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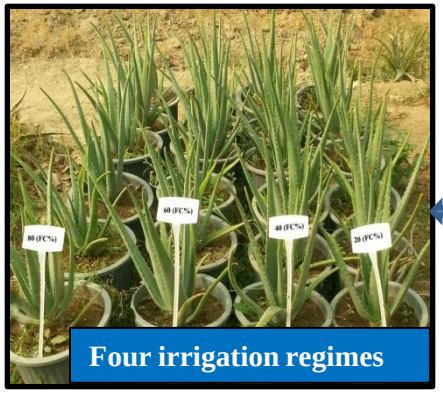

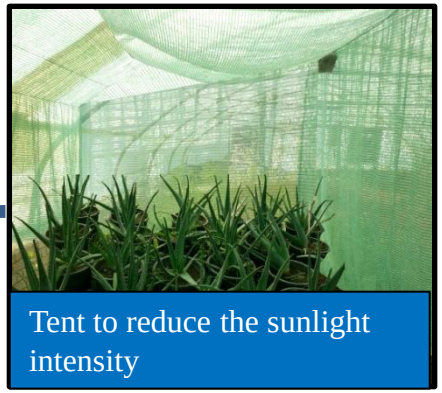

Chlorophyll fluorescence parameter measurements

Up

Decreased F_m, F_v/F_m, qP, Φ_{PSII}
Increased NPQ and F_0

Light intensity and water stress

Down

Increased F_m, F_v/F_m, Φ_{PSII} and qP
Decreased NPQ and F_0

Effects of Water Stress and Light Intensity on Chlorophyll Fluorescence Parameters and Pigments of *Aloe vera* L.

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Abstract

Aloe vera L. is one of the most important medicinal plants in the world. In order to determine the effects of light intensity and water deficit stress on chlorophyll (Chl) fluorescence and pigments of *A. vera*, a split-plot in time experiment was laid out in a randomized complete block design with four replications in a research greenhouse. The factorial combination of three light intensities (50, 75 and 100% of sunlight) and four irrigation regimes (irrigation after depleting 20, 40, 60 and 80% of soil water content) were considered as main factors. Sampling time was considered as sub factor. The first, second and third samplings were performed 90, 180 and 270 days after imposing the treatments, respectively. The results demonstrated that the highest light intensity and the severe water stress decreased maximum fluorescence (F_m), variable fluorescence (F_v)/F_m, quantum yield of PSII photochemistry (Φ_{PSII}), Chl and photochemical quenching (qP) but increased non-photochemical quenching (NPQ), minimum fluorescence (F_0) and Anthocyanin (Anth). Additionally, the highest F_m, F_v/F_m, Φ_{PSII} and qP and the lowest NPQ and F_0 were observed when 50% of sunlight was blocked and irrigation was done after 40% soil water depletion. Irradiance of full sunlight and water deficit stress let to the photoinhibition of photosynthesis, as indicated by a reduced quantum yield of PSII, Φ_{PSII}, and qP, as well as higher NPQ. Thus, chlorophyll florescence measurements provide valuable physiological data. Close to half of total solar radiation and irrigation after depleting 40% of soil water content were selected as the most efficient treatments.

Keywords: *A. vera*, Anthocyanin, Chl fluorescence, Light intensity, Water stress

Abbreviations: F_m, maximal fluorescence level from dark adapted leaves; F_m', maximal fluorescence level from leaves in light; F_0, minimal fluorescence level from dark-adapted leaves; F_v/F_m, maximal photochemical efficiency of the active center of PSII in the dark; F_0', minimal fluorescence level of leaves in light; F_s', Chl fluorescence yield during Actinic illumination irradiation; Chl, chlorophyll; Anth, anthocyanin; NPQ, non-photochemical quenching; qP, photochemical quenching; $\Phi_{PSII,}$ quantum yield of PSII photochemistry.

1. Introduction

Plants are exposed to different a biotic stresses such as high and low temperatures, water deficit, high light intensity, salinity, heavy metals and mechanical wounding under field conditions. In most cases, plants face several stresses at the same time and so they have developed sophisticated defense mechanisms to recognize and respond to a wide range of stresses (Mittler, 2006; Ibáñez et al., 2010). Thus, understanding their physiological processes and defense mechanisms of important for plant science research (Ibáñez et al., 2010; Wyka et al., 2012). Among environmental factors, light and moisture play major roles on plant growth and development (Jagtap et al.,1998). Light is known as the second most ecological factor affecting plant growth, production and survival and it is a major factor determining photosynthetic efficiency in plants, especially in the Crassulaceae family (Lüttge, 2004). In addition, water is another most important growth limiting factors in crop production and at the same time it is the most vital factors in physiological reactions.

