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Effects of mild and severe drought stress on the biomass, phenolic compounds production and photochemical activity of *Aloe vera* (L.) Burm.f.

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ABSTRACT

In this study, the biomass, compatible solutes, PSII functioning and phenolic profiles of *Aloe vera* (L.) Burm.f. leaves were investigated at different time intervals after drought stress (20, 40 and 80 % of the field capacity). While the impaired ability of leaves for synthesis of assimilates caused growth inhibition in *A. vera* under severe drought stress, we observed that the content of proline, soluble sugars, total phenolic and flavonoids tended to increase in plants treated with mild drought stress. Under mild drought stress, the increased leaf thickness correlated with the higher productivity in terms of leaf biomass and gel production. Also, mild drought stress enhanced photochemical activity in *Aloe* leaves, and changed the entire quantity of secondary metabolite of vanillic acid produced, which may be considered to obtain better growth and considerable secondary metabolite of the medicinal *Aloe* plants treated with mild drought stress.

Key words: *Aloe vera* leaves; carotenoids; leaf thickness; photosystem II performance index; vanillic acid; drought stress

IZVLEČEK

UČINEK BLAGEGA IN MOČNEGA SUŠNEGA STRESA NA BIOMASO, TVORBO FENOLNIH SNOVI IN FOTOKEMIČNO AKTIVNOST VRSTE *Aloe vera* (L.) Burm.f.

V raziskavi so bili preučevani biomasa, osmotiki, delovanje fotosistema II in profil fenolnih snovi v listih vrste *Aloe vera* v različnih intervalih po vzpostavitvi sušnega stresa (20, 40 in 80 % poljske kapacitete). Medtem, ko je bila rast zavrta zaradi zmanjšane fotosintetske sposobnosti listov, je bila pri blagem sušnem stresu povečana vsebnost prolina, topnih sladkorjev, celokupnih fenolov in flavonoidov. Pri blagem sušnem stresu je povečana debelina listov korelirala z večjo produktivnostjo glede na biomaso in tvorbo gela. Blag sušni stres je povečal fotokemično aktivnost listov in spremenil količino sekundarnega metabolita vanilijske kisline in njenih derivatov. Sklepamo lahko, da blag sušni stres poveča rast in tvorbo sekundarnih metabolitov pri tej zdravilni rastlini.

Ključne besede: *Aloe vera* listi; karotenoidi; debelina listov; indeks učinkovitosti fotosistema II; vanilijska kislina; sušni stres

1 INTRODUCTION

Environmental stresses, such as drought, low or high temperature and excessive salinity have negative influence on the plant, causing changes in its normal growth, development and metabolism (Bohenert et al., 1995; Kranner et al., 2010). Drought stress induces oxidative stress through enhancing the formation of reactive oxygen species (ROS). ROS can react with photosynthetic pigments, lipids, proteins and DNA (Ahmad et al., 2010), leading eventually to lipid peroxidation, membrane damage, inactivation of antioxidant enzymes and cell death (Gill and Tuteja, 2010). However, for the detoxification of excessively produced ROS, plants possess a developed antioxidative defense mechanism. ROS scavenging occurs by a large number of ROS detoxifying enzymes and by antioxidants (Gill and Tuteja, 2010; Mittler et al., 2011).

To survive at drought stress conditions and to achieve maximum drought resistance, plants have developed adaptation strategy, which is associated with physiological traits, e.g., increased osmoprotectant and accumulation of sugars (Kooyers, 2015). In addition, drought avoidance occurs when plants enhance water-use efficiency (WUE) by decreasing transpiration, or increasing root growth. Generally, CAM plants are the best accommodated to hot and dry climatic conditions,

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because they have higher water-use efficiency (WUE) than that of C_3 plants (Habibi, 2016). *A. vera* (L.) Burm. f. is a drought tolerant species that is considered as a constitutive CAM species (Silva et al., 2010). Nevertheless, drought stress can limit *A. vera* growth and production (Delatorre-herrera et al., 2010; Cousins and Witkowski, 2012). In *A.vera*, the synthesis of soluble sugars, proline and secondary metabolites are highly affected by water deficit stress (Lucini et al., 2013; Ray et al., 2013).

Phenolic compounds are the most widespread substantial groups of plant secondary metabolites that exhibit antioxidant properties (Quan et al., 2016). Several investigators have reported that the levels of phenolic compounds such as phenolic acids and flavonoids were influenced by drought stress in tobacco and wheat leaves (Ma et al., 2014).These compounds can scavenge ROS (Quan et al., 2016) and prevent lipid peroxidation, protein denaturation and DNA damage (Mittler, 2002; Król et al., 2014). Interestingly, the relationship between water availability and phenolic compounds synthesis is dependent on the plant species, treatments and/or experimental systems. Some studies have shown that environmental stress can cause a decline (Weidner et al., 2009). However, other studies have indicated that stress increases phenolic compounds accumulation (Weidner et al., 2009; Król et al., 2014). Thus, the exact mechanism of phenolic compounds accumulation in response to long and continuous stressor remains unknown.

