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The response of *Hordeum spontaneum* desert ecotype to drought and excessive light intensity is characterized by induction of O_2 dependent photochemical activity and anthocyanin accumulation

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ABSTRACT

The goal of the current research was to study the role of anthocyanin accumulation, O_2-related photochemical processes and non-photochemical quenching (NPQ) in the response of desert and Mediterranean plants to drought and excessive light.

Plants of *Hordeum spontaneum* were collected from Mediterranean and desert environments and were subjected to terminal drought for 25 days and then measured for PSII yield at 2 and 21% O_2, NPQ, net carbon assimilation, stomatal conductance, leaf relative water content (LRWC), anthocyanin concentration and leaf absorbance.

Under terminal drought, LRWC, carbon assimilation and stomatal conductance decreased similarly and significantly in both the Mediterranean and the desert ecotypes. Anthocyanin accumulated more in the desert ecotype than in the Mediterranean ecotype. NPQ increased more in the Mediterranean ecotype as compared with the desert ecotype. PSII yield decreased significantly in the Mediterranean ecotype under drought and was much lower than in the desert ecotype under drought. The relatively high PSII yield under drought in the desert ecotype was O_2 dependent.

The response of the *H. spontaneum* ecotype from a desert environment to drought stress was characterized by anthocyanin accumulation and induction of O_2 dependent photochemical activity, while the response of the Mediterranean ecotype was based on a higher induction of NPQ.

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1. Introduction

Since drought has major effects on plant growth and over a third of the earth's land area is under constant threat of drought, understanding the mechanisms of drought tolerance may help in the development and improvement of agricultural crops in water-limited conditions.

A major limiting factor in plant productivity and growth is the rate of photosynthesis, which can be slowed significantly under drought conditions [1]. The inhibition of photosynthesis under drought is caused by stomatal closure and a decrease in mesophyll conductance, which leads to relatively low availability of CO_2 in the chloroplast [2]. Drought can also inhibit cellular processes, such as ATP synthesis [3]. These combined limitations inhibit the assimilation of CO_2 in the Calvin cycle [4]. The combination of strong light and drought imposes additional challenges for plants since the Calvin cycle is inhibited, and an excess of electrons from the light reaction can form reactive oxygen species (ROS) that can inflict damage on the light reaction centers and on multiple cellular components, such as proteins, lipids and DNA [5].

Since solar irradiance is high in deserts, plants in these areas face, among other stresses, a combined stress of excessive light and drought conditions. In the leaves of many desert plants, there are dense and bright leaf trichomes, a high deposition of wax (or salt as in *Atriplex* genus (or simply bright leaves (usually as a result of low chlorophyll content) that enable plants to sustain high light intensities. All of these morphological features result in a higher reflection of incoming irradiance, thus allowing the plants to avoid excess radiation and to minimize water loss [6,7].

In addition to morphological adaptations, plants possess biochemical adaptation mechanisms to deal with excessive light. These adaptations are usually dynamic. Non-photochemical quenching (NPQ) is one of the most studied mechanisms by which plants protect themselves from high light. The NPQ mechanism works by down regulating the photochemical light reaction, dissipating the excess light energy as heat. Therefore, NPQ acts to prevent the creation of excess reductive energy that could be harmful to the plant. The main process that contribute to NPQ are the

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pH-dependent, enzymatic de-epoxidation of xanthophylls and conformational changes in PSII antenna proteins [8,9]. It has been shown in several different studies that under drought conditions, plants increase their level of NPQ [10–12]. In the desert perennial species *Yucca chidigera* and *Yucca brevifolia*, NPQ was found to be high during the summer and the winter months, which are either too hot or too cold to support growth; however, in the spring, NPQ was lower, allowing more light energy to be used in photochemical processes [13].

Other biochemical mechanisms used by plants to confront an excess of light are based on the use of alternative target molecules that receive the excess reductive energy formed. Oxygen serves as one of these molecules, mainly through the process of photorespiration. Photorespiration is a well-known and common process in most plants (C_3 plants); it is considered energetically unfavorable since it competes with carbon assimilation by consuming oxygen at the expense of CO_2 [14].

Photorespiration has been shown to function in photoprotection under excessive light conditions [15,16], to increase under drought [17,18], and to be higher in drought-tolerant transgenic plants [19]; photorespiration has been also suggested to have a role in nitrate assimilation [20]. Another process that targets oxygen for reduction is the water–water cycle (Mehler reaction), in which oxygen is directly reduced by electrons to produce water [21]. The function of the water–water cycle as a large electron sink in higher plants was suggested to be marginal [22,23]. The water–water cycle enables a transiently high electron transport flow, consequently creating a pH gradient across the thylakoid membrane, inducing the photo-protective response of NPQ [24]. It has been shown that oxygen-dependent reactions, such as photorespiration, increase under drought [17,18]; however, it is still unclear to what extent these reactions are used by desert plants in comparison with plants from other environments under drought and excess light.

