in number of leaves in *A.thaliana* results in decrease of photosynthesis capacity followed by biomass diminishment. It has previously been reported that the reduction of leaf growth after soil salinity increase is the reason of the salt osmotic effect around the roots. This dramatic enhancement in soil salinity results in leaf cells water loss. However, this loss is not permanent. In fact the cells are able to retain their original turgor and volume due to osmotic adjustment. It is noteworthy that despite the fact that cells regain their volume under salinity, but the rate of cell elongation is decreased (CRAMER, 2002). After a while, decrease in cell division and cell elongation result in smaller leaf size and slower appearance. The alteration in cell dimension and more reduction in area than depth are observed so the leaves get thicker and smaller (FRICKE and PETERS, 2002).

To see whether wild type and  $vte_4$  mutant *A.thaliana* have effect on the water relations parameters or not, these two plant types were examined. The wild type *A.thaliana* performed stronger under salt stress compared to  $vte_4$  mutant when relative water content was determined. Interestingly, the wild type and  $vte_4$  mutant *Arabidopsis* in terms of water potential were not significantly different. Previous study indicated that salinity can affect the water relations of most higher plants so this stress leads to water deficit (PANAHI, 2009).

The relative water content (RWC) recorded the highest value under control condition whereas the lowest value belonged to 100mM NaCl concentration. This outcome is in accordance with another study where the relative water content diminished under saline condition (CELA *et al.*, 2011). Therefore, the alterations in level of water availability integrated with salinity demonstrated the negative effect through reduction in relative water content.

In this study, both wild type and mutant (vte<sub>4</sub>) Arabidopsis plants were subjected to salt stress (different NaCl concentration) in order to understand the mechanism of the plant's response to this abiotic stress via morphological and physiological traits. After 11 days of stress initiation both plant types were affected negatively. However, the wild type plants recorded higher value in terms of growth and development compared to the mutants. Mutant A.thaliana  $vte_4$  recorded lower value regarding PSII (Fv/Fm) compared to wild type. Specific leaf area decreased in wild type and mutant plants when salt stress was imposed. It should be noted that after 11 days of stress onset the water potential was not significant based on plant types. By comparing wild type and mutant plants, the lack of  $\alpha$ -tocopherol seemed to affect plant mechanism against salt stress which emphasize on the fact that this gene is acting against oxidative stresses such as water and salt stress. While  $\alpha$ -tocopherol is regarded as the most active form of vitamin E protecting plants against abiotic stresses, the *vte*<sub>4</sub> mutant plants indicated more sensitivity towards salt stress compared to wild type A.thaliana. It is concluded that understanding the plant mechanism under oxidative stress via integrated platform information of both plant physiology and genetic engineering can lead us to generate more tolerant or/and resistant plants for more food production in future.

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## EFEKAT STRESA SOLIMA NA DIVLJI GENOTIP I VTE<sub>4</sub> MUTANT Arabidopsis thaliana: MODEL BILJKA ZA INŽENJERING TOLERANTNOSTI NA SOLI

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#### Izvod

Vršena su ispitivanja efekta zaslanjenosti na fiziološke i morfološke osobine divljeg genotipa (Colombia – 0) i *vte*<sub>4</sub> mutanta *Arabidopsis thaliana* pri različitim koncentracijama NaCl. Divlji genotip i mutant su izlagani abiotičkom stresu (zaslanjenosti) u toku 11 dana u cilju utvrđivanja parametara rasta,, razvoja i odnosa vode. Produženim vremenom tretmana i divlji genotip i mutant su imali pokazatelje negativnih efekata. Kada je ispitivan tip biljaka, divlji tip *A.thaliana* je imao više vrednosti, 0.82 gW/gFW za relativni sadržaj vode (RWC) u uslovima 50mM NaCl, dok su biljke mutanta dostigli vrednost 0.78 gW/gFW pod istim uslovima. Međutim, frakcija mase korena je pokazala povećanje kod biljaka divljeg genotipa i kod biljaka *vte*<sub>4</sub> mutanta 11 dana posle početka izlaganja stresu. Redukcija vodnog potencijala je utvrđena kod oba ispitivana genotipa. Na osnovu dobijenih rezultata zaključeno je da su biljke divljeg genotipa imale veći stepen tolerantnosti. Odsustvo gena za gama – tokoferol transferazu (gama-TMT) kod mutant *vte*<sub>4</sub> izgleda da utiče na izmenu odbrambenog mehanizma biljaka mutanta prema zaslanjenosti.

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