Aloe vera as a CAM plant and one of the most important medicinal plants grows in warm and dry regions. Several studies have been conducted focusing on *A. vera* adaptation to water deficit stress; however information is lacking on the role of phenolic compounds, compatible solutes as well as photochemical reactions in *Aloe vera* plants exposed to mild and severe water stress, which may be one of the most important approaches to overcome with water deficit stress. In this study, changes in compatible solutes, PSII functioning and individual phenolic acids in response to mild and severe drought stress in *A. vera* were investigated. We also hypothesized that regulation of water availability may be a promising way to obtain the highest concentrations of secondary metabolite in the medicinal plant.

2 MATERIALS AND METHODS

2.1 Plant material and treatments

The 15–17 cm pups (small plants growing from the sides of the mother plant) of *Aloe vera* (L.) Burm.f. plants were chosen and planted in top of the cylindrical plastic pots (18 cm in diameter and 45 cm in depth) containing 10 kg sandy loam soil (pH 7.3) for five months, and irrigated with distilled water every 10 days to maintain at 80 % field capacity (FC). Plants were grown under day/night temperature of 30-35/18-22 ºC, relative humidity of 50-55 % and daily photon flux density of about 500-600 µmol m^{-2} s^{-1} in an environmentally controlled growth chamber throughout the pre-experimental period. The treatments of drought stress were composed of control (80 % FC), mild drought stress (40 % FC) and severe drought stress (20 % FC). Finally, plants were harvested and analyzed in a temporal (on different days after imposition of drought stress) manner.

2.2 Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence transients (*OJIP* transients) were evaluated with a Packet-PEA chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, PE 32 1JL, England) in dark-adapted leaves for at least 20 min, using the *JIP*-test to analyse chlorophyll *a* fluorescence rises. Some groups of measured and calculated parameters using the *JIP*-test (Strasser et al., 2004) were described in the following section.

- F_v/F_m, the maximum PSII photochemical efficiency, namely the maximum quantum yield of primary photochemistry. Where F_m or F_{max} is maximal chlorophyll fluorescence intensity measured when all photosystem II (PSII) reaction centers are closed, F_v is variable chlorophyll fluorescence (F_m−F_o), F_o is minimal fluorescence (all PSII RCs are assumed to be open), respectively.
- PI_{abs}, the performance index that is calculated as: $(RC/ABS) \times (\varphi_{Po}/(1 - \varphi_{Po})) \times (\psi_o/(1 - \psi_o))$, where, RC is for reaction center; ABS is for absorption flux; φ_{Po} is for maximal quantum yield for primary photochemistry; and ψ_o is for the quantum yield for electron transport

2.3 Determination of total carotenoids, chlorophyll *a* and *b*

The leaf concentration of chlorophyll and carotenoids was analysed according to Lichtenthaler and Wellburn (1983). After centrifugation at 1000 rpm for one minute, supernatants were used for determination of photosynthetic pigments, and the absorbance was read at 400-700 nm on spectrophotometer. Leaf concentrations of chlorophylls and carotenoids were calculated as:

Chl a = 15.65 A666 - 7.340 A653
Chl b = 27.05 A653 - 11.21 A666
Total carotenoids = 1000 A470 - 2.860 Ca - 129.2 Cb/245

2.4 Estimation of total proline, soluble sugars and starch

Proline was determined by the method of Bates et al. (1973). Leaf samples from each group were homogenized in 3 % (w/v) sulphosalycylic acid and the homogenate was centrifuged at 3,000 g for 20 min. Mixture was boiled for 1 h in water bath after addition of acid ninhydrin and glacial acetic acid. Reaction was then stopped by ice bath, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve. For determination of total soluble sugars and starch contents, fresh leaves were extracted in 20 ml of 80 % (v/v) ethanol at 95 ºC for 1 h. After centrifugation at 10,000 g for 10 min, starch was determined in the pellet according to Jarvis and Walker (1993). Total soluble sugars were analyzed using anthrone reagent according to Irigoyen et al (1992).

2.5 Assay of phenylalanine ammonia-lyase (PAL) activity and related metabolites

To determine PAL activity, formation of cinnamic acid was recorded by spectrophotometry at 290 nm according to modified method of Zucker (1965). One unit (U) of PAL activity was defined as the amount of the enzyme that produced 1 nmol cinnamic acid per h. Total phenolic content was quantified by the method of Velioglu et al. (1998). Gallic acid was used for constructing the standard curve. Results were expressed as mg gallic acid (GA) per gram of the fresh mass. Total flavonoid content was assessed using the method adapted by Meda et al. (2005). Briefly, 5 ml of 2 % aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of leaf extracts (0.02 mg ml^{-1}). Absorption readings at 415 nm were taken after 10 minutes against a blank sample without $AlCl_3$. The total flavonoid content was calculated using a standard curve of quercetin and expressed as mg quercetin equivalent (QE)/100 g extract. Anthocyanin content was estimated according to the method of Krizek et al. (1993) using HCl-methanol solvent (1: 99, v: v), and the amount of anthocyanin was ranked from the absorbance at 550 nm.