An important mechanism dealing with excess light in plants involves the accumulation of anthocyanin. Anthocyanins can shield chlorophyll molecules by absorbing light, mainly in the green, but also in the red and blue parts of the spectrum, thus acting as sunscreen by preventing light from reaching the light reaction centers and preventing the formation of ROS [25]. Antocyanin can also serve as an efficient antioxidant and can help in the detoxification of ROS [26–28]. The function of anthocyanin as either an antioxidant or a light attenuator ("sunscreen") was found to be largely determined by the amount of anthocyanin and its spatial distribution within the leaves and cells [29]. Photorespiration and anthocyanin accumulation were both suggested to function in photoprotection; however, the interaction between anthocyanin accumulation and photorespiration has not been studied.

Wild barley (*Hordeum spontaneum* L. Koch) plants can be found in a wide range of environments that differ in their climatic conditions. Therefore, studying such species can make major contributions to the understanding of plant adaption mechanisms to abiotic stresses and to the process of generating stress-tolerant crops [30]. The growth characteristics under optimal conditions of different ecotypes of *H. spontaneum*, originated from different climatic regions, did not correlate with the specific average annual rainfall in the different climatic regions [31]; however, comparative studies of desert and non-desert ecotypes have revealed a number of morphological, phenological and life history differences [32] that were found to be adaptive [33,34]. The xeric ecotype from the Negev Desert in Israel (receiving around 90 mm of annual rainfall) exhibited lower competitive ability [35] but also a lower reduction in yield under low irrigation [36]. In addition, an earlier flowering was observed in the desert ecotype [37] as compared with the mesic ecotype from the Galilee region in Israel (receiving around 500 mm of annual rainfall). The above experiments did not examine ecotype differences in physiological mechanisms, such as photosynthesis, photorespiration, anthocyanin accumulation and NPQ.

Our overall goal was to study the contribution of oxygen-related photochemical processes, NPQ and anthocyanin accumulation, to the response of desert and Mediterranean ecotypes of wild barley plants to their environment of high light and drought. To achieve this goal, we set up experiments in which the above two ecotypes of wild barley were grown under terminal drought and compared to plants grown under well-irrigated conditions.

2. Materials and methods

2.1. Plant materials

Sampling of *H. spontaneum* was done in both Mediterranean and desert climatic zones of Israel (hereafter, mesic and xeric ecotypes), each represented by one population. The mesic ecotype (AM) was collected in the Upper Galilee (elevation 300 m, average annual precipitation around 500 mm), 1 km west of kibbutz Ammiad. The vegetation is Mediterranean grassland on terra-rossa soil. The xeric ecotype (SB) was sampled from a wadi (Arabic for ephemeral river valley) in the Negev Desert, 3 km south-west of kibbutz Sede Boqer (elevation 400 m, average annual precipitation around 90 mm) having sparse desert vegetation on loess soil.

2.2. Plant growth conditions

Seeds were germinated on wetted paper and then transferred to three-liter pots filled with washed and sterilized sand. The pots were in a greenhouse in which the photosynthetic photon flux density (PPFD) was between 600 and 700 (μE) at midday. The temperature was controlled at 25° C during the day and 10° C at night, and the relative humidity was 40–60% in midday. The plants were grown under continuous irrigation at full capacity for 25 days and were fertilized with a half Hoagland nutrient solution twice a week. After 25 days, irrigation was halted for half of the plants from each ecotype (drought treatment), while the other half was irrigated (irrigation treatment) twice a week to full soil capacity. Throughout the experiments, the plants were all at the same ontogenic state of vegetative growth. Experiments were repeated three times.

2.3. Photosynthesis and chlorophyll fluorescence measurements

All measurements were carried out in a darkened room. An IRGA Li-6400TX (Li-Cor Inc., NE, USA) was used to measure photosynthesis and chlorophyll fluorescence as described in the following. Plants were dark adapted for 30 min. After the initial dark period, green leaves were enclosed within the measuring chamber for an additional 5 min in the dark. Then the leaves were subjected to 15 min of light at a constant light intensity of 1500 PPFD. The time period of 15 min was chosen due to the steady state reached after this time period which was similar to after 25 min. [CO_2] was kept at 400 μmol/mol; leaf temperature at 25° C; and relative humidity (RH) between 40 and 55%. [O_2] was either atmospheric, 21%, or low, 2% in nitrogen. The gas exchange parameters measured were net CO_2 assimilation ($\mu mol\, CO_2\, m^{-2}\, s^{-1}$) and stomatal conductance ($mmol\, H_2O\, m^{-2}\, s^{-1}$).