Plants continuously adjust their growth and development to optimize photosyntheticactivity under fluctuating conditions. This developmental plasticity is achieved through the perception, transduction and integration of multiple environmental signals. For instance, energy lost in high light intensity is considerably more than in low light conditions (Valladares and Pearcy, 2002). It

has been reported that high light intensities cause irreparable photoinhibitory damages to plant, particularly when water deficit stress occurs at the same time (Hoch et al., 2001; Borkowska, 2002). High light intensities and water deficit stress negatively affect physiological processes (Thomas and Turner, 2001; Aranda et al., 2005; Zivcak et al., 2014). These factors affect photosynthesis and Chl fluorescence parameters directly or indirectly (Maxwell and Johnson, 2000). Although morphological and physiological responses to environmental stresses occur simultaneously, early detection of environmental stresses through physiological processes is possible (Naumann et al., 2007). In order to understand the physiological status of a certain plant and determine photosynthetic damage as affected by environmental stresses, Chl fluorescence assay is a rapid and, sensitive measure of photosynthetic competence in higher plants that can be used to detect the impact of such stresses on them (Baker and Rosenqvist, 2004; Calatayud et al., 2006). Under different environmental conditions, there are vast changes in Chl fluorescence. The light energy absorbed by Chl molecules can be directed in three ways: energizing photosynthesis, dissipation as heat or remission as fluorescence (Müller et al., 2001). It has been reported that, high light intensity causes a significant reduction in maximum fluorescence (F_m), variable fluorescence(F_v) and photochemical efficiency of photosystem II (Φ_{PSII}) (Figueroa et al., 2003). Photosynthetic efficiency of photosystem II, both in the light ($\Delta F/ F_m'$) and in a dark-adapted state (F_v/F_m) are the most widely used Chl fluorescence measuring parameter in plant research (Baker and Rosenqvist, 2004; Broetto et al., 2007). Under stressful conditions, there are several mechanisms to dissipate extra energy as heat or fluorescence (Naumann et al., 2007). Non-photochemical quenching (NPQ) is a protective mechanism that plants employ to dissipate excess light energy. Plants often absorb more light energy than they can process in photosynthesis. In this regard, it has been reported that high light intensity and water deficit

stress increase NPQ while reduce Φ_{PSII} and qP (Miyake et al., 2005; Naumann et al., 2007; Ashraf and Harris, 2013). Similar results have been found by Herrera (2000) and Adams et al. (1987) who studied the effect of water deficit stress and high light intensity on mentioned parameters in crassulaceae plants. On the other hand, environmental stresses including high light intensity affect photosynthetic pigments and can inhibit photosynthesis (Ashraf and Harris, 2013). Light absorption is the first stage in photosynthesis, which is carried out by light absorbing pigments such as Chl and Anths (Liu et al., 2004). Light absorption efficiency depends on pigments concentration and its structure (Horton and Ruban, 2005; Porcar-Castell et al., 2014). Chl concentration varies according to environmental conditions. Some pigments such as Anths and carotenoids have been shown to act as a "sunscreen", protecting plant cells from high light damage by absorbing blue-green and ultraviolet light, thereby protecting the tissues from photo-inhibition, or high-light stress (Steyn et al., 2002; Hormaetxe et al., 2005). Anths and carotenoids concentration would increase to protect the chloroplasts under water deficit and high light intensity conditions (Gould et al., 2000; Horton and Ruban, 2005; Hatier and Gould, 2008). Increase in Anth concentration under unfavorable environmental conditions has been reported in other studies (Chalker-Scott, 1999; Hughes et al., 2005; Albert et al., 2009). Increase in Anth and rhodoxanthin concentration due to high light intensity has been previously reported by Lüttge (2000) in *A. vera*. In this plant, leaves turn red or brown under environmental stress conditions (Cousins and Witkowski, 2012). Chl fluorescence responses to environmental stresses faster than Chl content, therefore study on fluorescence variations would help us to understand physiological status of the plants. There are fewer information on Chl fluorescence and pigments changes as affected by environmental stress in *A. vera*. Since this plant is a succulent species with crassulacean acid metabolism (CAM) photosynthetic CO_2 fixation pathway, it usually

grows in warm and dry regions where light intensity is extremely high. The plant at maturity stages requires more light than early stages of growth. Considering the photosynthetic CO_2 fixation pathway in *A. vera*, study on the effect of light intensities and water deficit stress would help us to understand physiological changes in this plant. While there are several studies focusing on light intensity or water deficit stress as separate factors (Paez et al., 2000; Rodríguez-García et al., 2007; Lucini et al., 2013); a comprehensive study to determine the outcomes and impacts of this two factor interaction has not been carried out so far. Hence, the current study was aimed to evaluate the effects of different light intensities and water deficit stress levels on Chl fluorescence parameters and pigments to find out their relationships during plant growth stages.

2. Material and methods

2.1. Experimental design, treatments and growth conditions

A split-plot in time experiment was laid out in a randomized complete block design with four replications in a research greenhouse situated in Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran during 2013 and 2014 growing seasons. The factorial combination of three light intensities (50, 75 and 100% of sunlight) and four irrigation regimes (irrigation after depleting 20, 40, 60 and 80% of soil water content) were considered as main factors. Sampling time was considered as sub factor. The first, second and third samplings were performed on 22nd September 2013, 20th January and 21st March 2014, respectively, at 90, 180 and 270 days after imposing the treatments. The 18-20 cm pups (small plants growing from the sides of the mother plant) were planted in plastic pots and placed in greenhouse for two months, irrigated equally. Thereafter, plants were transplanted into new pots filled with 18 kg homogeneous soil and then

irrigation regimes were imposed for the next 9 months. At the same time, plants were subjected to different light intensities by placing them under a nylon mesh tent to reduce the sunlight intensity by 50 and 75%. The light intensity under the tents was measured using portable solarimeter (118 HAENNI) at noon (Fig. 1). The greenhouse temperature was adjusted on 28 and 22 °C in days and nights, respectively.

