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Effect of Light and Water Deficiency on Growth and Concentration of Various Primary and Secondary Metabolites of *Aloe vera* L.

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ABSTRACT

The effects of abiotic stresses on medicinal plants metabolism are well known, but how plants respond to the interaction of these stressors is little understood. Therefore, the current experiment was aimed to investigate changes in growth and concentration of various primary and secondary metabolites of *A. vera* grown under water deficit and different light intensity conditions. A split-plot in time research was laid out in a randomized complete block design with four replications in a research greenhouse. The factorial combination of four irrigation regimes (irrigation after depleting 20, 40, 60, and 80% of soil water content) and three light intensities (50, 75, and 100% of sunlight) were considered as the main factors. Sampling time was considered as sub factor. The results showed that the highest leaf, gel, and peel fresh weights were observed when the plants were subjected to low light intensity and irrigation was done after depleting 20% soil water moisture. Plants developed under full sunlight produced more pups (4.30, 3, and 3.75 per plant, 90, 180, and 270 days, respectively) and leaves (14.25, 18, and 21.25 per plant, 90, 180 and 270 days, respectively) and showed the higher fresh (165.75 g per plant) and dry root (37.60 g per plant) weight. These traits decreased with increasing water deficit severity during all the sampling times. Glucose (79.30 mg g^{-1}DW, 270 days), fructose (233.50 mg g^{-1} DW, 270 days), aloin (27.68%, 90 days), proline (2.07 mg g^{-1} FW, 90 days) and Phosphoenolpyruvate Carboxylase (PEP-Case) (0.463 mmol NADH g^{-1} protein min^{-1}, 90 days) increased with increasing light intensity and water deficit severity during all the sampling times. Although high light intensity and water deficit led to yield and growth reduction, concentration of various primary and secondary metabolites increased. The results suggest that reduction in light intensity mitigates adverse effects of water deficit by inducing primary and secondary metabolites changes. It can be considered as an acclimation mechanism under water deficit conditions to avoid yield loss in *A. vera* production.

Keywords Aloin, Environmental stresses, Irrigation regime, Soluble sugars.

INTRODUCTION

Among the conditions affecting plant growth, water and light are the major factors controlling crops growth and production (Zhang *et al*., 2010). Experimental results revealed that evaluating the effect of a single stress factor alone without considering other factors would be insufficient, as plants are normally subjected to a combination of different abiotic or biotic stresses (Mittler, 2006). Furthermore, it should be taken into account that a combination of stresses can

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cause significantly higher harmful effects than each of them alone (Zhang *et al*., 2010; Tattini *et al.*, 2004). Water and light stresses represent an explicit instance of abiotic stress factors that often occur together (Mittler, 2006; Giraud *et al*., 2008; Jagtap *et al.*, 1998) and restrict crops growth and production (Giraud *et al*., 2008). Light intensity is one of the main factors affecting the biosynthesis rate of secondary metabolites in medicinal plants (Selmar and Kleinwachter, 2013a). Drought stress, which usually arises by water shortage, is caused by high solar radiation too, creating a condition that could be problematic for plants (Giraud *et al*., 2008). Drought stress, for example, results in metabolic driven responses because of stomatal closure and could increase the leaf internal temperature, avoiding thermal exchanges with the atmosphere at night. Subsequently, a huge oxidative stress occurs in plant, shifting the metabolic fluxes towards consumption of reduction equivalents for CO_2 fixation (Selmar and Kleinwachter, 2013a). This will result in an increased biosynthesis of secondary metabolites, such as alkaloids and terpenoids. When different environmental stresses happen at the same time, antagonistic responses might be observed. The plants acclimation to abiotic stresses, especially those that occur at the same time, would require an appropriate reaction tailored to each stressor, as well as customized to the need to compensate or adjust for some of the antagonistic aspects of the stress combination (Suzuki *et al*., 2014). The light intensity that exceeds the photosynthesis capacity of the leaves may be caused by the high incidence of the light radiation, as well as by the failure in photosynthetic carbon assimilation, due to stomatal closure. This process may be the directly light-dependent assimilation of CO_2 via rubisco in the chloroplasts, malate decarboxylation in the cytosol, or malate efflux from the vacuole (Mittler, 2006). Although the effects of water deficit vary from plant to plant, restriction of water losses is caused by water deficit under high light intensity. Regarding medicinal plants, both reduced light intensity and water deficit could alter secondary metabolites composition in *Ligustrum vulgare* (Tattini *et al*., 2004).

Aloe vera is a perennial plant with fleshy leaves that fix carbon through Crassulacean Acid Metabolism (CAM). *A. vera* originated from Africa, is widely cultivated in warm and dry regions of the world, and is adapted to dry conditions, where high light intensities dominate (Cousins and Witkowski, 2012). *A. vera* has more than 240 nutritional and medicinal constitutes including vitamins, minerals, enzymes, polysaccharides, lignin, saponins, sterols, amino acids, salicylic acid, and compounds found in the leaves and in the extracted gel (Ray *et al*., 2013; Murillo-Amador *et al.*, 2014).

The leaves have two main parts. The innermost part, which is clear, soft, and moist, consists of large thin-walled parenchyma cells. The shallowest part, which is called chlorenchyma, contains the main photosynthetic cells and forms the basic green tissue of the leaves. The skin is rich in 1, 8-dihydroxyanthraquinone derivatives and their glycosides, whereas the parenchyma is rich in complex carbohydrates (Newton, 2004). Moreover, polysaccharides, anthraquinones, enzymes, and different minerals are found in *A. vera* gel and play a critical role in expression of a diverse collection of the bio-active properties. *A. vera* is among the few medicinal plants used in food, cosmetic, and pharmaceutical industries. The gel is used by manufactures as a flavoring component or preservative agent (Christaki and Florou-Paneri, 2010).

Despite little information available on *A. vera* agronomic practices, its cultivation is growing in recent years (Ray and Gupta, 2013). On the other hand, little is reported about the effects of environmental stresses on phytochemical and biochemical characteristics of *A. vera*. Water deficits have a greater effects on growth, yield and nutritional composition of crops under high light than low light conditions (Tattini *et al*., 2004; Bernal *et al*., 2015). The accumulation of primary metabolites in plants is affected by water and light conditions (Ramakrishna and Ravishankar, 2011). The information on the effects of ecological stresses on *A. vera* is insufficient and limited to a single stress factor, for example, the effects of water (Rodríguez-García *et al.*, 2007; Delatorre-herrera *et al.*, 2010) or light intensity (Zapata *et al.*, 2013; Lucini *et al.*, 2013) in different studies. There are few reports in the literature focusing on the effects of high light intensities and water deficit, imposed at the same time, on *A. vera*. Hence,

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the aim of our study was to examine the effect of water deficiency and light on growth, concentration of various primary and secondary metabolites of *A. vera* growing in a greenhouse in Iran. The results will provide a snapshot of the influence of light intensities and water deficit on *A. vera* and will be used to define whether appropriate light control and water availability might increase yield and secondary metabolites synthesis of *A. vera* in greenhouse systems.