Chlorophyll fluorescence measurements and the assimilation measurements using an IRGA Li-6400TX were carried out simultaneously. The basic fluorescence parameters measured were: F_m – maximum fluorescence in a dark-adapted state, F_0 – minimal fluorescence in a dark-adapted state, F'_m – maximum fluorescence in a light-adapted state, and F_s – fluorescence in a light-adapted state.

The maximum photochemical potential of PSII (F_v/F_m) was calculated as follows: $F_v/F_m = (F_m - F_0)/F_m$. The efficiency of converting

light energy to photochemical processes by PSII, PSII yield, was calculated as PSII yield $= (F'_m - F_s)/F'_m$ [38].

Non-photochemical quenching (NPQ) was calculated as $(F_m - F'_m)/F'_m$ [39]; NPQ was measured simultaneously with the PSII yield measurements. An alternative method to calculate NPQ [40] was also used and presented as the efficiency of regulated thermal dissipation $\Phi NPQ = (F_s/F'_m) - (F_s/F_m)$.

In addition, photosynthesis measurements were also carried out in the greenhouse, at a midday natural light intensity of 700 PPFD; these measurements were carried out 25 days after the beginning of the drought treatment in order to assess the stomatal conductance, net CO_2 assimilation, PSII yield, NPQ and ΦNPQ under natural growth conditions. For these measurements, green leaves were enclosed in the IRGA Li-6400, under a light intensity of 700 PPFD [CO_2] of 400 μmol/mol, leaf temperature at 25 °C, and relative humidity (RH) between 40 and 55%, readings of photosynthetic parameters were taken after stable values were reached.

2.4. Leaf absorbance

Leaf absorbance was measured between the wavelengths of 400–700 nm, using an external integrating sphere (Licor, 1800–12s, USA), connected by optic fiber to an analytical spectral device spectrometer (FieldSpec Pro FR, USA) with a spectral range of 350–2500 nm [40].

2.5. The excitation distribution between photosystems I and II

The excitation distribution between photosystems I and II was measured as follows: leaves from light-exposed plants were taken from the greenhouse and were immediately frozen in liquid nitrogen. The leaves were then ground in an extraction buffer (330 mM Manitol, 30 mM HEPES, 2 mM EDTA, 2 mM $MgCl_2$ pH 7.8). A glass rod, pre-incubated in liquid nitrogen, was then immersed in the leaf extract, resulting in a uniform frozen coat around the rod. The sample was inserted into a glass Dewer vessel, which was placed in a FluooMax3 fluorometer (Jobin Yvon, France). The fluorescence spectra of the leaf extract was measured between 650 and 750 nm, following a 430 nm excitation beam. The excitation and emission slits were set at 5 nm, with an integration time of 0.25 s. The emission spectra gave two distinct peaks at 682 and 730 nm, resulting from the fluorescence of PSII and PSI chlorophyll antenna systems, respectively. The PSII excitation distribution was estimated based on the following calculations: PSII excitation distribution $= (F'_{682\,nm})/(F'_{682\,nm} + F'_{730\,nm})$.

2.6. Leaf relative water content

The leaf relative water content (LRWC) was measured following [41].

2.7. Chlorophyll and anthocyanin content

The chlorophyll concentration in the leaves was determined according to [42] and is presented as Chl (mg) per fresh weight (g), as it was determined in 1 ml of extraction solution (ethanol). The total anthocyanin content was determined according to [43] presented as OD 530 per fresh weight (g), as it was determined in 1 ml of extraction solution.

2.8. Plant dry weight

Plants were harvested 52 days after sowing (27 days from the beginning of the drought treatment); the different plants were separated into roots and shoots and were oven dried at 65 °C for 72 h, and then the samples were weighed.

Table 1
Dry weight of shoot and root. The effect of a 25 day drought on overall plant biomass and root/shoot ratio, $n = 7 \pm$ standard error.