2.2. Soil moisture content

Soil moisture content was monitored daily using a time domain reflectrometry (TDR) device (TRIM-FM 10776, Germany). A 20 cm three pointed rods probe was used to volumetrically measure the soil moisturecontent. The data were confirmed using gravimetric moisture. The following equation was used for this purpose.

Θ_v % $=\Theta_G \times P_S/P_W$

Where Θ_G is the gravimetric water content; Θ_V is the volumetric soil moisture; Pw is the water density; Ps is the density of soil.

Required water was supplied based on available water. In order to reduce evaporation, soil surface was covered by aluminum foil and drained water was collected and measured. Soil moisture at field capacity and wilting point were determined as 20.87 and 7.61%, respectively. Furthermore, pressure plate apparatus was used to determine soil pF and then soil moisture curve was plotted (Table 1).

2.3. Chlorophyll fluorescence parameters

Chl fluorescence parameters were assessed using a portable photosynthesis meter (Walz GmbH Eichenring, 691090 Effeltrich, Germany) at the end of summer, autumn and winter.

Minimal fluorescence, F_0, was measured in 30 min dark-adapted leaves and maximal fluorescence, F_m, in the same leaves in full light-adapted conditions. Maximal variable fluorescence ($F_v=F_m–F_0$) and the photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves were also calculated from the measured parameters (Maxwell and Johnson, 2000). In light adapted leaves (for 15 min) steady state fluorescence yield (F_s'), maximal fluorescence (F_m') after 0.8 s saturating white light pulse and minimal fluorescence (F_0')were measured when actinic light was turned off and further calculation was made by using the equation $F_0' = F_0/ (F_v/F_m+ F_0/ F_m')$ (Oxborough and Baker, 1997). Quenching value due to non-photochemical dissipation of absorbed light energy (NPQ) was determined at each saturating pulse in accordance with the equation $NPQ= (F_m-F_m')/ F_m'$. The coefficient for photochemical quenching, qP, which represents the fraction of open PSII reaction center, was calculated as $qP= (F_m' – F_s') / (F_m' – F_0')$ (Maxwell and Johnson, 2000), Furthermore, F_v' /F_m', F_q' and F_q' /F_m' values were also determined. Photochemical efficiency of photosystem II (Φ_{PSII}) was calculated as follow (Genty et al., 1989).

$\Phi_{PSII}= (F_m'– F_s')/ F_m'$

2.4. Pigments assay

Chl from the leaf samples was extracted in 80% acetone solution, following the method of Arnon (1949). Extracts were filtrated and total Chl content was measured spectrophotometryically at 645 and 663 nm, respectively. The Chl content was expressed as mg g^{-1} fresh weight.

For the determination of Anth concentration, 0.2 g fresh leaves were taken and extracted in 15 ml glass centrifuge tubes containing 10 ml of acidified methanol (methanol: HCl, 99: 1, v: v) and

kept overnight in the dark. The samples were brought up to volume, and the absorbance value at 550 nm was determined spectrophotometrically. Anth concentration was calculated using an extinction coefficient of 33000 mol^{-1} cm^{-1} (Krizek et al., 1993).

2.5. Statistical analysis of data

Main and interaction effects of experimental factors 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 0.05 probability level was used to check significant differences between means. When an *F*-test indicated statistical significance at $P < 0.01$ or $P < 0.05$, the protected least significant difference (protected LSD) was used to separate the means of main effect and the significant interactions were separated by slicing method.

3. Results

3.1. Analysis of variance

Analysis of variance showed that irrigation regime, light intensity and sampling time significantly affected all measured traits. The two-way interaction between sampling time and irrigation regime was significant for Φ_{PSII} and qP. The sampling time × light intensity interaction was significant for F_v, Φ_{PSII}, qP, NPQ and Chl (Table 2). Furthermore, there was a significant three-way interaction between irrigation regime, light intensity and sampling time on F_v/F_m, F_0 and Anth (Table 2). Interaction and main effects are discussed below in the order of their statistical significance, which ranges from the highest-level interactions to the main effects of

treatments. When two- or three-way interactions are present for each trait measured, it means that interpretation of the main effects is incomplete or avoiding.

3.2. Chlorophyll fluorescence parameter measurements

3.2.1. F_0, F_m, F_v/F_m and F_v parameters

Chl fluorescence was affected by light intensity and water deficit stress in different stages of growth. The highest and lowest values for F_m, F_0 and F_v were obtained in the last of summer and winter, respectively (Table 4). According to the results, the highest F_0 was observed when plants were subjected to full sunlight and irrigated after depleting 80% of soil water content. By contrast, the lowest value was related to plants treated by reduced light intensity (50% full sunlight) and irrigated after depleting 20% of soil water content. Full sunlight and severe water deficit stress (irrigation after depleting 80% of soil water content) increased F_0 by 23, 23 and 55% in summer, autumn and winter, respectively (Table 7). F_m value of *A. vera* decreased with increasing light intensity, whereas the highest F_m value was related to plants subjected to 50% of full sunlight treatment at all three sampling times. There was no significant difference in F_m value measured between 50 and 75% light intensity (Fig. 2). As shown in Table 3, the irrigated after depleting 40% of soil water content had the highest F_m.