MATERIALS AND METHODS

Experimental Design, Treatments, and Growth Conditions

The experiments were implemented in a randomized complete block design arranged as split-plot in time with four replicates in a greenhouse located at Faculty of Agriculture, University of Tarbiat Modares, Tehran, Iran, in 2013 (summer, autumn) and 2014 (winter) growing seasons. Four irrigation regimes (irrigation after depleting 20, 40, 60, and 80% of the Field Capacity (FC); the total available water determined by the difference between FC and the Permanent Wilting Point (PWP). Before the experimental period, plants were grown for a period of 2 months to the same environmental conditions and irrigated with 80% FC; the irrigation treatment began in 20 June 2013, leading to a total number of irrigations during the experiment period of 75. The irrigation treatments were combined with three light levels (50, 75, and 100% of full sun), allocated in four randomized blocks (main plots) with different sampling times assigned in split-plots. The samples were collected 90, 180, and 270 days after the application of the treatments. Small plants (18-20 cm tall) developing from the sides of the mother plants were transplanted after two months into pots containing 18 kg soil. The plants were under the different sunlight treatments to irrigation regimes for 9 months. The plants were shaded placed under nylon mesh tents to reduce the light level by 50 or 75%. The light level was measured daily at noon using a portable solarimeter (118 HAENNI). There were 344, 232, and 198 sunshine hours in summer, autumn, and winter, respectively. The greenhouse temperature was adjusted to 28°C in the greenhouse during the day and 22°C during night. From each treatment and replicate, four plants were randomly selected and the number of leaves and pups counted. The harvested plants were transferred to the laboratory and gel and peel fresh weight were recorded after eliminating upper and lower parenchyma.

At the end of the experiment, three plants from each treatment were harvested to determine fresh and dry root weight. The plants were separated into roots and shoots, the roots were washed and dried in an oven at 60°C for 3 days.

Soil Moisture Content

A Time Domain Reflectometry (TDR) device (TRIM-FM 10776, Germany) equipped with a 20 cm three-pointed probe was used daily to monitor soil moisture content. Gravimetric moisture was calculated as follows.

$$\Theta_v\ (\%) = \Theta_G \times P_S / P_W$$

Where, Θ_G is the Gravimetric water content, Θ_V is the Volumetric soil moisture, P_W is the Water density, and P_S is the density of the Soil.

Irrigation was applied based on available soil water. Aluminum foil was used to reduce soil evaporation and water drained from plots was measured. Soil moisture at field capacity and wilting point was 20.9 and 7.6% volumetric soil moisture, respectively, using a pressure plate apparatus and soil moisture retention curve (pF curve).

Proline Accumulation

The method of Bates *et al*. (1973) was used to determine proline content in *A. vera* samples. The samples were homogenized with 3 ml sulphosalicylic acid (3% w/v), and then centrifuged at 18,000×g for 15 minutes. The supernatant (2 mL) was mixed with 2 mL glacial acetic acid and 2 mL freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6M orthophosphoric acid) in test tubes. The tubes

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were incubated in a water bath for 1 hour at 100°C and then cooled to room temperature. Toluene (4 mL) was added to the tubes and mixed on a vortex mixer for 20 seconds. The tubes were allowed to stand for at least 10 minutes, to allow separation of the toluene and aqueous phases. The toluene was carefully pipetted out into new tubes and its absorbance measured at 520 nm in a spectrophotometer. The proline content was calculated using a standard curve and expressed as mg g^{-1} Fresh Weight (FW).

Concentration of Soluble Sugars

Sugars (glucose, sucrose, maltose, fructose, and xylose) were determined according to the Sturm *et al*. (2003) with some minor modifications. Gel was extracted from the leaves manually in the laboratory, then, samples were freeze-dried (Labogene ScanVac Cool Safe Freeze Dryer System (CS55-4, Lynge, Denmark)) for 48 hours. Twenty mg gel powder of *A. vera* was diluted to 2 mL with deionized water and clarified by centrifugation at 6,000 rpm for 18 minutes at 4°C. The supernatant was isolated, and extract was passed through 0.45 µm Millipore filters and a 20-µL sample was used for current HPLC analysis of sugars in triplicate.

High Performance Liquid Chromatograph (HPLC) (Agilent Technologies 1200 Series) equipped with refractive index detector RID and Zorbax Carbohydrate 5 Micron column (4.6×250 mm) was used. Test conditions were mobile phase mixture of acetonitrile with deionized water in ratio 65:35 (v/v); flow rate 0.8 mL min^{-1}; temperature of column and detector 30°C. Pure sugars were used as external standards. Peaks generated from the *A. vera* gel were identified by comparison of their rotation times. Chromatograph software calculated the concentration of the sugars comparing chromatograms of samples with standard curves of respective sugars.

Concentration of Aloin

Aloin was determined using the methods of Waller *et al.* (2004). After cutting the leaf, the yellow syrup that leaked from wound was collected and stored in liquid nitrogen until analyzed. The samples were freeze-dried (Labogene ScanVac Cool Safe Freeze Dryer System (CS55-4, Lynge, Denmark)) for 24 hours. Aloin was determined using high-performance liquid chromatography (Waters, USA; 4.6×250 mm, dp 10 µm column, µ Bondapack C18). Standard samples were purchased from Sigma-Aldrich, USA. Stock solution was prepared by dissolving aloin into water-methanol solvent (1:1 v:v) and used to make standard solutions. The concentration was calculated using external standard and aloin standard curves.

Activity of Phosphoenolpyruvate Carboxylase

PEP-Case activity was measured spectrophotometrically as described by Murillo -Amador *et al*. (2014) at 340 nm by coupling the reaction to the oxidation of NADH in the presence of Malate Dehydrogenase (MDH). The standard assay medium was a mixture of enzyme extract, 10 units of MDH, 0.1 mM NADH, 2.5 mM $MgSO_3$ and 5 mM NaHCO in a total volume of 2.95 mL 50 mM Tricine buffer (pH 8.8). The reaction was started by adding 50 mL 2.2 mM PEP. The NADH oxidation rate was measured every 15 seconds for 3 minutes. The reaction was observed using the visual display of the spectrophotometer to confirm adequate mixing of the cuvette contents and that NADH oxidation reaction was linear. The assays were done in triplicate.