2.6 HPLC analysis

For the sample preparation and calibration curves, the powdered leaves (0.5 g) were extracted with methanol (5 ml) in a shaking incubator for 8 hours at room temperature. The supernatant was centrifuged at 3000 g for 3 minutes, and then filtered prior to HPLC analysis. For the calibration curve, the stock solutions of the identified phenolic compounds (chlorogenic acid, syrginic acid, gallic acid, rosmarinic acid, vanillic acid and luteolin) were equipped with methanol to obtain a 1 mg ml^{-1} concentration, and the calibration curves for standard samples were fabricated by plotting the peak area of the identified phenolic compounds against their concentrations through dilution of each stock solution in methanol to six concentrations (0.78 ppm, 1.58 ppm, 3.12 ppm, 6.25 ppm and 25 ppm). Vanillic acids were identified at 245 nm, gallic and syrginic acids at 275 nm, chlorogenic and rosmarinic acids at 320 nm and luteolin at 350 nm. The correlation coefficients (r^2) of all phenolic standards were higher than 0.993.

The HPLC analysis was done using a Knauer liquid chromatography apparatus Shimadzu HPLC instrument (a 1000 Smartline Pump, a 5000 Smartline Manager Solvent Organizer and a 2800 Smartline Photodiode Array Detector). Separation achieve on a 25 cm × 4.6 mm with a pre-column, Eurospher 100-5 C18 analytical column provided by Knauer (Berlin, Germany). Data acquisition and integration perform with EZchrom Elite software. A 20 µl sample of the methanol extract of *Aloe vera* leaves was injected into an HPLC column through a 3900 Smartline Auto-sampler injector equipped with a 100 µl loop. Separation was performed using 0.02 % trifluoroacetic acid in water (elution A) and methanol (elution D). The total running time was 55 minutes at a flow rate of 0.5 ml min^{-1}, and the oven temperature was 20 ^oC.

2.7 DPPH assay

The antiradical activity was estimated by using the method described by Yen and Chen (1995). Briefly, the mixture of methanolic extract and DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution was left in the dark at room temperature for 20 min, and then absorbance was recorded at a wavelength of 517 mm.

2.8 Statistical analysis

Experiments were performed in complete randomized block design (RBD) with 4 replications. Statistical analysis was carried out using sigma stat (3.5) with Tukey test ($P < 0.05$). Correlation analysis using Spearman Rank Order Correlation in sigma stat (3.5) was applied to determine the relationship between parameters. Chlorophyll fluorescence data were analyzed and conducted using the PEA Plus V1.10 software.

3 RESULTS AND DISCUSSION

3.1 Biomass and gel production and water relations under mild and severe water stress

Drought is one of the major abiotic stresses affecting plant growth worldwide (Wu et al., 2018). However *Aloe vera* is a promising crop for arid zones, with high yield of leaf biomass and gel production under mild water stress conditions (Silva et al., 2010), but it has been demonstrated that severe drought stress decreases leaf yield and growth of *Aloe vera* (Silva et al., 2014; Hazrati et al., 2017). This is confirmed by the present study showing that the production of leaves biomass and gel was not influenced by mild drought stress and only the severe drought stress caused significant biomass reduction (Table 1). In plants treated with drought for 180 days, leaf thickness tended to increase under mild drought stress, while leaf thickness and relative water content (RWC) were reduced under severe drought stress. In these conditions, *A. vera* exhibited morphologic plasticity to adapt to water deficit. Indeed, succulent plants can exhibit plasticity in their photosynthesis depending on the presence of thick leaves (Habibi and Ajori, 2015, Habibi, 2016).

Table 1: Effects of mild and severe drought stress on the leaf biomass (g leaf^{-1}), relative water content (RWC, %) and leaf thickness (mm) of *Aloe vera*. Measurements were performed 180 d after drought treatments. Data of each row within each defined plant part indicated by the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 8).

	Control	Mild drought stress	Severe drought stress
Fresh leaf biomass	550±29 ^{a}	526±35 ^{a}	352±38 ^{b}
Photosynthetic tissue	185±21 ^{a}	197±24 ^{a}	145±28 ^{b}
Gel	365±17 ^{a}	329±29 ^{a}	207±23 ^{b}
Dry leaf biomass	45±6.2 ^{a}	44±3.7 ^{a}	32±3.2 ^{b}
Photosynthetic tissue	19±4.4 ^{a}	21±4.0 ^{a}	15±3.3 ^{a}
Gel	26±2.3 ^{a}	23±3.6 ^{a}	17±2.9 ^{b}
RWC	88±3.7 ^{a}	84±4.5 ^{a}	71±2.2 ^{b}
leaf thickness (mm)	13.2±1.14 ^{ab}	16.0±2.23 ^{a}	10.4±1.07 ^{b}