The highest F_v was related to plants subjected to reduced sunlight, and irrigation supplied after depleting 40% of soil water content. By contrast, the lowest value was corresponded to the full sunlight intensity and irrigation after depleting 80% of soil water content (Tables 3 and 6). There was no significant difference in F_v values between 50 and 75% light intensities as well as between 20, 40 and 60% irrigation regimes in all the sampling times (Tables 3 and 6). F_v/F_m ratio was significantly affected by three-way interaction of irrigation regime × light intensity ×

sampling time (Table 2). The F_v/F_m ratio significantly decreased with increasing light intensity and water deficit stress severity during all the sampling times (Table 7). The highest and lowest ratios were recorded in autumn and winter, respectively (Table 4). In addition, the lowest F_v/F_m ratio was related to combination of full sunlight and severe water deficit stress (Table 7).

3.3. Φ_{PSII}, *qP and NPQ* parameters

The results demonstrated that Φ_{PSII} and qP values decreased with increasing light intensity and water deficit stress severity during all the sampling times (Tables 5 and 6). The highest values for Φ_{PSII} and qP were observed in summer when plants were subjected to 50% of sunlight and irrigated after depleting 20% of soil water content (Tables 5 and 6). There was no significant difference in both Φ_{PSII} and qP values between 20 and 40% irrigation regimes during all the sampling times (Table 5). Interestingly, reduction in Φ_{PSII} and qP values were more pronounced when high light intensity was combined with severe water deficit stress compared with high light intensity only (data not shown). The NPQ value considerably increased with increasing light intensity during all the sampling times. According to the results, the highest NPQ value was observed when plants were subjected to full sunlight in summer, whereas the lowest value with the reduced light intensity (50% full sunlight) was obtained in winter (Table 6). Also NPQ value increased with increasing water deficit stress. The highest and lowest NPQ value was observed in plots irrigated after depleting 80 and 40% of soil water content, respectively (Table 3). Furthermore, no significant difference in NPQ values between light intensity of 50 and 75%, also between 20 and 40% irrigation regimes were observed (Tables 3 and 6).

3.4. Chlorophyll content

The highest chlorophyll content was observed when plants were subjected to 50% of full sunlight during all the sampling times. By contrast, the lowest value related to full sunlight (Table 6). Chl content was higher in irrigated after depleting 60% of soil water content than in other irrigation regimes (Table 3). Irrigated after depleting 80% of soil water content significantly decreased Chl content. There was no significant difference in Chl content of plants grown between 20 and 40% irrigation regimes (Table 3). Furthermore, the highest (0.666 mg g^{-1}) and lowest (0.399 mg g^{-1}) Chl content was observed in winter and summer, respectively (Table 4). Increase in light intensity and water deficit stress severity significantly decreased Chl content in all the sampling times (Tables 3 and 6).

3.5. Anthocyanin

The results showed that Anth accumulation considerably varied with light intensity and water deficit stress severity (Table 7). This content increased due to high light intensities and water deficit stress. The highest Anth accumulation (0.529 mg g^{-1}) was observed when plants were subjected to full sunlight and irrigated after depleting 80% of soil water content in summer, whereas that of lowest value (0.199 mg g^{-1}) corresponded to the reduced light intensity (50% full sunlight) and irrigated after depleting 20% of soil water content in winter (Table 7). As regards sampling time, the highest (0.390 mg g^{-1}) and lowest (0.339 mg g^{-1}) Anth accumulation was related to summer and winter, respectively. No significant difference in Anth content between autumn and winter sampling times was observed (Table 4). In case of irrigation regimes, no significant difference was detected between 20 and 40% as well as between 60 and 80% soil water depletion. Moreover, there was no significant difference between 50 and 75% of sunlight treatments during all the sampling times (Table 7). Application of high light intensity combined

with water deficit stress caused more Anth accumulation compared with their individual effect. Generally, high light intensity and water deficit stress severity increased Anth synthesis during plant growth period. Conversely Anth content decreased on account of lower light intensities or mild water deficit stress.

4. Discussion

The F_0, F_m, F_v, F_v/F_m, NPQ, qP and Φ_{PSII} values are the most important Chl fluorescence parameters which are broadly used in plant stress physiology studies (Thomas and Turner, 2001; Baker and Rosenqvist, 2004; Fu et al., 2012; Murchie and Lawson, 2013). F_0 is minimal fluorescence levels when all antenna pigment complexes associated with the photosystem are assumed to be open (dark adapted) (Gorbe and Calatayud, 2012). Increase in F_0 represents any difficulty and degradation in photosystem II (D1 protein and other part of PS) or any disruption in energy transfer into the reaction center (Calatayud et al., 2006). It has been reported that F_0 would increase under full stress conditions, but the F_v/F_m ratio would be reduced (Maxwell and Johnson, 2000). In the current study, F_0 significantly increased due to high light intensity and severe water deficit stress. Similar results have been found by other researchers (Baker and Rosenqvist, 2004; Calatayud et al., 2006; Fu et al., 2012). Increase in F_0 is mainly associated with two factors: 1: reduction in plastoquinone electron receptors and incomplete oxidation due to retardation, which results into electron transfer chain delay in PSII and 2: light- harvesting Chl a/b protein complexes separation in PSII (Baker, 2008; Ashraf and Harris, 2013). Totally, increase in F_0 lead to reduced photochemical capacity of PSII (Calatayud et al., 2006). It has been stated that there is an association between F_0 value and Chl content in plants (Fu et al., 2012). In the current study, F_0 decreased with increasing Chl content in the leaves.