Statistical Analysis

Main and interaction effects of irrigation and light were determined from Analysis Of Variance (ANOVA) using the General Linear Model (GLM) procedure in Statistical Analysis System (SAS) software. The PROC UNIVARIATE within SAS was used to test the assumptions of ANOVA, and residuals were normally distributed. Least Significant Difference (LSD) test at the P= 0.05 level was

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used to check significant differences among means.

RESULTS

Plant Growth and Leaf Yield

In all sampling rounds, there were no significant differences between light intensity of 75 and 50% as well as between 20 and 40% moisture depletion treatments (Table 1 and Figure 1). Leaf, gel, and peel fresh weight generally decreased with increasing light level and water deficit (Table 2). The lowest yields occurred when full sun light and severe water deficit were simultaneously imposed. In all sampling rounds, the maximum leaf fresh weight (325.22, 405.80 and 680.52 g per leaf, 90, 180, and 270 days after applying the treatments, respectively), gel fresh (218.80, 241.30 and 438.87 g per leaf, 90, 180, and 270 days after applying the treatments, respectively) and peel fresh (107.67, 162.40 and 241.65 g per leaf, 90, 180, and 270 days after applying the treatments, respectively) were obtained when 50% of sunlight was blocked and irrigation was done after depleting 20% soil water content. By contrast, the minimum values were obtained when the plants were irrigated after depleting 80% soil water content and grown under full light. In comparison with the maximum values, leaf, gel, and peel fresh weight decreased by 49, 50, and 36% in the first sampling, 45, 42, and 50% in the second sampling, and 45, 56, and 42% in the third sampling, respectively.

Fresh and Dry Root Weight

Irrigation regime and light intensity significantly affected roots fresh and dry weight, at the end of 9 months of growth (Figures 2-A and -B). The plants under full sunlight and irrigated after depleting 20% soil water content produced more fresh weight (165.75 g per plant) and dry root weight (37.60 g per plant) (70 and 75%, respectively) compared with those plants that were grown under 50% of irradiation and irrigated after depleting 80% soil water content. The root weight increased with increasing light intensity and decreased with increasing water

Table 1. Effect of different irrigation regimes and light intensities on leaf number per plant, gel and peel fresh weight of *A. vera.*[a]

Light intensity (%)	Irrigation regime (after depleting %FC)	Days after treatment (Date)								
		90 (22 September) or summer			180 (21 December) or autumn			270 (21 March) or winter		
		Leaf number	Gel	Peel	Leaf number	Gel	Peel	Leaf number	Gel	Peel
						g per leaf				
Full sun	20	13bc	176.92abcd	105.55a	17.50ab	174.25def	133.55cd	21.25a	349.38de	210.25bc
	40	13bc	150.13bcde	98.03ab	17bc	226.40ab	141.35abc	20b	375.92bcd	211.72bc
	60	10.50e	133.98cde	66.08cde	14.50gh	161.00ef	110.53def	18.50d	299.80ef	181.08d
	80	10.25e	108.58e	58.20e	14h	139.00f	82.50g	17.75d	191.25g	140.80f
75	20	13.75ab	186.55abc	90.98abc	17bc	187.38cde	126.30cde	19.75bc	357.15cd	199.98bcde
	40	14.25a	177.23abcd	84.87abcd	18a	220.60ab	139.45bc	19.75bc	417.33ab	172.33de
	60	11.50d	144.68cde	74.78bcde	16.25cd	178.10de	127.73cde	18.75cd	296.33ef	187.53cd
	80	10.75de	127.40de	70.18cde	15ef	144.20f	90.53fg	17.75d	287.23f	147.13ef
50	20	13.25bc	218.80a	105.42a	16.25cd	241.30a	164.50a	20.25ab	438.88a	241.65a
	40	12.75c	200.73ab	107.68a	15.75de	219.23abc	162.40ab	20b	381.95bcd	223.65ab
	60	10.75de	184.78abc	87.35abcd	14.50h	206.18bcd	127.15cde	18.25d	410.25abc	179.63d
	80	11de	163.00bcd	62.63de	14.75f	165.38ef	105.60efg	18d	338.98def	192.60cd

[a] Means within a column followed by the different letters are significantly different ($P< 0.05$).

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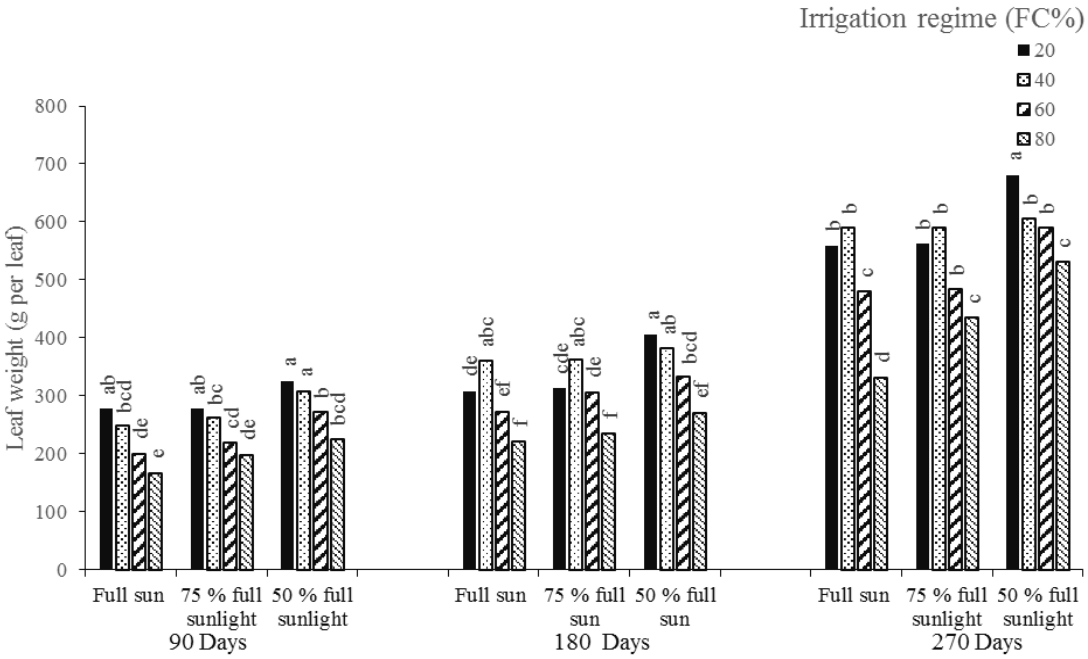

Figure 1. Effect of light and water depleting on leaf weight in *A. vera*. Means within a column followed by the different letter are significantly different at P< 0.05. FC (%): Irrigation after depleting 20, 40, 60, or 80% of the Field Capacity.

deficit severity. Full sunlight tended to increase the root weight in all irrigation regimes.