Ecotype	Treatment	Total dry biomass (g)	Root/shoot dry mass ratio (g/g)
AM	Drought	4.2 ± 1.66	0.83 ± 0.1
	Irrigation	13.7 ± 1.13	0.6 ± 0.06
SB	Drought	3.11 ± 1.06	0.78 ± 0.05
	Irrigation	9.1 ± 0.33	0.4 ± 0.1

2.9. Statistical analysis

Statistical analysis was done using the Tukey–Kramer HSD test with $\alpha = 0.05$ after a one-way ANOVA (JMP6 statistical software, AS Institute, Cary, NC, USA).

3. Results

3.1. Effect of prolonged drought on plant biomass

In both ecotypes, drought caused a similar and significant decrease of ~67% in overall dry biomass; the root to shoot biomass ratio increased in both ecotypes (Table 1).

3.2. Changes in leaf pigmentation in response to drought

Under drought conditions, the SB plants (xeric ecotype) produced purple-colored leaves; however, in the AM plants (mesic ecotype), there was only a slight change in leaf color (Fig. 1). The concentration of total anthocyanin under drought increased by 230 and 30% in the leaves of the SB and AM plants, respectively, as compared with the irrigated control (Fig. 2a). The increase in leaf anthocyanin concentration was not accompanied by a decrease in the total chlorophyll concentration (Fig. 2b).

The changes in leaf pigmentation had an influence on the absorptive properties of the leaves, namely that the absorbance in the wavelength region of 500–600 nm increased in the leaves of the drought-treated SB plants (Fig. 3a); however, the increase of absorbance in this region was probably due to the increase of anthocyanin, which absorbs light in this part of the spectrum. The overall absorbance of the drought-treated SB ecotype was higher than that of the AM ecotype (Table 2). The light distribution between PSII and

Fig. 1. Leaves of the Mediterranean and desert ecotype of *H. spontaneum*. *H. spontaneum* leaves of the Mediterranean AM ecotype and the desert SB ecotype that were subjected to either constant irrigation or 25 days of complete drought. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

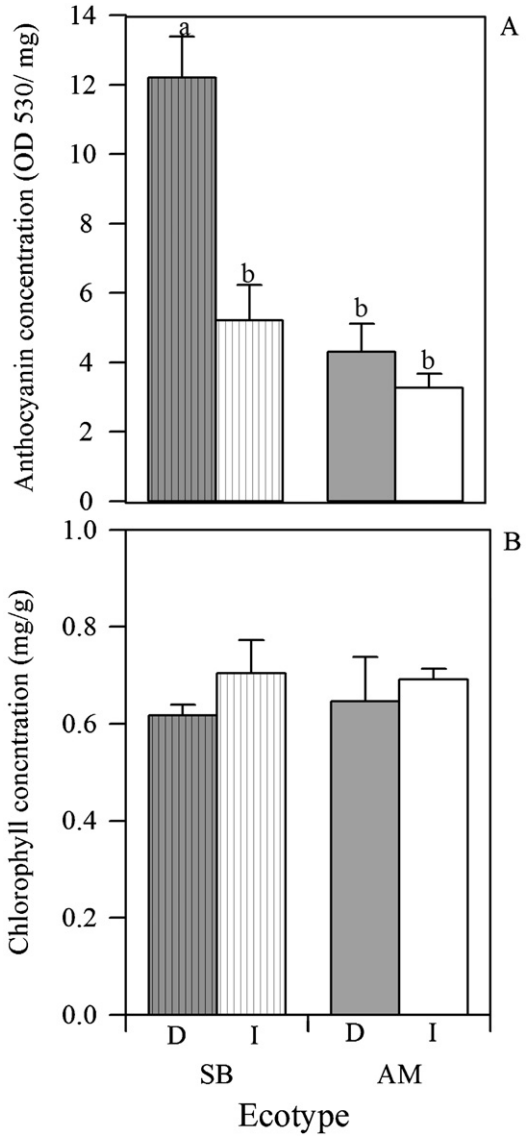

Fig. 2. Anthocyanin and chlorophyll concentrations. The different ecotypes, SB and AM, under the irrigation (I) or drought (D) treatments. (a) Anthocyanin concentration in fresh weight of leaves, presented as optical density (OD) at 530 nm, which is the typical absorbance peak of anthocyanin molecules in an acidic extraction buffer (pH 1), $n = 6$. (b) Total chlorophyll (a + b) concentration in an ethanol extraction of fresh leaves, as determined by absorbance at 648 and 665 nm, $n = 6$. Groups that do not share a common letter are statistically different ($\alpha = 0.05$), standard error bars are shown.