3.2 Possible importance of compatible solutes in the responses of *A. vera* plants to mild drought stress

After 120 and 180 days of exposure, the content of proline and soluble sugars was increased by mild drought stress. The content of starch, however, was not affected in plants treated for 60, 120 and 180 days with both mild and severe drought stress (Fig. 1). CAM plants ability to maintain growth under drought stress is based on the efficient synthesis of sugars, polysaccharides and other osmolytes, such as proline and glycine betaine (Delatorre-herrera et al., 2010; Salinas et al., 2016) in order to adaptation to water deficit. Similarly, our results showed that mild drought stress significantly increased proline and soluble sugars contents, which might have a scavenger function and act as an osmolyte (Sankar et al., 2007; Salinas et al., 2016). Nevertheless, under the severe drought stress, the content of proline and soluble sugars did not continue to increase probably due to source limitations. In this study, the maintenance of dry matter production under mild drought stress may have contributed to the enhanced levels of compatible solutes during the experiment.

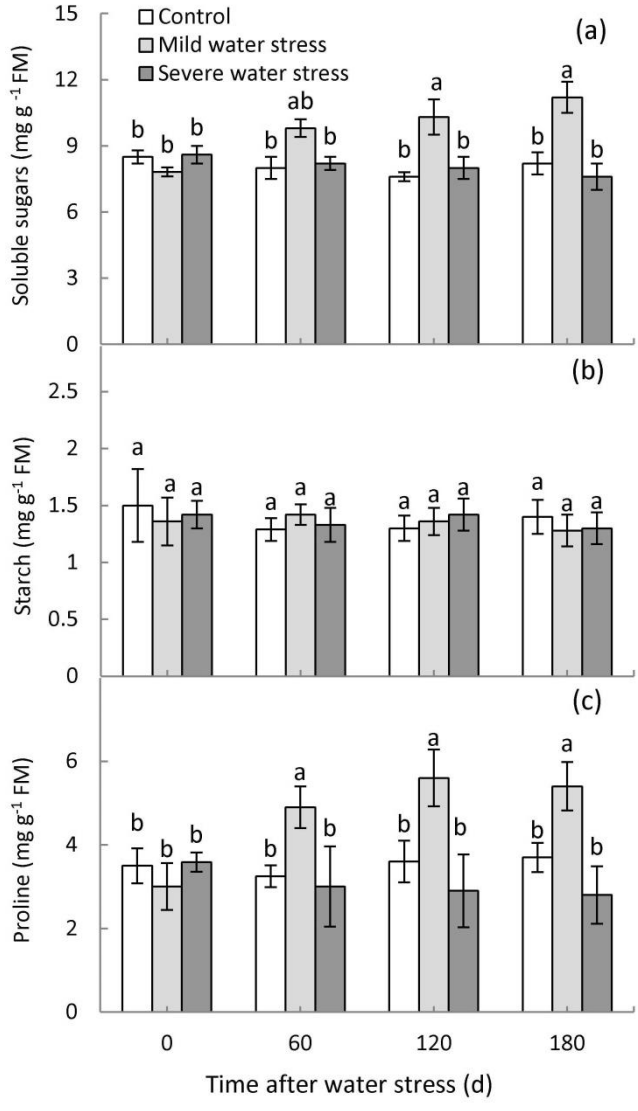

Figure 3: Effects of mild and severe drought stress on the concentration of soluble sugars, starch and proline in *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

3.3 Treatment with mild drought stress enhances photoprotection activity in *Aloe* leaves ISTT

Chlorophyll *a* and b contents were not affected significantly by various drought levels in *Aloe vera* plants (Fig. 2). After 120 days treatment, however, carotenoid content of leaves was enhanced only under the mild drought stress (Fig. 2). Under mild drought stress, an increase in carotenoid content can perform an important role as a non-enzymatic antioxidant in the photoprotection of photosynthesis (Miura and Tada, 2014; Habibi and Ajory, 2015) as well as in the dissipation of absorbed light energy as thermal energy (qE) (Cazzonelli and Pogson, 2010). Indeed, when *Aloe vera* plants were exposed to mild drought stress, the accumulation of protective pigments such as carotenoids in leaves developed an effective photoprotection mechanism, as demonstrated by the maintenance of the maximum quantum yield of photosystem II (F_v/F_m) and photosystem performance index (PI_{abs}) in plants treated with mild drought stress (Fig. 3). In contrast, after 120 and 180 days of treatment, F_v/F_m and PI_{abs} exhibited a significant decrease only in plants subjected to severe drought stress, suggesting a photoinhibitory effect (Diao et al., 2014). Since the shape of the OJIP curve is very sensitive to environmental stress (Strasser et al., 2004), we measured chlorophyll fluorescence to determine the precise effects of drought stress on the photosynthetic

apparatus (Su et al., 2015) using the JIP-test (the analysis of the fluorescence rise OJIP). Under mild drought stress conditions, the OJIP chlorophyll fluorescence curve obtained from the *Aloe vera* leaves revealed a normal fluorescence rise when compared to control plants (Fig. 4), indicating that reaction centers behave almost normally (Kalaji et al., 2011; Zhang et al., 2014). In this study, however, a quicker fluorescence rise in the *J* step was perceived in response to severe drought stress, which is related to deactivation of the reaction center leading to drastic reduction in photochemistry through a blockage of electron flow (Strasser et al., 2004; van Heerden et al., 2007; Kalaji et al., 2011).