The F_m is maximal fluorescence level when a high intensity flash has been applied. All antenna sites are assumed to be closed, reflecting a state of electrical transfer when passed PSII (Baker and Rosenqvist, 2004). In this study, F_m decreased in response to high light intensities and water deficit stress, which is due to deactivation of proteins in Chl structure.

The variable fluorescence, F_v, calculated as F_m-F_0, represents the maximum quantum yield of Chl fluorescence (Baker and Rosenqvist, 2004). The F_v/F_m value is the ratio of variable fluorescence to maximal fluorescence and calculated as F_m-F_0/F_m. It measures the maximum efficiency of PSII (the efficiency if all PSII centers were open) (Murchie and Lawson, 2013). This value can be used to estimate the potential efficiency of PSII by taking dark-adapted measurements (Hura et al., 2007; Gorbe and Calatayud, 2012). Increase in F_v/F_m reflects more light use efficiency in plants (Baker, 2008; Li et al., 2015). In addition, increase in F_v/F_m is also correlated with reduced energy loss as heat(Jagtap et al., 1998; Jeon et al., 2006; Broetto et al., 2007). Our results are in agreement with Figueroa et al. (2003) who reported that increase in light intensity caused reduced F_v/F_m, indicating more energy dissipated as heat. Similar results have been found in other plants in crassulaceae family by other authors (Foyer et al., 1994; Hurst et al., 2004; Broetto et al., 2007). Reduction in F_v/F_m is due to reduced photo-damaged in PSII which lead to reduce PSII maximum efficiency (Porcar-Castell et al., 2014).

The qP value approximates the proportion of PSII reaction centers that are open. In other words, qP represents the energy consumed in photosynthesis. On the other hand NPQ is the amount of dissipated excessive irradiation into heat. NPQ represents effective way how photosynthetic organisms can dissipate excessive irradiation into heat (Pinnola et al., 2013). Study on NPQ can help to understand xanthophyll cycle activity (Ralph and Gademann, 2005).

The Φ_{PSII} value gives an estimation of the efficiency (Baker and Rosenqvist, 2004); it represents the photochemistry at different photon flux density (Maxwell and Johnson, 2000). There is an inverse relationship between qP and NPQ as well as Φ_{PSII} and NPQ (Maxwell and Johnson, 2000; Fu et al., 2012; Massacci et al., 2008). In our study, the Φ_{PSII} and qP decreased with increasing value of NPQ and were in line with the results reported by Fu et al. (2012). Under favorable conditions, almost all the absorbed light is consumed in photochemical reactions (Porcar-Castell et al., 2014). There is a negative correlation between Φ_{PSII} and NPQ (Fu et al., 2012). In addition, it has been reported that there is a linear relationship between Φ_{PSII} and CO_2 absorption (Baker, 2008; Martins et al., 2013). In a study on *Peperomia carnevalii*, water deficit stress decreased Φ_{PSII} and qP but increased NPQ (Herrera, 2000). In this study, the NPQ value increased with increasing light intensity and water deficit stress severity. Environmental stresses, especially high light intensities make the electron transfer chain saturated and increase proton accumulation, therefore NPQ would increase (Müller et al., 2001; Lambrev et al., 2012; Porcar-Castell et al., 2014). The higher value of NPQ indicates the ability to mitigate the negative effects of environmental stress at the chloroplast level, as these organelles have the ability to dissipate the excess excitation energy (Kościelniak et al., 2006; Li et al., 2014; Ismail et al., 2014). According to the previous studies, it is said that combining two ecological factors would result into a negative response by the plant that eventually leads to increased NPQ (Miyake et al., 2005; Fu et al., 2012; Costa et al., 2015). This finding was confirmed by the reductions in F_v/F_m ratios found in these plants. High light intensity decreased Chl content in *A. vera* plants. Moreover, combination of high light intensity and water deficit stress increased Chl degradation during different growth stages. The Chl degradation has negative effect on photosynthesis efficiency (Sakuraba et al., 2014). One of the possible reasons for this reduction

under high light intensities is prohibited chloroplast formation. Under low light intensities chloroplasts get larger with high concentration of Chl (Fu et al., 2012). Our results indicated that F_0 would decrease with increasing Chl content. In fact, increase in Chl content, improves PSII efficiency (Abadía et al., 1999). Increase in F_0 is also known as a mechanism to diminish reactive oxygen species activity (Fu et al., 2012). An increase in Chl content was observed in Doritaenopsis when plants were subjected to low light intensities (Jeon et al., 2006). It is also true that plants grown in shade produce more Chl to absorb more light (Li et al., 2014).

Anths are water-soluble vacuolar pigments that may appear red, purple, or blue in color depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid pathway (Hatier and Gould, 2008); and they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation. In photosynthetic tissues, Anths have been shown to act as a "sunscreen", protecting cells from high-light damage by absorbing ultraviolet light, thereby protecting the tissues from photoinhibition, or high-light stress (Gould et al., 2002; Steyn et al., 2002; Albert et al., 2009; Zhou and Singh, 2004; Hoch et al., 2003). The light is one of the most important factors in Anth biosynthesis (Zhou and Singh, 2004). It has been reported that there exists a positive correlation between light intensity and Anth biosynthesis (Ramakrishna and Ravishankar, 2011). In our study, high light intensity and water deficit stress increased Anth biosynthesis. The highest Anth content was observed in summer one of the reasons for this could be the higher radiation during summer compared with other seasons. Similar results have been found by other researchers (Hughes et al., 2005; Ramakrishna and Ravishankar, 2011). It has been hypnotized that increase in Anth biosynthesis is parallel with Chl reduction as Anths absorb blue and red light (Gould et al., 2000). In addition, it has been proven that Anths protect chloroplast against high light intensities (Hughes et al.,

2005; Gould et al., 2000; Merzlyak et al., 2008; Gould et al., 2010). Anths prevent extra electron transfer and energy loss (Demmig-adams and Adams, 1992).