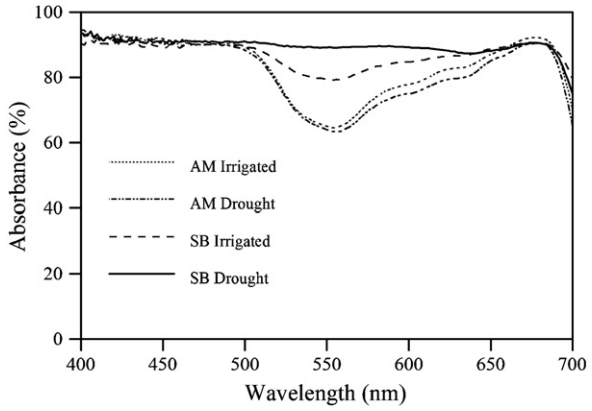

Fig. 3. Leaf absorbance. Leaf absorbance in the photosynthetically active part of the spectrum (400–700 nm), as measured by an integrated sphere device; each curve is representative of three repeats.

PSI, as reflected in measurements by fluorescence at 77 K, revealed a statistically significant higher distribution to PSII in the SB plants ($P < 0.05$); however, the difference in the values was less than five percent (Fig. 4b).

3.3. Leaf water status and CO_2 assimilation

Under drought, LRWC decreased significantly ($P < 0.05$), compared with the irrigated control, in both ecotypes: from 93.7 and 91% to 66.8 and 65.2% in the SB and AM ecotypes, respectively (Fig. 4a). Under a light intensity of 1500 PPFD, stomatal conductance decreased significantly ($P < 0.05$), by 83.6 and 75%, in the drought-treated AM and SB ecotypes, respectively (Fig. 4b). CO_2 assimilation decreased significantly ($P < 0.05$), compared with the irrigated control, by 89 and 82.4% in the AM and SB ecotypes, respectively (Fig. 4c). Similar effects were also measured when stomatal conductance and assimilation were measured under lower light intensity (700 PPFD) at the natural light intensity in the greenhouse, after 4 h of exposure (Table 3). These results suggest that in both the Mediterranean and the desert ecotypes, severe drought leads to leaf dehydration, thereby causing an almost complete closure of stomata and inhibition of carbon assimilation.

3.4. Photochemical reaction of photosystem II and its dependence on atmospheric O_2

Under drought conditions, PSII yield (at 1500 PPFD) decreased significantly ($P < 0.05$), by 49%, in the AM ecotype as compared with the irrigated plants; however, in the SB ecotype, there was no significant change in PSII yield. The PSII yield in the SB drought-treated plants was significantly ($P < 0.05$) higher (77%) than in that of the AM ecotype under drought (Fig. 5b). When PSII yield was measured

Table 2
Absorbance of photosynthetic active radiation and light excitation distribution to photosystem II in light-exposed leaves. The average absorbance, between 400 and 700 nm, was measured using an integrated sphere device, $n = 5 \pm$ standard error. Light energy distribution to PSII, as was measured in light-exposed leaves, by fluorescence at 770 K. PSI fluorescence peaked at 730 nm, while PSII peaked at 682 nm, excitation light was at 430 nm, $n = 5 \pm$ standard error.

Ecotype	Treatment	Average absorbance ± SE	Fraction of excitation distribution to PSII
AM	Drought	83.780 ± 3.410	0.582 ± 0.016
	Irrigation	83.050 ± 3.350	0.590 ± 0.0210
SB	Drought	90.240 ± 0.900	0.620 ± 0.003
	Irrigation	86.490 ± 3.490	0.589 ± 0.004

Table 3
Net assimilation and stomatal conductance in plants grown and measured under natural light intensity of 700 PPFD. Measurements were taken in the greenhouse, at 11:00 AM, about 5 h after sunrise, light intensity was 700 PPFD, $n = 5 \pm$ standard error.

Ecotype	Treatment	Net assimilation (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s
AM	Drought	0.42 ± 0.08	11 ± 1.3
	Irrigation	5.63 ± 0.34	113 ± 11
SB	Drought	0.81 ± 0.08	12 ± 1.5
	Irrigation	7.32 ± 0.41	186 ± 4.6