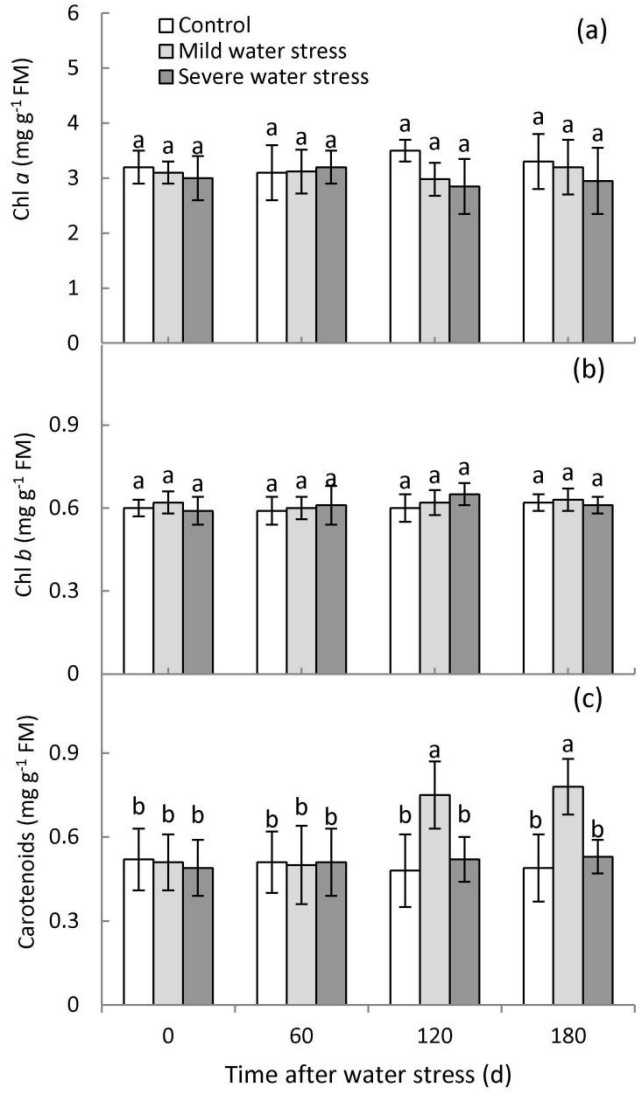

Figure 2: Effects of mild and severe drought stress on the content of chlorophyll *a*, *b* and total carotenoids in *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

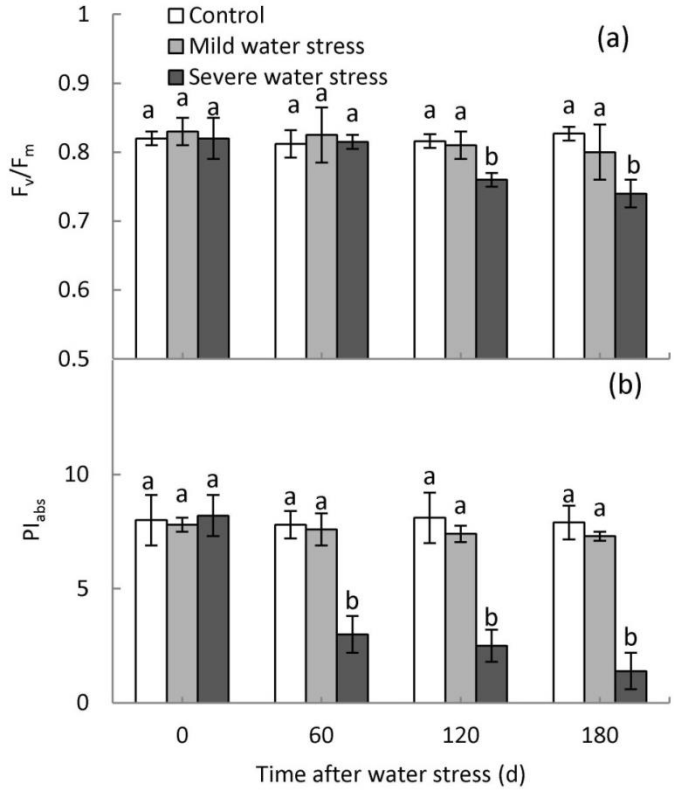

Figure 3: Effects of mild and severe drought stress on the maximum quantum yield of PSII (F_v/F_m) and the Performance Index (PI_{abs}) in *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