5. Conclusions

A. vera is an important industrial-medicinal plant. Light and water are two of the most important factors needed for plant growth and development. In the present study, the effect of light and water was determined on Chl fluorescence parameters, Chl and Anth contents of *A. vera* in different times. Results showed that the use of the physiological information from Chl fluorescence can give further insights for understanding the responses of plants to environmental stresses and enable a selection of favorable conditions for at least partial removal of photoinhibitory damage. F_0 and NPQ values increased with increasing light intensity and water stress severity. By contrast, F_m, F_v. F_v/F_m, Φ_{PSII} and qP decreased. Anth and Chl content increased and decreased, respectively, on account of high light intensities and water deficit stress. We noted that photosynthetic efficacy of *A. vera* would be decreased due to high light intensities and water deficit stress. When high light intensities and water deficit stress were applied simultaneously, the adverse effects of them were even more pronounced. The high light intensity and water deficit stress has inhibitory effect on physiological and biochemical parameters, such as $F_{0,}$ F_v/F_m and Anth. Therefore, it can be concluded that *A. vera* resistance to water deficit stress is more than to high light intensities. In addition, the reduction in light intensity could increase photosynthetic efficiency. *A. vera* plants showed increased ability for photosynthetic acclimation under intermediate light intensities as observed through fluorescence parameters. Plants grown under decreasing light intensities and without water stress showed higher F_v/F_m, Φ_{PSII} , qP and Chl, whereas that plants exposed to high irradiance and water stress

exhibited low values of F_v/F_m and high values of NPQ, which indicated the occurrence of photoinhibition under these conditions. Finally, 50% of sunlight intensity and irrigation after depleting 40% of soil water content was selected as the best treatments during all growth periods.

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Table1

Physical and chemical properties of soil used in pot experiments.

Texture	Field capacity	Wilting point	pH	EC	O.C[1]	T.N[2]	P	M.	K	Sulfur
	% by volume			(mmhos/cm)	(%)				(mg/kg)	
Sandy loam	20.87	7.61	7.6	1.76	1.03	0.1	20	20	422	49

1, 2 and 3 denotes the organic matter, total nitrogen and mineral nitrogen, respectively.

Table 2

Analysis of variance (mean squares) for the effects of light intensity and irrigation regime on Chl fluorescence and pigments in *A. vera.*

Source of variation	df	F_m	F_v	F_v/F_m	F_0	Φ_{PSII}	NPQ	qP	Chl	Anth
Irrigation regime (I)	3	0.036**	0.067**	0.0381**	0.0094**	0.0179**	0.0211**	0.0255**	0.059**	0.072**
Light intensity (L)	2	0.083**	0.225**	0.1707**	0.0364**	0.0182**	0.0368**	0.0114**	0.464**	0.229**
L*I	6	0.005 ^{NS}	0.005 ^{NS}	0.0031**	0.0004**	0.0005 ^{NS}	0.0018 ^{NS}	0.0013*	0.024**	0.007**
Main error	33	0.004	0.004	0.0007	0.0002	0.0003	0.0009	0.0006	0.005	0.002
Time	2	1.231**	0.919**	0.0995**	0.0403**	0.0116**	0.1322**	0.0311**	1.018**	0.037**
Time*I	6	0.003 ^{NS}	0.003 ^{NS}	0.0042**	0.0006**	0.0006*	0.0011 ^{NS}	0.0011*	0.007 ^{NS}	0.004**
Time*L	4	0.004 ^{NS}	0.018**	0.0411**	0.0058**	0.0023**	0.0046**	0.0017*	0.112**	0.007**
Time*L*I	12	0.002 ^{NS}	0.003 ^{NS}	0.0024**	0.0005**	0.0001 ^{NS}	0.0009 ^{NS}	0.0003 ^{NS}	0.003 ^{NS}	0.002**
Sub error	72	0.003	0.004	0.0006	0.0001	0.0002	0.0009	0.0005	0.005	0.001
C.V. (%)		3.49	7.22	3.48	4.32	13.62	15.27	13.58	14.16	8.84

F_0, minimum Chl fluorescence yield obtained with dark-adapted leaf; F_m, maximum Chl fluorescence yield obtained with dark-adapted leaf; F_v, variable fluorescence of the dark-adapted samples; F_v/F_m , maximal photochemical efficiency; NPQ, non-photochemical quenching; qP , photochemical quenching; Φ_{PSII}, yield of PSII photochemistry; Chl, chlorophyll; Anth, anthocyanin.

* Significant at the 0.05 probability levels.

** Significant at the 0.01 probability levels .

NS, not significant .

Table 3

Main effects of irrigation regime on F_m, F_v, NPQ and Chl in *A.vera.*

Irrigation regime (after depleting %FC)	F_m	F_v	NPQ	Chl (mg g^{-1})
20	0.822a	0.595a	0.182c	0.491b
40	0.850a	0.607a	0.186c	0.518ab
60	0.830a	0.576a	0.217b	0.539a
80	0.775b	0.510b	0.232a	0.445c

F_m, maximum Chl fluorescence yield obtained with dark-adapted leaf; F_v, variable fluorescence of the dark-adapted samples; NPQ, non-photochemical quenching; Chl, chlorophyll. Means within a column followed by the same letter are not significantly different at the level of 5%.