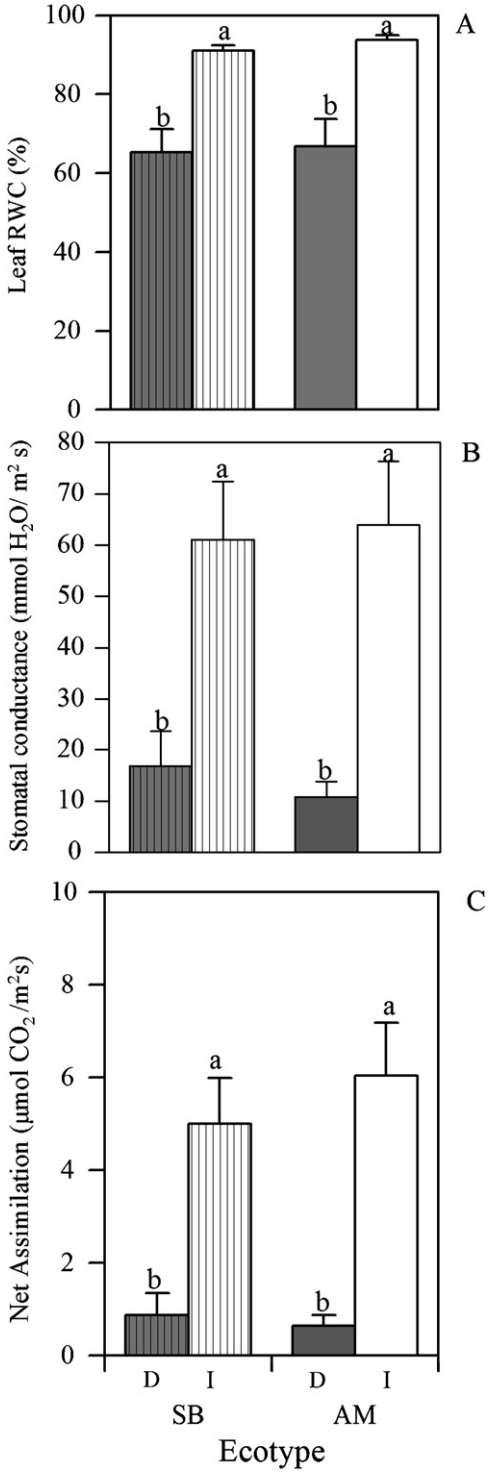

Fig. 4. Leaf relative water content, stomatal conductance and carbon assimilation. The different ecotypes, SB and AM, under the irrigation (I) or drought (D) treatments. (a) Leaf relative water content (LRWC) in, $n > 10$. (b) Stomatal conductance to water vapor as measured at a light intensity of 1500 PPFD, 8 min of light period, $n > 8$. (c) Carbon assimilation, as was measured at a light intensity of 1500 PPFD, $n > 10$. Measurement conditions for assimilation and stomatal conductance were: 8 min of light period, 1500 PPFD, and 400 μmol/mol CO_2. Groups that do not share a common letter are statistically different ($\alpha = 0.05$), standard error bars are shown.

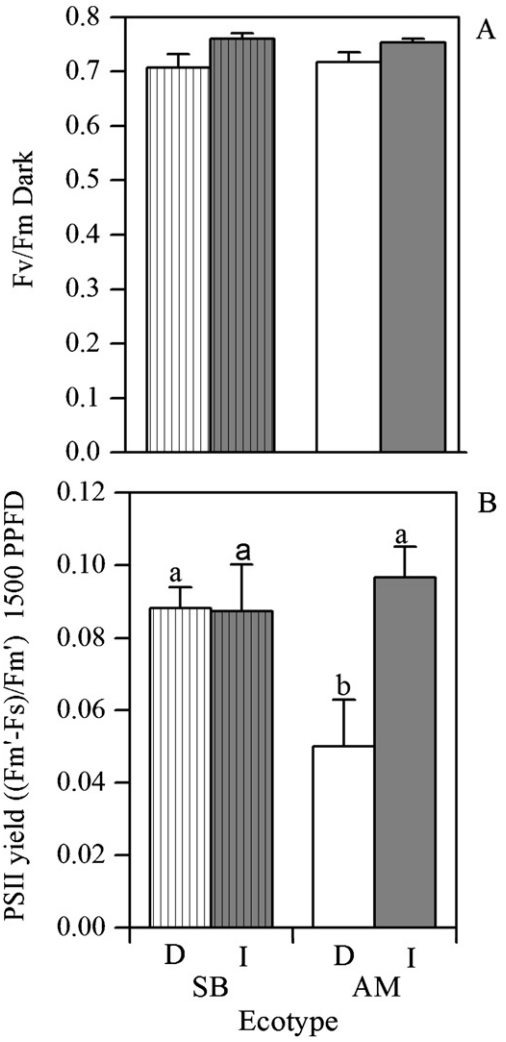

Fig. 5. Maximum efficiency and PSII yield. The different ecotypes, SB and AM, under the irrigation (I) or drought (D) treatments. (a) Maximum efficiency of photosystem II (F_v/F_m) was measured in dark-adapted leaves, $n > 10$. (b) Photosystem II yield measurement conditions were: 8 min of light period, 1500 PPFD, and 400 μmol/mol CO_2, $n > 10$. Groups that do not share a common letter are statistically different ($\alpha = 0.05$), standard error bars are shown.

in the greenhouse (light intensity of 700 PPFD) at midday, PSII yield was lower in the SB drought-treated plants as compared with the control plants; however, it was still twice as high as that of the AM drought-treated plants (Table 4).

