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 stresses 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, 3.6, 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
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 CO₂ 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 CO₂ 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-dihydroxyantraquinone derivatives and their glycosides, whereas the parenchyma is rich in complex carbohydrates (Newton, 2004). Moreover, polysaccharides, antraquinones, 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 (Rodriguez-Garcia 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,
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, \(\Theta_G\) is the Gravimetric water content, \(\Theta_v\) 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
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 at 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\(_4\) and 5 mM NaHCO\(_3\) 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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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 moisture depletion treatments (Table 1 and Figure 1). The lowest yields occurred when full sun light and severe water deficit were simultaneously imposed. In all treatments, 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) and peel fresh weight (218.80, 241.30, and 438.87 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 2A and B). The plants under full sunlight (Figures 2A and B) and 50% of sunlight produced more fresh and dry root weight (37.60 g per plant) (70 and 50% in the second sampling, and 45, 42, and 49% in the third sampling, respectively).

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

<table>
<thead>
<tr>
<th>Light intensity (%)</th>
<th>Irrigation regime (after depleting %FC)</th>
<th>Days after treatment (Date)</th>
<th>Leaf number</th>
<th>Gel peel fresh weight of leaf per plant</th>
<th>Leaf number</th>
<th>Gel peel fresh weight of leaf per plant</th>
<th>Leaf number</th>
<th>Gel peel fresh weight of leaf per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90 (22 September) or summer</td>
<td>180 (21 December) or autumn</td>
<td>270 (21 March) or winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>Gel</td>
<td>Peel</td>
<td>Leaf</td>
<td>Gel</td>
<td>Peel</td>
<td>Leaf</td>
</tr>
<tr>
<td>Full sun</td>
<td></td>
<td>20</td>
<td>13bc</td>
<td>176.92abcd</td>
<td>105.55a</td>
<td>17.50ab</td>
<td>174.25def</td>
<td>133.55cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>13bc</td>
<td>150.13bcde</td>
<td>98.03ab</td>
<td>17bc</td>
<td>226.40ab</td>
<td>141.35abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>10.50e</td>
<td>133.99cde</td>
<td>66.08cde</td>
<td>14.50gh</td>
<td>161.00ef</td>
<td>110.83def</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>10.25e</td>
<td>108.58e</td>
<td>58.20e</td>
<td>14h</td>
<td>139.00e</td>
<td>82.50g</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>20</td>
<td>13.75ab</td>
<td>166.55ab</td>
<td>90.98ab</td>
<td>17bc</td>
<td>187.38cde</td>
<td>126.30cde</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>14.25a</td>
<td>177.23ab</td>
<td>84.87abcd</td>
<td>18a</td>
<td>220.60ab</td>
<td>139.45bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>11.50d</td>
<td>144.68cde</td>
<td>74.78bcde</td>
<td>16.25cd</td>
<td>178.10d</td>
<td>127.73cde</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>10.75de</td>
<td>127.40de</td>
<td>70.18cde</td>
<td>15e</td>
<td>144.20f</td>
<td>90.53g</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>20</td>
<td>13.25be</td>
<td>218.80a</td>
<td>105.42a</td>
<td>16.25cd</td>
<td>241.30a</td>
<td>164.50a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>12.75c</td>
<td>200.73ab</td>
<td>107.68a</td>
<td>15.75de</td>
<td>219.23abc</td>
<td>162.40ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>10.75de</td>
<td>184.78abe</td>
<td>87.35bcd</td>
<td>14.50h</td>
<td>206.13bc</td>
<td>127.15cde</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>11de</td>
<td>163.00feb</td>
<td>62.63de</td>
<td>14.75f</td>
<td>165.38ef</td>
<td>105.60efg</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Days after treatment (Date)</th>
<th>Leaf number</th>
<th>Leaf fresh weight</th>
<th>Gel fresh weight</th>
<th>Peel fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 (22 September) or summer</td>
<td>100</td>
<td>11.68b 3.39a</td>
<td>223.24b 142.40b</td>
<td>81.96a 15.75b 2.37a</td>
</tr>
<tr>
<td>180 (1 December) or autumn</td>
<td>75</td>
<td>12.56a 1.53b</td>
<td>239.16b 158.96b</td>
<td>80.19a 16.56a 0.75b</td>
</tr>
<tr>
<td>270 (21 March) or winter</td>
<td>50</td>
<td>11.93b 1.24b</td>
<td>282.91a 191.83a</td>
<td>85.14a 15.31b 0.68b</td>
</tr>
</tbody>
</table>