Table 4

Main effects of sampling times on Chl fluorescence and pigments in *A. vera*.

Sampling time	F_m	F_v	F_0	F_v/F_m	Φ_{PSII}	NPQ	qP	Chl (mg g^{-1})	Anth (mg g^{-1})
90 (22 September) or summer	0.938a	0.658a	0.280a	0.700b	0.127a	0.265a	0.194a	0.430b	0.390a
180 (21 December) or autumn	0.883b	0.646a	0.237b	0.726a	0.100b	0.178b	0.144c	0.399c	0.346b
270 (21 March) or winter	0.637c	0.413b	0.225c	0.638c	0.099b	0.170b	0.159b	0.666a	0.339b

F_m, maximum Chl fluorescence yield obtained with dark-adapted leaf; F_v, variable fluorescence of the dark-adapted samples; F_0, minimum Chl fluorescence yield obtained with dark-adapted leaf; F_v/F_m , maximal photochemical efficiency. NPQ, non-photochemical quenching; qP , photochemical quenching; Φ_{PSII}, yield of PSII photochemistry; Chl, chlorophyll; Anth, anthocyanin. Means within each column followed by the same letter are not significantly different at the level of 5%.

Table 5

Irrigation regime × sampling time interaction on Φ_{PSII} and qP in *A. vera* (sliced by time sampling).

Irrigation regime (after depleting %FC)	Days after treatment (date)					
	90 (22 September) or summer		180 (21 December)or autumn		270 (21 March) or winter	
	Φ_{PSII}	qP	Φ_{PSII}	qP	Φ_{PSII}	qP
20	0.153a	0.224a	0.109a	0.153ab	0.126a	0.190a
40	0.144a	0.217a	0.117a	0.162a	0.112a	0.179a
60	0.113b	0.176b	0.096b	0.140b	0.087b	0.141b
80	0.096c	0.157c	0.079c	0.120c	0.070c	0.126b

qP, photochemical quenching; Φ_{PSII}, yield of PSII photochemistry. Means within a column followedby the same letter are not significantly different at the level of 5%.

Table 6

Light intensity× sampling time interaction on F_v, $\Phi_{PSII,}$ NPQ , qP and Chl in *A.vera* (sliced by sampling time).

Light intensity (%)	Days after treatment (date)														
	90 (22 September) or summer					180 (21 December)or autumn					270 (21 March) or winter				
	F_v	Φ_{PSII}	NPQ	qP	Chl (mg g^{-1})	F_v	Φ_{PSII}	NPQ	qP	Chl (mg g^{-1})	F_v	Φ_{PSII}	NPQ	qP	Chl (mg g^{-1})
100	0.61b	0.11c	0.31a	0.18b	0.36b	0.58b	0.09b	0.20a	0.14b	0.36b	0.29c	0.06c	0.19a	0.13c	0.46c
75	0.67a	0.13b	0.27b	0.19b	0.46a	0.66a	0.10a	0.17b	0.15a	0.39b	0.46b	0.10b	0.16b	0.16b	0.70b
50	0.69a	0.14a	0.22c	0.21a	0.47a	0.69a	0.11a	0.16b	0.15ab	0.45a	0.49a	0.13a	0.16b	0.18a	0.84a

F_v, variable fluorescence of the dark-adapted samples; Φ_{PSII}, yield of PSII photochemistry; NPQ, non-photochemical quenching; qP, photochemical quenching; Chl, chlorophyll. Means within a column followed by the same letter are not significantly different at the level of 5%.

Table 7

Interaction effect of light intensity × irrigation regime × sampling time on F_0, F_v/F_m and Anth in *A. vera* (sliced by time sampling).

Treatments		Days after treatment (date)								
Light intensity (%)	Irrigation regime (after depleting %FC)	90 (22 September) or summer			180 (21 December) or autumn			270 (21 March) or winter		
		F_0	F_v/F_m	Anth (mg g^{-1})	F_0	F_v/F_m	Anth (mg g^{-1})	F_0	F_v/F_m	Anth (mg g^{-1})
100	20	0.290abcd	0.690d	0.449b	0.235cd	0.722b	0.369cd	0.248c	0.588f	0.342 bcd
	40	0.295abc	0.680de	0.410bc	0.238cd	0.733abc	0.369cd	0.273b	0.545g	0.370b
	60	0.300a	0.668ef	0.528a	0.265b	0.655d	0.416b	0.283b	0.507h	0.457a
	80	0.308a	0.650f	0.529a	0.283a	0.645d	0.479a	0.313a	0.375i	0.446a
75	20	0.235f	0.737ab	0.317cd	0.225de	0.748abc	0.306ef	0.198fg	0.695cd	0.226f
	40	0.273de	0.718bc	0.360bc	0.248c	0.745abc	0.360de	0.208ef	0.700bcd	0.326bcde
	60	0.290abcd	0.698c	0.359bc	0.248c	0.725abc	0.360de	0.210ef	0.693cd	0.364bc
	80	0.280bcd	0.688d	0.391bc	0.245c	0.705c	0.409bc	0.230d	0.640e	0.454a
50	20	0.240f	0.745a	0.281d	0.213f	0.763ab	0.249g	0.143h	0.783a	0.199f
	40	0.258ef	0.735ab	0.342c	0.213f	0.770a	0.245g	0.190g	0.718bc	0.281e
	60	0.280cd	0.723bc	0.354bc	0.218ed	0.773a	0.319e	0.210g	0.730b	0.314cde
	80	0.298ab	0.675de	0.359bc	0.220de	0.740abc	0.269fg	0.190e	0.680d	0.297d

F_0, minimum Chl fluorescence yield obtained with dark-adapted leaf; F_v/F_m, maximal photochemical efficiency; Anth, Anthocyanin. Means within a column followed by the same letter are not significantly different at the level of 5%.