Regulated thermal dissipation, NPQ, increased under drought in both ecotypes; however, this increase was insignificant (Fig. 6a, $P > 0.05$). In the drought-treated plants, ΦNPQ, regulated thermal dissipation efficiency, increased in both ecotypes as compared with the control; however, ΦNPQ levels of the SB drought-treated plants were significantly lower ($P < 0.05$) than that of the drought-treated AM plants (Fig. 6b). Higher values of NPQ (50% more) and ΦNPQ were also measured in the drought-treated AM ecotype after 5 h under sunlight in the greenhouse (Table 3). Since assimilation was very low in the SB ecotype drought-treated plants, we hypothesized that the high PSII yield maintained in these plants could have

Table 4
PSII yield, NPQ and ΦNPQ at growth light intensity of 700 PPFD. Measurements were taken in the greenhouse, at 11:00 AM, about 5 h after sunrise, light intensity was 700 PPFD, $n = 4 \pm$ standard error.

Ecotype	Treatment	PSII yield	NPQ	ΦNPQ
AM	Drought	0.066 ± 0.023	3.48 ± 0.21	0.623 ± 0.01
	Irrigation	0.266 ± 0.070	0.888 ± 0.02	0.260 ± 0.02
SB	Drought	0.133 ± 0.030	2.371 ± 0.23	0.525 ± 0.067
	Irrigation	0.307 ± .049	0.697 ± 0.15	0.210 ± 0.028

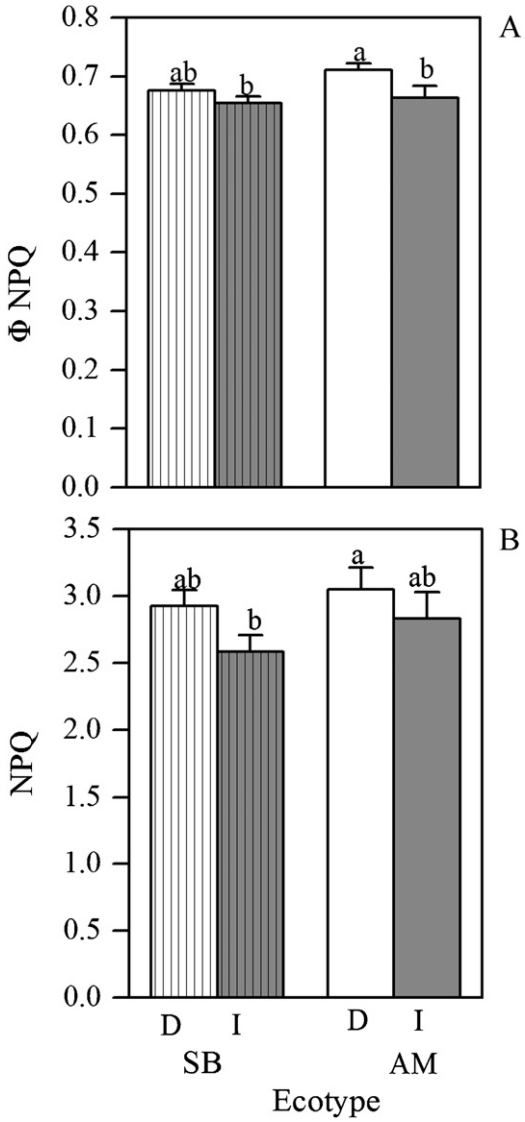

Fig. 6. Non-photochemical quenching efficiency of non-photochemical quenching. The different ecotypes, SB and AM, under the irrigation (I) or drought (D) treatments. (a) Non-photochemical quenching (NPQ) calculated according to the formula $NPQ = F_m - F'_m/F'_m$. (b) The efficiency of NPQ – ΦNPQ was calculated according to the formula $\Phi NPQ = (F_s/F'_m) - (F_s/F_m)$. Measurements were taken after 8 min of exposure to 1500 PPFD and 400 μmol/mol CO_2, $n > 10$. Groups that do not share a common letter are statistically different ($\alpha = 0.05$), standard error bars are shown.