Leaf and Pup production

Leaf and pup production were significantly affected by light intensity and water deficit treatments. At all growing stages, and under consistent light intensity, leaf production decreased due to increased water deficit. The maximum leaf number in the first (14.25 per plant) and second sampling rounds (18 per plant) were obtained when the plants were subjected to 75% sunlight intensity and 20 or 40% moisture depletion treatment. In the third sampling round, the maximum leaf number (21.25 per plant) was related to 20% moisture depletion and full sunlight treatment. Generally, leaf production was better with a 20 or 40% depletion of soil moisture than with a 60 or 80% (Table 1).

According to Figure 3, irrespective of water deficit, increase in light intensity increased pup production. On the contrary, increase in water deficit severity caused a significant decrease in pup production. The maximum pup number per plant (11.8, average of three sampling rounds) was obtained when full sun was applied and irrigation was performed after depleting 40% soil water content. On the other hand, the minimum pup number per plant (2, average of three sampling rounds) was obtained when 50% of sunlight was blocked and irrigation done after depleting 20% soil water content. There was a significant difference among seasons in terms of pup production. The maximum (2.05 per plant) and minimum (1.23 per plant) pup number were observed in summer (90 days after applying the treatments) and autumn (180 days after applying the treatments), respectively (Table 2). Nonetheless, there was no difference between 180 and 270 days after applying the treatments in terms of pup production.

Concentration of Aloin Concentration

Different levels of water deficit and light intensity significantly affected aloin concentration (Figure 4), and the highest concentration (27.68 %) was recorded when the plants were subjected to full sunlight and

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Table 2. Main effects of irrigation regimes and light intensities on growth and yield in *A. vera.*[a]

Days after treatment (Date)														
90 (22 September) or summer					180 (21 December) or autumn					270 (21 March) or winter				
Leaf number	Pup number	Leaf fresh weight	Gel fresh weight	Peel fresh weight	Leaf number	Pup number	Leaf fresh weight	Gel fresh weight	Peel fresh weight	Leaf number	Pup number	Leaf fresh weight	Gel fresh weight	Peel fresh weight
Light intensity (%)				(g per leaf)			Per plant			(g per leaf)		Per plant		
100	11.68b	3.39a	223.24b	142.40b	81.96a	15.75b	2.37a	290.39b	175.16b	116.98b	19.37a	3.02 a	490.42.b	304.09c	185.96b
75	12.56a	1.53b	239.16b	158.96ab	80.19a	16.56a	0.75b	304.01b	182.57b	121b	19a	0.94b	517.43b	339.51b	176.74b
50	11.93b	1.24b	282.91a	191.83a	85.14a	15.31b	0.68b	347.93a	208.02a	139.91a	19.12a	0.62c	601.89a	392.51a	209.38.b
Irrigation regime (After depleting %FC)															
20	13.30 a	2.11b	293.57a	194.09a	93.15a	17.25a	1.25b	342.43a	200.97b	141.45a	20.41a	1.44bc	600.76a	381.80a	217.29a
40	13.30a	2.40a	272.88a	176.06b	96.58a	16.58b	1.58a	368.06a	222.07a	147.73a	19.91a	1.83a	594.71a	391.76a	202.56a
60	10.91b	1.92bc	230.54b	154.48bc	76.06b	15.98c	1.16a	303.56b	181.75c	121.8b	18.50c	1.75a	518.20b	335.46b	182.74b
80	10.66b	1.78c	196.74c	132.99c	64.63b	14.58c	1.08b	242.40c	149.52d	92.87c	17.40c	1.08c	432.66c	272.48c	160.17c
General means	12.06C	2.05A	248.43c	164.40C	84.31C	15.87B	1.27C	314.11B	188.58B	125.96B	19.17A	1.53B	536.58A	345.37A	190.69A

[a] A-C and a-c Means within a column followed by the same letter are not significantly different ($P \leq 0.05$). Different capital letters amongst seasons show significant differences.

irrigated after depleting 80% soil moisture and harvested 90 days after applying the treatments (summer). By contrast, the lowest concentration (14.33 %) was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil water content and harvested 270 days after applying the treatments (winter). Reduction in light intensity not only mitigated negative effects of water deficit, but also decreased aloin concentration, which was highly variable. The highest (23.39 % and lowest (14.13 %) concentrations were obtained in summer (90 days after applying the treatments) and winter (270 days after applying the treatments), respectively (Table 4 and Figure 4).

Soluble Sugars

Only fructose and glucose were detected in the dried gel samples with no xylose and sucrose. Overall, gel samples contained more fructose than glucose. The concentration of these two sugars generally improved with increasing water deficit severity under the three different light regimes. In all sampling rounds, the highest glucose and fructose concentrations were found when the plants were exposed to full sunlight and watered after depleting 80% soil water content. By contrast, the lowest concentration was detected when 50% of sunlight was blocked and irrigation was done after depleting 20% soil water content. It should be noted that there was significant difference among sampling rounds in term of glucose and fructose concentrations so that the maximum (73.18 and 26.89 mg g^{-1}) and minimum (62.90 and 20.15 mg g^{-1}) values were recorded 270 (the third sampling round) and 90 (the first sampling round) days after applying the treatments, respectively (Tables 3 and 4).

Phosphoenolpyruvate Carboxylase (PEP-Case)

The activity of PEP-Case generally increased with increasing water deficit under the three different light regimes. The highest activity (0.463 mmol NADH g^{-1} protein min^{-1}) was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil

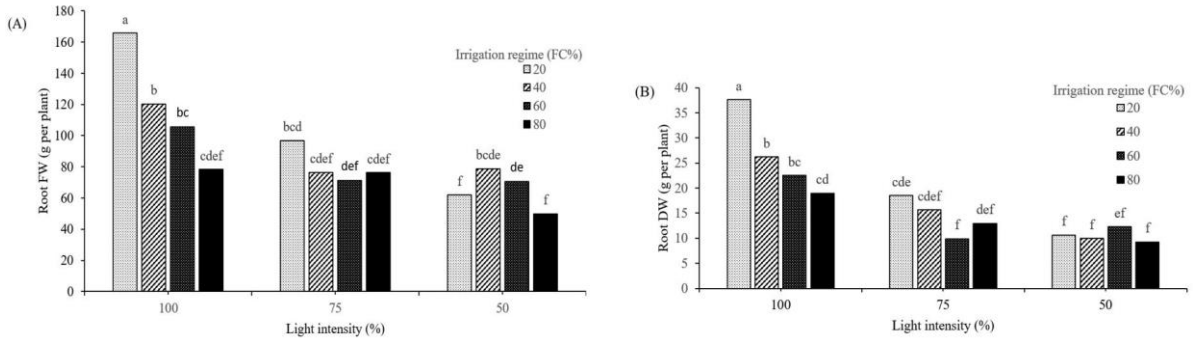

Figure 2. Effect of light and soil water depletion on root fresh (A) and dry (B) weight in *A. vera* after 270-days. Means within a column followed by the different letter are significantly different at P< 0.05. FC (%): Irrigation after depleting 20, 40, 60, or 80% of the Field Capacity.