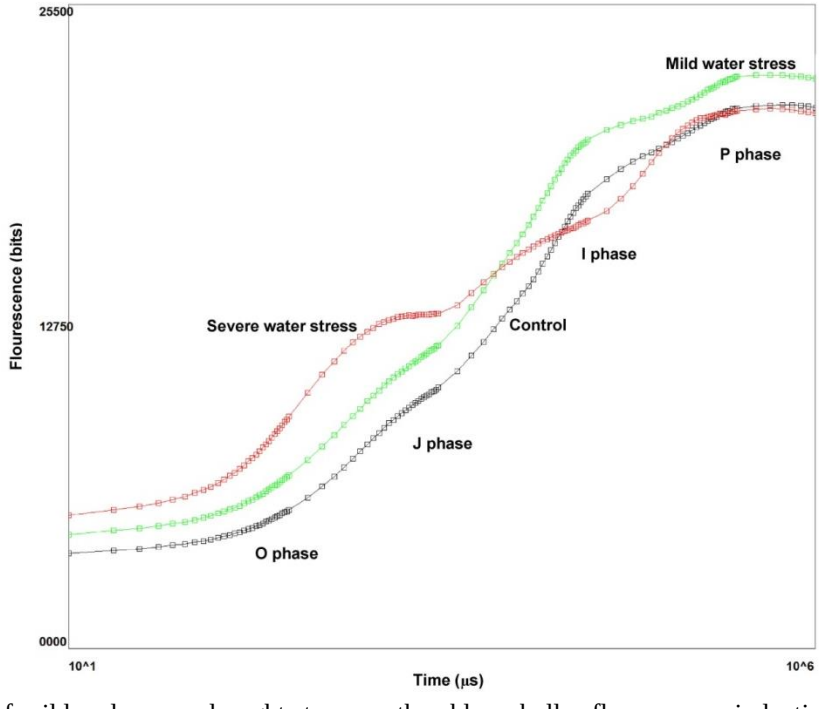

Figure 4: Effects of mild and severe drought stress on the chlorophyll *a* fluorescence induction curve of *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

3.4 Mild drought stress increases phenolic compounds production in *Aloe* leaves

Aloe leaves contain remarkably high amounts of phytochemical contents with therapeutic and preventive properties for human beneficial health effects. Phenolic compounds are the second major substances found in *Aloe vera*, which are used for medicinal purposes (Sathyaprabha et al., 2010; Lopez et al., 2013). Numerous studies have revealed that the quantity of phenolic acids in natural plants was affected by various environmental stresses (Lee et al., 2012; Zhang et al., 2015). In plants treated with drought stress for 180 days, total phenolic and flavonoids was increased under mild drought stress, while their contents remained unchanged under severe drought stress conditions (Fig. 5). After 120 and 180 days of exposure, however, an increase in anthocyanin levels was observed only in plants treated with severe drought stress. These result showed that long-term water loss may be necessary but not sufficient to induce anthocyanin compounds. In our experiments, accumulation of phenolic compounds in treated plants (Fig. 5) was correlated with PAL activation in the aloe leaves (Fig. 6). Under sever dfrought stress PAL activity suppressed, resulting in reduced phenolic compounds in the aloe leaves. Since phenolic compounds accumulation is associated with antioxidant activity (Aladedunye et al., 2008; Zhou et al., 2014; Lee et al., 2017), we investigated antioxidant properties including DPPH radicals in the present research. In more detail, the highest scavenging effect was observed under mild drought stress. As a result, the antioxidant activities of *Aloe* leaves against DPPH radicals were correlated with higher metabolite contents. In confirmation of this, there was a linear and positive correlation ($r = 0.77$, $P < 0.01$) between total phenol content and antioxidant activities against DPPH radicals in plants subjected to mild drought stress (Fig. 7). In addition, due to the protective effect of phenolic compounds in the screening of photoradiation (Takahashi and Badger, 2011) as well as their function as a non-enzymatic antioxidant during exposure to drought stress (Quan et al., 2016), this higher phenolic compounds accumulation in plants subjected to mild drought stress, may be an important protection mechanism for photosynthetic primary reactions of *Aloe* leaves under drought stress. This was corroborated by a linear and positive correlation ($r = 0.73$, $P < 0.01$) between total phenol content and PI_{abs} in plants subjected to mild drought stress (Fig. 7).

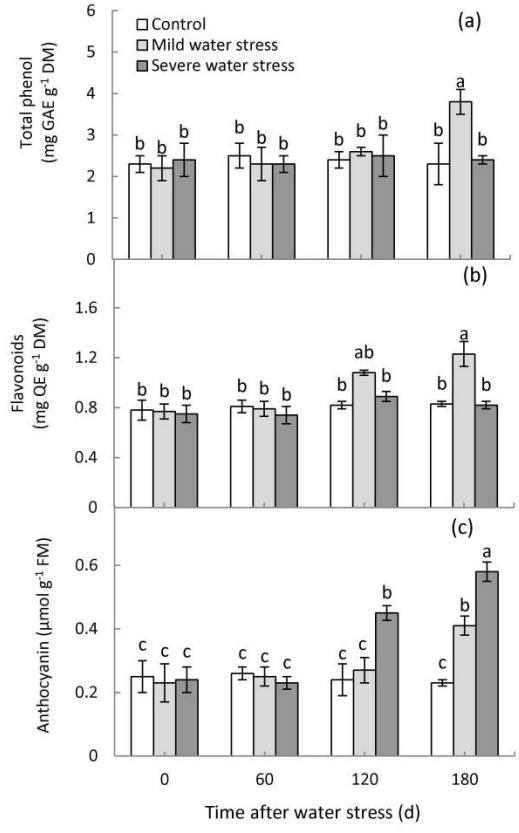

Figure 5: Effects of mild and severe drought stress on the total phenol, flavonoids and anthocyanin content in *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