**Irrigation regime (After depleting %FC):**

- **20%:** 20.13a 2.11b 293.57a 194.09a 93.15a 17.25a 1.25b 342.43a 209.97b 141.45a 20.14a 1.44bc 600.76a 381.80a 217.29a
- **50%:** 13.30a 2.40a 272.88a 176.06a 96.58a 16.58a 1.58a 368.06a 222.07a 147.73a 19.91a 1.83a 594.71a 391.76a 202.56a
- **80%:** 10.91b 1.92bc 230.54b 154.48b 76.06b 15.98c 1.16a 302.56b 181.75c 121.85b 18.50c 1.75a 518.20b 335.46b 182.74b

**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-C and a-c Means within a column followed by the same letter are not significantly different (P ≤ 0.05). Different capital letters amongst seasons show significant differences.

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**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 moisture. The lowest activity (0.048 mmol NADH g⁻¹ protein min⁻¹) was recorded when the plants were exposed to 50% sunlight and watered after reducing 20% soil moisture. By contrast, the highest concentration of glucose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of glucose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture. The highest concentration of fructose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of fructose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture. The highest concentration of sucrose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of sucrose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture. The highest concentration of glucose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of glucose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture. The highest concentration of fructose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of fructose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture. The highest concentration of sucrose was recorded when 50% of sunlight was blocked and irrigation was done after depleting 20% soil moisture. The lowest concentration of sucrose was recorded when the plants were exposed to full sunlight and watered after reducing 80% soil moisture.
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.

<table>
<thead>
<tr>
<th>Days after treatment (Date)</th>
<th>90 (22 September) or summer</th>
<th>180 (21 December) or autumn</th>
<th>270 (21 March) or winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity (%)</td>
<td>Irrigation regime (After depleting % FC)</td>
<td>Fructose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Full sun</td>
<td>20</td>
<td>33.60 tgh</td>
<td>15.12 d</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>37.40 fg</td>
<td>19.83 de</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>79.33 d</td>
<td>30.85 c</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>198.17 a</td>
<td>64.38 a</td>
</tr>
<tr>
<td>75</td>
<td>20</td>
<td>24.00 ghi</td>
<td>7.21 f</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>19.22 i</td>
<td>5.47 f</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>63.23 e</td>
<td>15.76 ed</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>130.67 b</td>
<td>35.52 b</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>8.33 l</td>
<td>2.66 f</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20.01 h</td>
<td>4.69 f</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45.88 f</td>
<td>17.55 ed</td>
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<tr>
<td></td>
<td>80</td>
<td>95.07 c</td>
<td>22.77 d</td>
</tr>
</tbody>
</table>

*Means within a column followed by the different letters are significantly different (P < 0.05).*
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Water moisture. On the other hand, the lowest activity (0.170 \( \mu \text{mol NADH per g protein per min} \)) 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).

**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 \( \text{CO}_2 \) 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.\(^a\)

<table>
<thead>
<tr>
<th>Light intensity (%)</th>
<th>Irrigation regime (after depleting %FC)</th>
<th>Proline (mg g(^{-1}) FW)</th>
<th>PEP-Case (mmol NADH g(^{-1}) protein min(^{-1}))</th>
<th>Proline (mg g(^{-1}) FW)</th>
<th>PEP-Case (mmol NADH g(^{-1}) protein min(^{-1}))</th>
<th>Proline (mg g(^{-1}) FW)</th>
<th>PEP-Case (mmol NADH g(^{-1}) protein min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>0.90d</td>
<td>0.318de</td>
<td>0.723g</td>
<td>0.295c</td>
<td>0.829ef</td>
<td>0.225de</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.99c</td>
<td>0.323d</td>
<td>1.023bcd</td>
<td>0.293cd</td>
<td>1.042cd</td>
<td>0.228cde</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.48b</td>
<td>0.355e</td>
<td>1.186ab</td>
<td>0.325b</td>
<td>1.357ab</td>
<td>0.253c</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>2.07a</td>
<td>0.463a</td>
<td>1.255a</td>
<td>0.383a</td>
<td>1.391a</td>
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</tr>
<tr>
<td>75</td>
<td>20</td>
<td>0.90d</td>
<td>0.303de</td>
<td>0.681g</td>
<td>0.258ef</td>
<td>0.706f</td>
<td>0.193fg</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.89d</td>
<td>0.298e</td>
<td>0.895def</td>
<td>0.245f</td>
<td>0.979de</td>
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</tr>
<tr>
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<td>0.325d</td>
<td>1.092abc</td>
<td>0.288cd</td>
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<td>0.235cd</td>
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<tr>
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<td>0.395b</td>
<td>1.243a</td>
<td>0.328b</td>
<td>1.141cd</td>
<td>0.323a</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>0.72d</td>
<td>0.245g</td>
<td>0.644g</td>
<td>0.195g</td>
<td>0.626g</td>
<td>0.170g</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.00c</td>
<td>0.265fg</td>
<td>0.802efg</td>
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<td>0.830ef</td>
<td>0.178g</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.01c</td>
<td>0.273f</td>
<td>0.901def</td>
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</tr>
<tr>
<td></td>
<td>80</td>
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<td>0.315de</td>
<td>0.977cde</td>
<td>0.270de</td>
<td>0.997de</td>
<td>0.280b</td>
</tr>
</tbody>
</table>