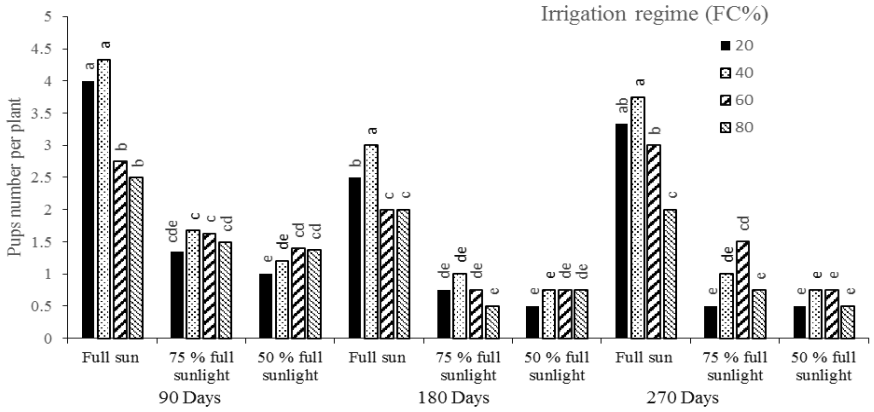

Figure 3. Effect of light and soil water depletion on pups number in *A. vera*. Means within a column followed by the different letter are significantly different at P< 0.05. FC (%): Irrigation after depleting 20, 40, 60, or 80% of the Field Capacity.

Table 3. Soluble sugar of *A. vera* subjected to different irrigation regime and light intensity conditions at different growth periods.[a]

		Days after treatment (Date)					
		90 (22 September) or summer		180 (21 December) or autumn		270 (21 March) or winter	
Light intensity (%)	Irrigation regime (After depleting %FC)	Fructose	Glucose	Fructose	Glucose	Fructose	Glucose
		mg g^{-1} DW					
Full sun	20	33.60fgh	15.12d	35.14gh	18.65ef	40.94fg	23.05f
	40	37.40fg	19.83de	41.35fg	24.07de	47.81ef	29.05de
	60	79.33d	30.85c	83.52d	32.17c	93.97c	38.99c
	80	198.17a	64.38a	202.95a	69.65a	233.54a	79.83a
75	20	24.00ghi	7.21f	25.40hi	8.07g	29.15gh	10.52g
	40	19.22i	5.47f	20.28i	5.65g	22.66hi	8.02gh
	60	63.23e	15.76ed	65.35e	17.18f	63.64d	20.89f
	80	130.67b	35.52b	133.94b	38.44b	148.65b	44.57b
50	20	8.33l	2.66f	9.43l	3.09g	12.39i	4.21h
	40	20.01h	4.69f	20.97hil	5.45g	25.54h	7.58gh
	60	45.88f	17.55ed	49.64f	21.15def	54.73de	25.04ef
	80	95.07c	22.77d	98.70c	26.41d	105.26c	31.01d

[a] Means within a column followed by the different letters are significantly different (P< 0.05).

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Table4. Main effects of irrigation regimes and light intensities on primary and secondary metabolites in *A. vera*.[a]

	Days after treatment (Date)													
	90 (22 September) or summer					180 (21 December) or autumn					270(21 March) or winter			
	Aloin	Fructose	Glucose	Proline	PPE	Aloin	Fructose	Glucose	Proline	PEP	Aloin	fructose	Glucose	Proline	PEP
Light intensity (%)	%	mg g^{-1} DW		(mg g^{-1} FW)	(NADH g^{-1} protein min^{-1})	%	mg g^{-1} DW		(mg g^{-1} FW)	(mmol NADH g^{-1} protein min^{-1})	%	mg g^{-1} DW		(mg g^{-1} FW)	(mmol NADH g^{-1} protein min^{-1})
Full sun	25.09a	87.12a	32.54a	1.36a	0.36a	21.77a	90.74a	36.13a	1.04a	0.32a	22.12a	104.06a	42.73a	1.15a	0.26a
75	23.75b	59.28b	15.99b	1.14b	0.33b	18.63c	61.24b	17.33b	0.98b	0.28b	19.40b	66.02b	21b	1.01b	0.24b
50	21.14c	32.42c	11.92c	0.97c	0.27c	20.87b	44.68c	14.02c	0.84c	0.22c	19.05b	49.48c	16.96c	0.85c	0.21c
Irrigation regime (After depleting %FC)															
20	21.98b	21.97c	8.33c	0.84b	0.28c	19.53b	23.32c	9.93c	0.68c	0.24c	15.76d	12.59c	27.49c	0.72c	0.19c
	22.07b	25.54c	9.99c	0.96b	0.29c	21.03a	27.53c	11.72c	0.91b	0.25c	19.46c	14.88c	32c	0.95b	0.20c
60	24.68a	62.82b	21.38b	1.31a	0.31b	21.43a	66.17b	23.49b	1.06a	0.28b	28.31b	70.78b	23.14a	1.17a	0.24b
80	24.58a	141.30a	40.89a	1.51a	0.39a	18.37c	145.19a	44.83a	1.16a	0.32a	51.80a	162.48a	22.41b	1.18a	0.31a
General mean	23.33A	62.90	20.15	1.15A	0.32A	20.09B	65.55B	22.47B	1.01B	0.27B	20.19B	73.18A	26.89A	0.96B	0.23C

[a]A-C and a-c Means within a column followed by the same letter are not significantly different ($P \leq 0.05$). Different capital letters amongst seasons show significant differences.

water moisture. On the other hand, the lowest activity (0.170 µmol NADH per g protein per min) was observed when 50% of irradiation was blocked and irrigation was done after 20% soil water reduction. When light and water deficit severity were considered as fixed effects, PEP-Case activity increased with increasing water deficit severity and decreased with reducing light intensity, respectively. The maximum PEP-Case activity was recorded 90 days after applying the treatments. In comparison with 180 and 270 days samplings, 15 and 17% increase was found in 90 days sampling (Tables 4 and 5).

Proline

The response of proline was similar to that recorded for PEP-Case activity (Tables 4 and5). The highest (20.17 mg g^{-1} FW) and lowest (0.62 mg g^{-1} FW) proline contents were detected when the plants were exposed to full sunlight and watered after reducing 80% soil water content (90 days after applying the treatments) and when 50% of sunlight was blocked and irrigation was done after depleting 20% soil water content (270 days after applying the treatments), respectively. As it can be seen from the results, the increase in water deficit severity and light intensity raised proline accumulation in the leaves (Table 5).