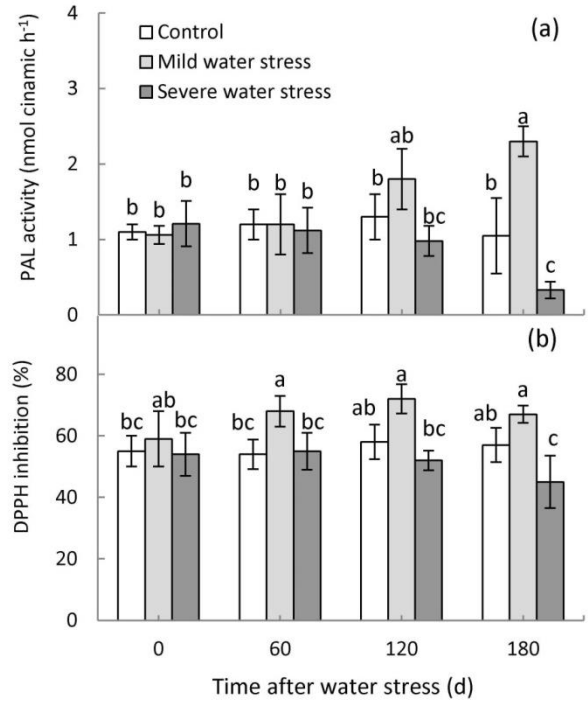

Figure 6. Effects of mild and severe drought stress on the activity of phenylalanine ammonia-lyase (PAL) and the antioxidant activities against DPPH radicals in *Aloe vera* plants. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

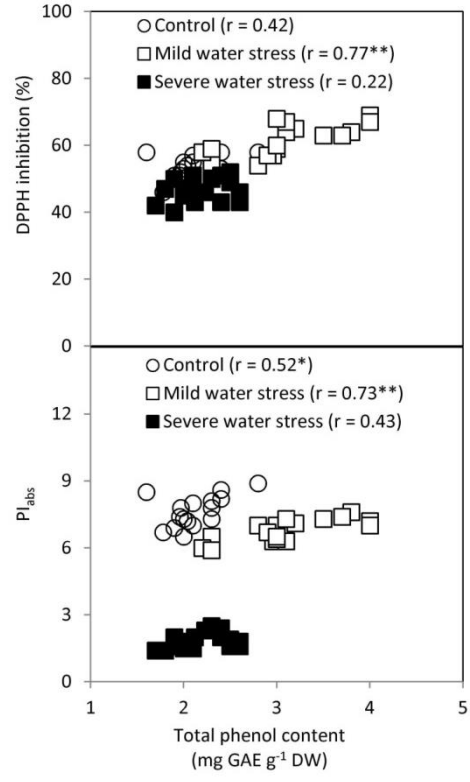

Figure 7: Effects of mild and severe drought stress on the correlation between values of performance index (PI_{abs}) or DPPH inhibition and total phenol content recorded in *Aloe vera* plants: ns, non-significant, * and **, significant at the 5 % and 1 % levels of probability, respectively.

Additionally, we assessed the main phenolic acids in the methanol extract of drought-stressed *Aloe* leaves using HPLC analysis. The typical chromatograms of *Aloe* leaves are presented in Fig. 8 and 9. The identification of each peak in the sample was performed using the retention time in the chromatogram. Since methanol is an appropriate solvent for the maximum extraction of phenolic compounds (Naczk and Shahidi, 2004), we used this solvent for extraction of phenolic acids in leaves. The quantification of each phenolic acids peak was done using the calibration curves in the range of 0.78-25 ppm, and their equations were measured as y = 130951x-143924 (chlorogenic acid, r^2 = 0.992), y = 97759x-97156 (vanillic acid, r^2 = 0.995) and y = 81796x-95603 (rosmarinic acid, r^2 = 0.998). This study revealed three major phenolic acid including rosmarinic acid, chlorogenic acid and vanillic acid in leaves. The metabolite profiles exhibited similar patterns except vanillic acid peak. Chlorogenic acid showed little differences with 500 µg g^{-1} (control) and 494 µg g^{-1} (mild drought stress), respectively. Moreover, no significant difference was observed in rosmarinic acid content of leaf among all treatments (control 547 µg g^{-1} and mild drought stress 551 µg g^{-1}). Under mild drought stress, vanillic acid content was significantly increased in drought-stressed leaves (845 µg g^{-1}) as compared with the control leaves (682 µg g^{-1}). Vanillic acid and chlorogenic acid are secondary metabolites, which have anti-inflammatory and anti-oxidative and anticancer properties (Chiang et al., 2003; Leal et al., 2011; Gengmao et al., 2015). For this reason, accumulation of vanillic and chlorogenic acid in the *Aloe* leaves under mild drought stress conditions was measured in this experiment. Here we showed that content of secondary metabolite of vanillic was highest at mild drought stress, suggesting that the level of vanillic acid like other plant secondary metabolites was influenced by the availability of water.