\(^a\)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
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),
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
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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REFERENCES


تأثیر نور و تنش کم آبی روی رشد و غلظت متابولیتهای اولیه و ثانویه گیاه صبرد ی (Aloe vera L.)

س. حضرتی، ز. طهماسبی سروستانی، ز. نیکولا، آ. بیرق دار کشکولی، ف. حیبی - زاده، ح. مصیبی، و. مختصی بیذگلی

چکیده

مطالعات زیادی در زمینه تاثیر ناشی‌های غیرنده روی متابولیسم گیاهان در سطح گسترش آنها، از مورد چگونگی واکنش گیاهان نسبت به اثرات تغییرات آب و هوا و تغییرات در غلظت متابولیتهای اولیه و ثانویه گیاه صبرد رشد یافته در تنش کم آبی و اثرات موجود هنگام تربیت گیاهان تحت شرایط مختلف تنش ناشی از تغییرات در غلظات و نرخ‌های طبیعی موجود در سطح نور می‌باشد. برای تفسیر یک آزمایش اسپلیت پلات در زمان دیگر طریق‌ها باید به طرف زیادی کامل تصادفی با چهار تکرار در غلظات تحصیلی اجرای گردید. ترکیب فاکتوری شامل چهار رنگ آبی (آبیاری پس از تخلیه 20، 40، 60 و 80 درصد نور و رشد خاک) و سطح تنش نور (0، 0.5، 1 و 1.5 درصد در نسبت نور خورشید به عنوان فاکتورهای اصلی و زمان‌های مختلف برداشت به عنوان فاکتور فرعی مورد بررسی قرار گرفت. نتایج نشان داد که بیشترین مقادیر وزن تبلک در سطح نور بین 0 و آبیاری پس از تخلیه 20 درصد رطوبت صورت قرار گرفتند، بدست آمد. گیاهان رشدی‌های تحت شرایط نور کامل بیشتری تعداد باشند (4/30 و 27/35 درصد از حضور پس از بیشتری و با گره‌ای از 4/15 و 18/25 و 30/27) عدد در هر بیشتری به ترتیب 400 و 200 و 70 روز یک سطح از اعمال تیمارها و همچنین بیشترین وزن تریش از 4/75 در بیشتری و وزن خشک ریشه (4/37 در بیشتری) را اتخاذ نمودند که این صفات از افزایش شد نش کم آبی در همه مراحل رشد کاهش پیدا نمود. میزان گلوکر (79/30، 76/30، 77/30، 75/30) در بیشتری، وزن خشک (323/50، 69/30، 70/30، 72/30) در بیشتری، آب‌بر (786/70، 90/70) در بیشتری و برخی از پرورش‌های گرم پروتونیک نور (786/70، 90/70) در بیشتری، افزایش شدند. نتایج نشان داد که گیاهان رشدی‌های تحت شرایط مختلف تنش ناشی از تغییرات در سطح متابولیتهای اولیه و ثانویه افزایش داده شدند. این می‌تواند به عنوان یک مکانیسم سازگاری تحت شرایط کم آبی به منظور بالا برد در کاهش عملکرد در گیاه صبرد در نظر گرفته شود.