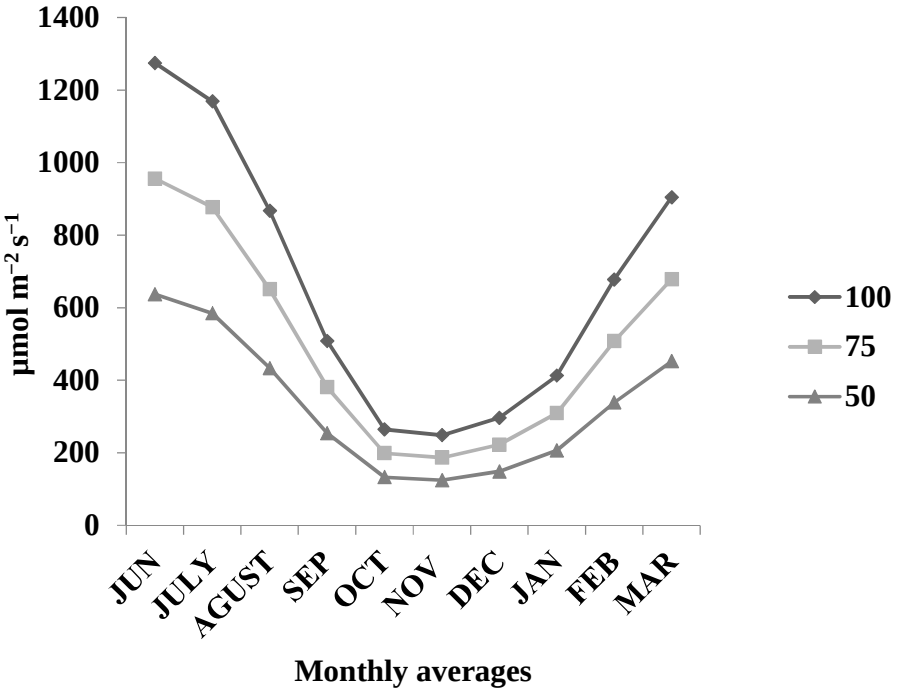

Fig. 1. Monthly averages of light intensity during growth of *A. vera.*

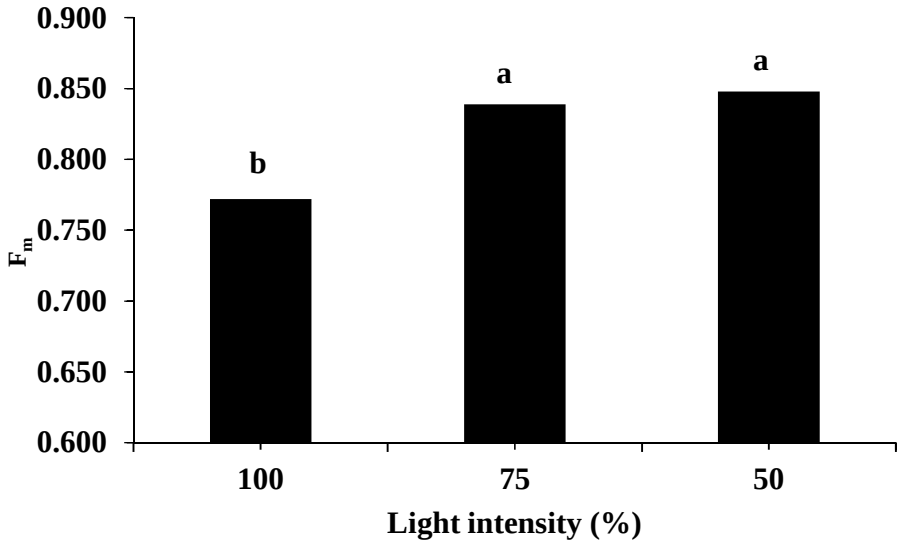

Fig. 2. Effect of light intensity on F_m in *A. vera*.

F_m, maximum Chl fluorescence yield obtained with dark-adapted leaf. Means within a column followed by the same letter are not significantly different at the level of 5%.

Highlights

- The highest light intensity decreased F_m, F_v/F_m, Φ_{PSII} and increased NPQ and F_0.
- Severe water deficit stress reduced F_m, F_v/F_m, Φ_{PSII} and qP increased NPQ and F_0.
- Higher light intensity and water stress decreased Chl and increased Anth content.
- Negative effects of water stress reduced by reducing light intensity.

Author contributions

S.H., Z.T.S., A.M and S.A.M.M.S designed the experiments, cultivated the plants, S.H and A.M performed the fluorescence measurements and analysed the data. S.H., Z.T.S and S.N wrote the paper. All authors read and revised the manuscript, provided helpful discussions and approved its final version.