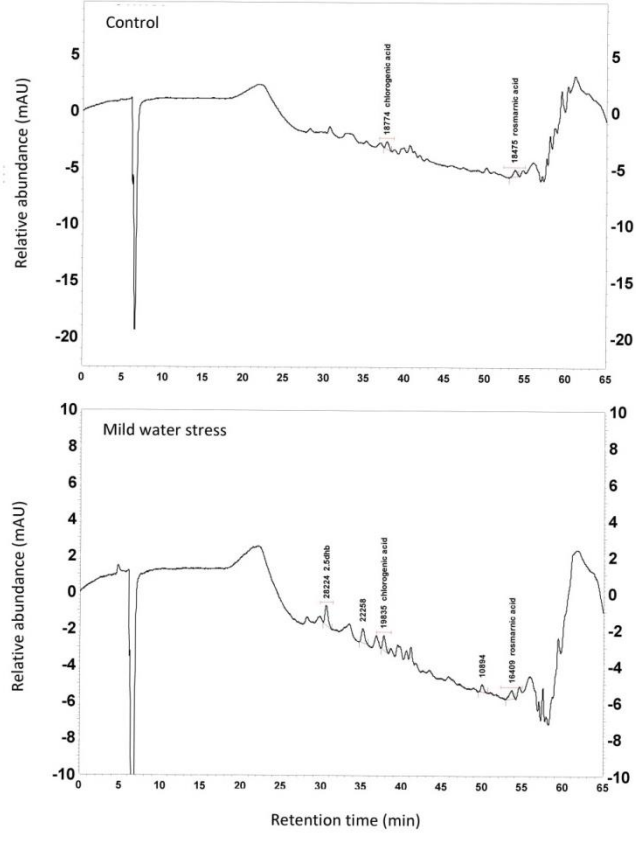

Figure 8: Effects of mild and severe drought stress on the representative chromatogram of prepared extract of *Aloe vera* leaves for chlorogenic acid and rosmarinic acid measurements.

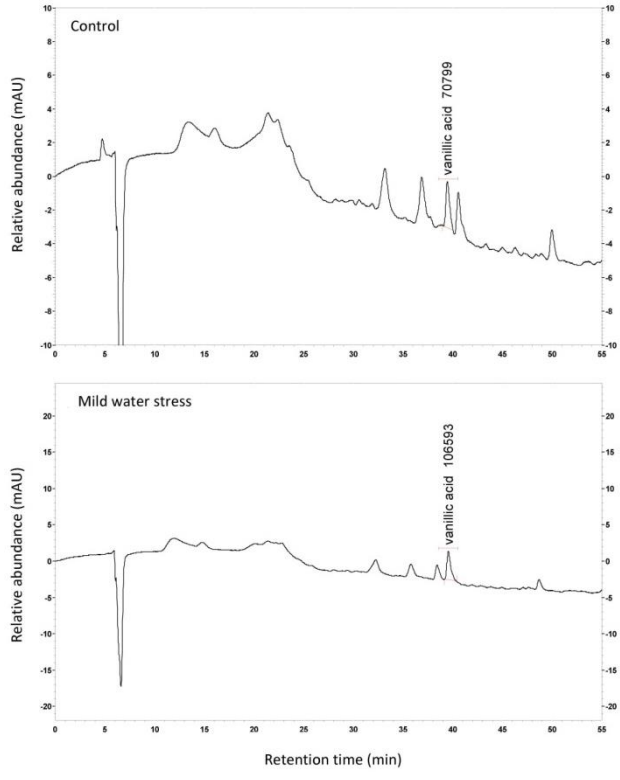

Figure 9: Effects of mild and severe drought stress on the representative chromatogram of prepared extract of *Aloe vera* leaves for vanillic acid measurement.

4 CONCLUSION

After 180 days of exposure, leaf thickness, biomass and gel production were reduced only under severe drfought stress. However, in line with physiological observations, medicinal *Aloe* plant adapted to mild drought stress during a long-term growth is mainly depended on compatible solutes adjustment, improvement of phenolic compounds production and presence of thick leaves. Mild drought stress improved growth by increasing photosynthesis via enhancing PI_{abs}. This improvement of PSII activity is coupled with the higher carotenoid and phenol production. In this study, the level of vanillic acid like other plant secondary metabolites was influenced by the availability of water, indicating that fluctuation in the content of vanillic acid was a plant response to environmental factors and part of an adaptative strategy leading to tolerance to abiotic stresses. However, considering conditions of natural habitat, biosynthesis and accumulation of vanillic acid by *Aloe* leaves in response to mild drought stress is not yet completely clear.

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