DISCUSSION

A. vera yield (leaf and gel fresh weights) significantly decreased due to high light intensity and water deficit. An increase in light intensity may be associated with a raise in leaf temperatures and in a decline of the water potential, which can alleviate the water deficit. According to the data mentioned, the highest gain in *A.vera* leaf and gel weight was not achieved in the treatments with full sun light and maximal irrigation. This, however, means that although CAM plants close their stomata during the day, apparently, the photosynthesis rate is significantly lower than in corresponding assays with lower light intensities. This indicates that under full sunlight also in *A. vera* plants, a significant loss of CO_2 occurred which might be due to enhanced photorespiration, despite

Table 5. Proline and PEP-Case of *A. vera* subjected to different irrigation regime and light intensity conditions at different growth periods.[a]

		Days after treatment (Date)					
		90 (22 September) or summer		180 (21 December) or autumn		270 (21 March) or winter	
Light intensity (%)	Irrigation regime (after depleting %FC)	Proline (mg g^{-1} FW)	PEP-Case(mmol NADH g^{-1} protein min^{-1}	Proline (mg g^{-1} FW)	PEP-Case(mmol NADH g^{-1} protein min^{-1}	Proline (mg g^{-1} FW)	PEP-Case(mmol NADH g^{-1} protein min^{-1}
Full sun	20	0.90d	0.318de	0.723fg	0.295c	0.829ef	0.225de
	40	0.99c	0.323d	1.023bcd	0.293cd	1.042cd	0.228cde
	60	1.48b	0.355c	1.186ab	0.325b	1.357ab	0.253c
	80	2.07a	0.463a	1.255a	0.383a	1.391a	0.323a
75	20	0.90d	0.303de	0.681g	0.258ef	0.706f	0.193fg
	40	0.89d	0.298e	0.895def	0.245f	0.979de	0.205ef
	60	1.44b	0.325d	1.092abc	0.288cd	1.203bc	0.235cd
	80	1.32bc	0.395b	1.243a	0.328b	1.141cd	0.323a
50	20	0.72d	0.245g	0.644g	0.195g	0.626g	0.170g
	40	1.00c	0.265fg	0.802efg	0.185g	0.830ef	0.178g
	60	1.01c	0.273f	0.901def	0.235f	0.973de	0.228cde
	80	1.16bcd	0.315de	0.977cde	0.270de	0.997de	0.280b

[a] Means within a column followed by the different letters are significantly different (P< 0.05).

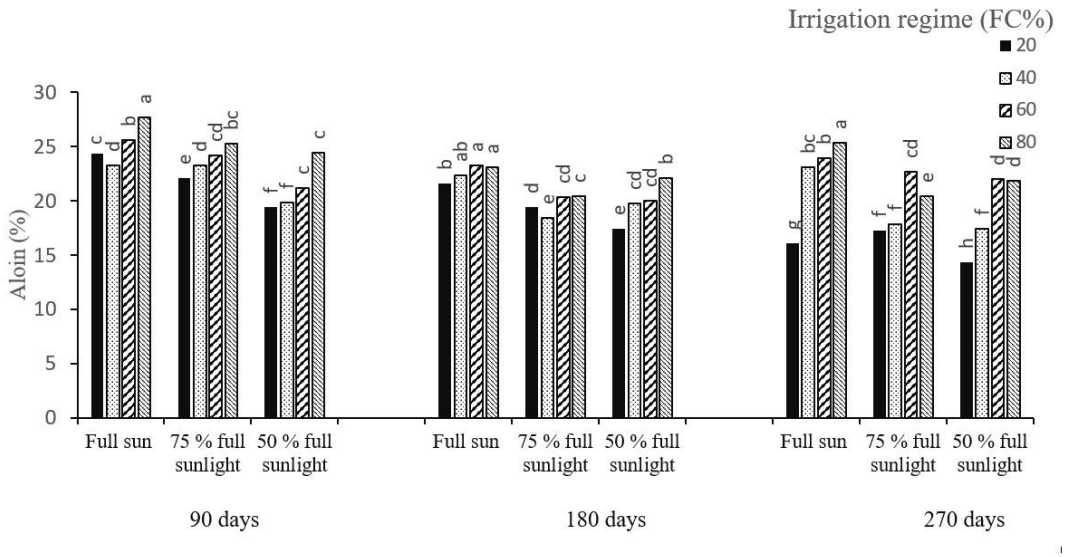

Figure 4. Effect of light and soil water depletion on aloin concentration in *A. vera*. Means within a column followed by the different letter are significantly different at P< 0.05. FC (%): Irrigation after depleting 20, 40, 60, or 80% of the Field Capacity.

putatively enhanced energy dispersing mechanisms, i.e., xanthophyll cycle and non-photochemical quenching. This phenomenon occurs despite a high activity of PEP carboxylase, which should re-fix (also during the day) this CO_2 (Selmar and Kleinwächter, 2013b). However, reduction in light intensity in all sampling rounds could considerably increase *A. vera* yield. For example, the maximum leaf, gel, and peel fresh weigh were achieved when light intensity was reduced and no water deficit was imposed. According to the results, better yield of shaded *A. vera* could be related to better humidity and temperature conditions in the shade (Carneiro *et al*., 2015).

According to the results, soil water content and light intensity are the most important factors affecting *A. vera* growth and yield. According to

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previous findings, to gain the best results in *A. vera* production, soil water content should not be higher than field capacity during *A. vera* growth (Silva *et al*., 2010; Delatorre-herrera *et al*., 2010). Possibly, as for other plants, *A. vera* growth would be reduced by decreasing soil water content; however, this reduction is less than in other plants knowing that *A. vera* is a succulent species and takes advantage from the CAM photosynthesis pathways (Delatorre-herrera *et al*., 2010).

In the current study, full irrigation and sunlight condition increased the root weight, which might be related to higher growth and carbon assimilation that affects plant development and acclimation (Valladares and Niinemets, 2008). When shaded *A. vera* plants were subjected to water deficit, biomass allocation to roots was reduced; root fresh and dry weight under full sunlight were much higher than 50% sunlight. The decreased biomass allocation to roots could also be caused by holding of assimilated carbon in leaves at the expenses of carbon in roots. Similar outcomes have been described by Paez *et al.* (2000). A reduction in carbon allocation to roots in plants grown under shade has also been defined in other species such as *Glycyrrhiza uralensis* Fisch. (Hou *et al*., 2009) and *Jatropha curcas* (Carneiro *et al*., 2015).