been due to the use of O_2 as an electron acceptor. In order to test this hypothesis, we measured the PSII yield at a low concentration of O_2 (2%), at which photorespiration is inhibited. The PSII yield, under low O_2, decreased by 35% ($P < 0.05$) in the SB drought-treated ecotype plants. This decrease was much larger than the relatively small decreases of 17.5, 20.5 and 15.7% in the SB irrigated, AM irrigated and AM drought-treated plants, respectively (Fig. 7). By comparing the PSII yield values of the AM ecotype drought-treated plants and the SB ecotype drought-treated plants, it is estimated that the O_2-dependent photochemical activity is responsible for 67% of the difference in the PSII yield between the two ecotypes under drought.

4. Discussion

Severe drought caused a significant and similar decrease in growth in both ecotypes; this was also reflected in the physiology of single leaves: leaf RWC, stomatal conductance and carbon

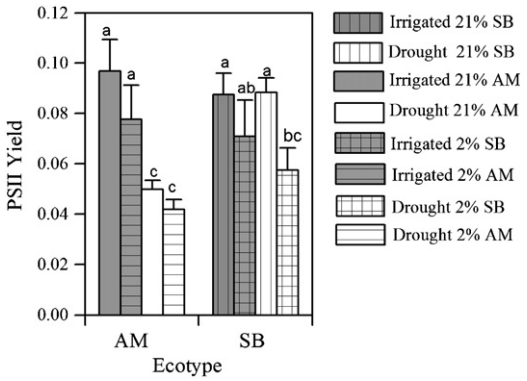

Fig. 7. Photosystem II yield at low and ambient oxygen concentrations. Plant leaves were exposed to either low (2%) or ambient (~21%) oxygen, at light intensity of 1500 PPFD, and 400 μmol/mol CO_2. Groups that do not share a common letter are statistically different ($\alpha = 0.05$), $n > 7$, standard error bars are shown.

assimilation decreased similarly and significantly in both ecotypes. The ability to assimilate carbon dioxide under low stomatal conductance is considered an important adaptation to drought conditions [44,45]. In our experiment, net carbon assimilation was inhibited almost completely in both ecotypes, under severe drought. Under such conditions, the potential damage caused by excess light can be high since the repair cycle of PSII reaction centers might be impaired due to lack of carbohydrates. Multiple ways exist in which plants protect themselves from photo-damage; among them are NPQ, anthocyanin accumulation and photorespiration.

Increasing the photoprotection of plants by NPQ under drought is well documented [46,47]. In our research, both ecotypes of *H. spontaneum* reacted to drought by increasing their NPQ; however, NPQ in the desert ecotype was significantly lower than in that of the Mediterranean ecotype in both drought and irrigated treatments. Therefore, we suggest that stronger induction of NPQ, under drought, is not an adaptive mechanism of the desert ecotype of *H. spontaneum* to its environment.

Anthocyanin molecules can absorb light in the visible range, therefore lowering the excitation pressure on photosystem II and preventing photo damage [48]. It has been suggested that anthocyanin can serve in photoprotection, in conjunction with other photo protective processes, such as NPQ [50]. However, our results indicated that higher induction of NPQ was recorded in the drought-treated Mediterranean ecotype, which accumulated a low amount of anthocyanin, while lower NPQ levels were recorded in the drought-treated desert ecotype. Anthocyanin accumulated mainly in the desert ecotype under drought and significantly less in the Mediterranean ecotype. These findings can be explained by the light absorptive function of anthocyanin that lowers the excitation pressure on PSII and, therefore, lowers the activation of NPQ in the desert ecotype.

We have found that the O_2-dependent PSII yield was a major contributor to the linear electron flow in the drought-treated desert ecotype of *H. sponataneum*. This finding suggests that adaptation to desert environments may be related to an increase in the capacity of the O_2-dependent PSII photochemical yield under drought, and supports previous observations of the role of photorespiration under drought [18,49,51].

Overall, the results suggest that, under conditions of drought, excessive light and the inhibition of carbon assimilation, the desert SB ecotype of *H. spontaneum* accumulated high concentrations of anthocyanin and maintained high levels of O_2-dependent photochemical activity, while the Mediterranean ecotype had higher NPQ. In conclusion, our results suggest that ecotypes of *H. spontaneum* from Mediterranean and desert origins have different

alternative strategies to mitigate the damage of drought and high light intensity.

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