The results revealed that root growth was decreased when *A. vera* plants were grown under water deficit condition. Root development is strongly affected by water stress. Roots are much more exposed to water deficit than aboveground parts, when soil water content is not optimal, reductions in root growth are seen (Vandoorne *et al*., 2012). The combined influence of water deficit and low light intensity reduced the dry and fresh root weight.

In the present study, the maximum leaf number (21.25) significantly decreased with increasing water deficit severity, a result in agreement with Rodriguez-Garcia *et al*. (2007) findings. Leaf size was significantly reduced in plants grown under full sunlight and water stress relative to that of plants grown in shade, but leaf number increased in plants grown under full sun light. The higher leaf area in shade-grown *A. vera* indicated resource allocation for optimizing light interception than leaf number.

According to previous studies, increase in available water negatively affects leaf growth and optimum leaf number. It has been reported that the optimum leaf number for *A. vera* is 21 leaves during the whole growing season. Under water deficit conditions, leaf production would be decreased (Silva *et al*., 2010).

According to the results, pup number increased with increasing soil moisture content and light intensity. Pup number decreased under severe water deficit. Similar results have been found by Silva *et al.* (2010). There are several findings that support the role of environmental conditions in pup production in *A. vera*. For instance, Rahi *et al*. (2013) have reported that pup number increased with increasing sodium content in the soil.

The results indicated that pup production is significantly affected by light intensity at all growth stages. There was a direct correlation between pup number and light intensity, in other words, pup number increased with increasing light intensity. Considering that light intensity during the first 90 days (during summer) was at the maximum levels, the maximum pup number was obtained from full sunlight treatment at all growth stages. Similar results have been reported by Paez *et al.* (2000) on *A. vera*. However, pup production declined in plants subjected to shade and water stress because of shift in biomass allocation to stems and leaf area, since plants grown in full sun do not need to invest heavily in vertical growth for light interception because light is not a scarce resource.

Aloin concentration was different in various growth stages. More aloin was produced in young plants, which were harvested 90 days after applying the treatments in summer, than in old plants, which were harvested in winter. Aloin concentration also varies from season to season and highly depends on the age of the plants (Bozzi *et al*., 2007). In a study, the highest and lowest aloin concentrations were obtained in summer and winter, respectively (Zapata *et al*., 2013). An increase in aloin concentration in warm seasons is due to higher ambient temperature and increased light intensity, which alter secondary metabolite synthesis (Beppu *et al*., 2004). In the present study, it was found that water deficit and high light intensity improve aloin synthesis in *A. vera* leaves. In most cases, environmental stresses could significantly enhance aloin concentration. Aloin accumulation during salinity (Rahimi-Dehgolan *et al*., 2012),

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high light intensity (Lucini *et al*., 2013) and salt stress (Rahi *et al*., 2013) has been well established. A change in secondary metabolites synthesis is an important mark of protecting plant against unfavorable environmental conditions (Ramakrishna and Ravishankar, 2011). When it comes to medicinal plants, reduced water deficit and light intensity have been found to alter essential oil levels and compositions (Tattini *et al*., 2004). According to the previous evidence, secondary metabolites synthesis in *A. vera* would increase under drought stress conditions (Delatorre-herrera *et al*., 2010). Enhanced synthesis of aloin may contribute to prevent injury caused by radicals advanced due to the stress conditions. This increase could either be due to a stress-related decline in dry matter production or a reliable enhancement of the total aloin content (Selmar and Kleinwächter, 2013a). This leads to defense in contradiction of UV light or too high light intensities (Kleinwächter and Selmar, 2014)

Fructose and glucose were found to be the most dominant soluble sugars in *A. vera* gel samples. Similar results were found by Paez *et al*. (2000). In some studies, maltose has been reported as the abundant sugar in the gel, which is inconsistent with the present study. This may be due to the extraction method. Here, we show that in all three sampling rounds, glucose concentration was found to be higher than fructose. In addition, the results showed that high light intensity increases glucose and fructose concentration in the *A. vera* gel. An increase in polysaccharides concentration in *A. vera* gel due to high light intensity has been reported by several authors (Ray and Gupta, 2013; Lucini *et al*., 2013). The capacity for accumulation of soluble carbohydrates in *A. vera* gel depends on seasonal factors affecting water soluble carbohydrate concentrations, such as air temperature, photoperiod and atmospheric carbon dioxide concentration (Ray *et al*., 2013). In the present study, soluble sugar concentration varied from season to season such that the maximum amount of soluble sugars was found in winter or 270 days after applying the treatments. The obtained results demonstrated that glucose and fructose synthesis increased with increasing light intensity and water deficit severity. Furthermore, it has been stated that light intensity and available water content affect soluble sugars accumulation (Paez *et al*., 2000; Lucini *et al*., 2013). Taking advantage from CAM pathway and being able to synthesize osmolytes helps the *A. vera* plants to overcome the water deficit (Delatorre-herrera *et al*., 2010). As mentioned earlier, soluble sugars concentration increased due to high light intensity and water deficit, thus, it seems that increasing the soluble sugars concentration favors an osmotic adjustment that improves drought tolerance in *A. vera* plants. This rise could be due to reduced mobilization, increased synthesis, or a combination of both processes. In consequence, sugars and thus polysaccharides are efficiently synthesized, serving as molecules to store water during the moisture stress periods in the arid regions (Salinas *et al*., 2016)

Our results indicate that, at all growing stages, PEP-Case activity improved with increasing light intensity and water deficit severity. The fact that PEP-Case has an important role in CAM plant metabolism suggests that this enzyme is related to adaptation when plants experience stress conditions. Numerous studies have illustrated the effects of environmental stresses on PEP-Case (the most abundant and important enzyme that plays a key role in carbon dioxide fixation in CAM plants) synthesis and activity. (Lüttge, 2004). An increase in PEP-Case activity due to salt stress in *A. vera* has been documented by Murillo-Amador *et al*. (2014). In sorghum, a significant increase in PEP-Case activity was observed when the plants were subjected to water deficit, high light intensity, and high temperatures simultaneously (Jagtap *et al*., 1998). Considering the fact that PEP-Case is a determining factor involved in photosynthesis of CAM plants and light is a primary requirement for photosynthesis, it is not surprising that an increase in light intensity contributes to an increase in PEP-Case activity (Jagtap *et al*., 1998). In the present study, it was found that light has a significant effect on PEP-Case activity, as mentioned by McElwain *et al.* (1992). Moreover, when the plants were subjected to high light intensity and water deficit at the same time, an increase in PEP-Case activity was more obvious. It seems that this increase could help the plants to deal with stressful conditions.

Results in current study show a gain in PEP-Case activity in *A. vera* plants exposed to water

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stress and high light intensity conditions, thereby indicating a role for this enzyme and its regulatory phosphorylation in response to plants under stress.

The physiological role of proline, as an adaptive response to environmental stresses, is the same in all plant species: an increase in proline accumulation in A. *vera* due to high light intensity water deficit is an expectable incident, as we also found such results. In our study, it was found that proline increased in A. vera plants with increasing water deficit and light intensity. These results suggest that, probably, proline is an important component in *A. vera* for osmotic adjustment during the high light intensity and water deficit conditions, tissue water content would be decreased, but increased proline accumulation protects against possible injury. This is supported by Delatorre-herrera *et al*. (2010) on *A. vera* under water deficit. It has been reported that proline can act as an electron acceptor during photo-inhibition and save photosystems against reactive oxygen species, and conserve $NADPH^+$ pool during oxidation-reduction cycles (Liang *et al*., 2013). Díaz *et al*. (2005) found that high light intensity and water deficit could increase proline accumulation on *Lotus corniculatus*. Free proline accumulation is the common and most important response of plants exposed to abiotic stresses in order to reduce injury to cells (Claussen, 2005).

CONCLUSIONS

In agricultural production, final produce extremely depends on environmental conditions such that abiotic stresses are the main limiting factors. On the other hand, light and water are the main contributors towards the plants growth and development, and play a main role in biochemical and phytochemical processes. In the current study, the effect of light and water on growth, yield, and concentration of various primary and secondary metabolites responses of *A. vera* were investigated during different growth stages. In general, the results indicated that high light intensity and severe water deficit significantly affect plant growth, yield, and concentration of various primary and secondary metabolites synthesis. Based on the results, although growth and yield decreased, the plants showed a high tolerance to severe water deficit and high light intensity. Root fresh and dry weight, leaf, and pup number significantly increased when plants were subjected to full sunlight treatment compared with other light intensities. By contrast, water deficit decreased root weight, leaf, and pup production. Reduced light intensity in well-irrigated plants caused significant increase in leaf, gel, and peel fresh weight. However, in all sampling rounds, the highest growth rate and yield were obtained when 50% of sunlight was blocked and irrigation was done after depleting 40% soil water content. The maximum aloin and soluble sugars concentration, PEP-Case activity, and proline accumulation were found when the plants were exposed to full sunlight and watered after depleting 80% soil moisture. In general, osmolyte contents increased in *A. vera* leaves to protect the plants against light and water deficit. Furthermore, sampling time showed a significant effect on concentration of various primary and secondary metabolites such that the maximum aloin percentage, PEP-Case activity, and proline accumulation were related to the first sampling in summer. In addition, the maximum glucose and fructose concentrations were related to the third sampling in winter. In general, it was found that *A. vera* plants are able to increase concentration of various primary and secondary metabolites synthesis to protect themselves against unfavorable conditions. The results showed that reduction in light intensity mitigated adverse effects of water deficit, which can be considered as a management strategy under water deficit conditions to avoid yield loss in *A. vera* production. Accumulation of various primary and secondary metabolites will provide the osmotic adjustment for *A. vera* plants to mitigate the effects of environmental stresses.

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تاثیر نور و تنش کم‌آبی روی رشد و غلظت متابولیت‌های اولیه و ثانویه گیاه صبرزرد (*Aloe vera* L.)

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چکیده

مطالعات زیادی در زمینه‌ی تاثیر تنش‌های غیرزنده روی متابولیسم گیاهان دارویی انجام گرفته، اما در مورد چگونگی واکنش گیاهان نسبت به اثرات متقابل بین تنش‌ها، مطالعات محدودی صورت گرفته است. بنابراین، هدف از این مطالعه ارزیابی رشد و تغییرات در غلظت متابولیت‌های اولیه و ثانویه‌ی گیاه صبرزرد رشد یافته تحت تنش کم‌آبی و شدت‌های مختلف تابش نور می‌باشد. بدین منظور، یک آزمایش اسپلیت پلات در زمان در قالب طرح پایه بلوک‌های کامل تصادفی با چهار تکرار در گلخانه تحقیقاتی اجرا گردید. ترکیب فاکتوریل شامل چهار رژیم آبیاری (آبیاری پس از تخلیه‌ی ۲۰، ۴۰، ۶۰ و ۸۰ درصد محتوی رطوبت خاک) و سه سطح تابش نور (۵۰، ۷۵ و ۱۰۰ درصد شدت تابش نور خورشید) به عنوان فاکتورهای اصلی و زمان‌های مختلف برداشت به عنوان فاکتور فرعی مورد بررسی قرار گرفت. نتایج نشان داد که بیشترین مقدار وزن تر برگ، ژل و پوست برگ زمانی که گیاهان تحت شدت نور پایین و آبیاری پس از تخلیه‌ی ۲۰ درصد رطوبت صورت قرار گرفتند، بدست آمد. گیاهان رشدیافته تحت شدت نور کامل بیشترین تعداد پاجوش (۴/۳۰، ۳ و ۳/۷۵ عدد در هر بوته به ترتیب ۹۰، ۱۸۰ و ۲۷۰ روز پس از اعمال تیمارها) و برگ‌ها (۱۴/۲۵، ۱۸ و ۲۱/۲۵ عدد در بوته، به ترتیب ۹۰، ۱۸۰ و ۲۷۰ روز پس از اعمال تیمارها) و همچنین بیشترین وزن تر ریشه (۱۶۵/۷۵ گرم در بوته) و وزن خشک ریشه (۳۷/۶۰ گرم در بوته) را تولید نمودند که این صفات با افزایش شدت تنش کم‌آبی در همه‌ی مراحل رشد کاهش پیدا نمود. میزان گلوکز (۷۹/۳۰ میلی‌گرم بر گرم وزن خشک، ۲۷۰ روز)، فروکتوز (۲۳۵/۵۰ میلی‌گرم بر گرم وزن خشک، ۲۷۰ روز)، آلوئین (۲۷/۶۸ درصد، ۹۰ روز)، پرولین (۲/۰۷ میلی‌گرم در وزن خشک، ۹۰ روز) و فسفواینول پیروات کربوکسیلاز (۰/۴۶۳ میلی‌مول NADH بر گرم پروتئین در دقیقه، ۹۰ روز) با افزایش شدت نور و تنش کم آبی در همه مراحل رشد افزایش پیدا کرد. اگر چه افزایش شدت تابش نور و تنش کم‌آبی منجر به کاهش رشد و عملکرد شد، اما میزان متابولیت‌های اولیه و ثانویه افزایش پیدا نمود. نتایج نشان داد که کاهش شدت تابش نور، اثرات تنش کم‌آبی را با تغییرات در سنتز متابولیت‌های اولیه و ثانویه افزایش داد. این می‌تواند به عنوان یک مکانیسم سازگاری تحت شرایط کم‌آبی به منظور جلوگیری از کاهش عملکرد در گیاه صبر زرد در نظر گرفته شود.

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