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BRIEF COMMUNICATION

Photosynthesis and photoprotection under drought in the annual desert plant *Anastatica hierochuntica*

A. EPPEL+ and S. RACHMILEVITCH

French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel

Abstract

Anastatica hierochuntica is an annual desert plant, which was recently shown to have unusually low nonphotochemical quenching (NPQ) and a high PSII electron transport rate (ETR). In the current study, we examined how these unusual characteristics are related to a lack of CO_2 and inhibition of net photosynthetic rate (P_N). We compared the photosynthetic and photoprotective response of *A. hierochuntica* and sunflower (*Helianthus annuus*), under conditions of photosynthetic inhibition, with either low CO_2 or drought. We found that under nonsteady state conditions of low CO_2 availability, *A. hierochuntica* exhibited about half of the NPQ values and almost twice of the ETR values of *H. annuus*. However, the long-term inhibition of P_N under drought caused a similar increase in NPQ and a decrease in ETR in both *A. hierochuntica* and *H. annuus*. These results suggest that the unusually low NPQ and high ETR in *A. hierochuntica* are not directly related to a response to drought conditions.

Additional key words: abiotic stress; relative water content; stomatal conductance.

Drought effects on plants are initiated by the suppression of cell growth, which is closely followed by stomatal closure (Saab and Sharp 1989, Tardieu 2012). Stomatal closure prevents excessive water loss, which can ultimately lead to plant dehydration and death (Chaves *et al.* 2003). Drought induces stomatal closure and the reduction of mesophyll conductance. As a consequence, the diffusion of CO_2 into the chloroplasts is lowered, and the assimilation of CO_2 in the Calvin cycle is inhibited (Flexas *et al.* 2006). This type of limitation on P_N is termed stomatal or diffusive limitation. Diffusive limitation can be transiently reversed by applying a high CO_2 concentration to the leaf (Flexas *et al.* 2004).

Stomatal closure caused by drought limits CO_2 availability for the Calvin cycle. Under these conditions, light energy can often exceed the energy that is needed to assimilate carbon in P_N. Therefore, drought conditions usually induce photoprotective mechanisms in order to avoid photodamage (Chaves *et al.* 2009). Photorespiration becomes a favorable process under conditions of low CO_2 concentrations, which occur during stomatal closure. Photorespiration was shown to increase under drought (Lovelock and Winter 1996, Wingler *et al.* 1999). Drought tolerance was also shown to be related to high rates of photorespiration in a drought-tolerant transgenic plant that had delayed senescence under drought (Rivero *et al.* 2009).

Nonphotochemical quenching (NPQ) is a well-studied and common mechanism of photoprotection in plants. NPQ dissipates excess energy from light directly into heat (Bilger and Björkman 1990). The dynamic component of NPQ, q_E, is driven by a reduction of pH in the chloroplast lumen, which in turn triggers both the activation of the xanthophyll cycle and structural changes in the light-harvesting complex of PSII (Muller *et al.* 2001). NPQ was also shown to increase under drought (Montanaro *et al.* 2007, Naumann *et al.* 2007) and due to salinity (Sperling *et al.* 2014). The induction of NPQ under drought has been suggested to be caused by a decrease in ATP synthase activity (Kohzuma *et al.* 2009).

It has been suggested that under drought, P_N is impaired due to an impairment of ATP synthesis (Tezara *et al.* 1999). This limitation on P_N was termed metabolic limitation. Metabolic limitation cannot be reversed by supplying leaves with transiently high CO_2 concentrations, in contrast to diffusive limitation (Flexas *et al.* 2006).

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+Corresponding author; e-mail: amireppel@gmail.com

Abbreviations: ETR – photosystem II electron transport rate; g_s – stomatal conductance; IRGA – infrared gas analyzer; NPQ – nonphotochemical quenching; P_N – net photosynthesis; RWC_{leaf} – leaf relative water content.

The importance of metabolic limitation for the inhibition of P_N under drought was demonstrated in sunflower (*Helianthus annuus*) (Tezara *et al.* 1999). However, other studies have demonstrated that the high CO_2 concentration can reverse this inhibition and restore carbon assimilation, both in *H. annuus* (Wise *et al.* 1990, Cornic and Fresneau 2002) and in other plant species (Flexas *et al.* 2006).

Anastatica hierochuntica is an annual desert plant (family Brassicaceae) found in the hot deserts of the Middle East and North Africa. In these areas, water is available only for a short period of time during the year, and therefore, annual plants have a limited time to complete their life cycle and produce seeds. *A. hierochuntica* plants have a fixed and opportunistic developmental pattern, in which flowering and seed production start after four or five true leaves have emerged, regardless of day length (Shem-Tov and Gutterman 2003). This development pattern is an adaptation to the desert environment that enables the plants to maximize resource utilization during a relatively brief window of opportunity (Shem-Tov and Gutterman 2003). *A. hierochuntica* has an uncommon photoprotective/photochemical response during short exposures to low CO_2 concentrations. In *A. hierochuntica*, the PSII ETR remains relatively high, and NPQ is only slightly induced (Eppel *et al.* 2014). In plants, such as *Arabidopsis thaliana*, *Helianthus annuus*, and *Thellungiella salsuginea*, a stronger induction of NPQ and a more significant downregulation of ETR was observed upon short-term exposure to low CO_2 concentrations (Eppel *et al.* 2014).

In this study, we examined, in *A. hierochuntica* and *H. annuus*, the relation between the short-term response to the inhibition of P_N (low CO_2 concentrations), and the long-term response to the inhibition of P_N that occurs under drought. We hypothesized that *A. hierochuntica* maintains low NPQ and a high ETR, in contrast to *H. annuus*, under drought conditions.

Anastatica hierochuntica and *H. annuus* plants were grown in a controlled growth chamber (*Percival*, Perry, Iowa, USA), in plastic pots filled with standard potting soil (*Matza Gan*, Israel). The daily light intensity schedule was: 30 min under PPFD of 10 μmol(photon) m^{-2} s^{-1}, 1 h of 200 μmol(photon) m^{-2} s^{-1}, 9 h of 600 μmol(photon) m^{-2} s^{-1}, 1 h of 200 μmol(photon) m^{-2} s^{-1}, 30 min of 10 μmol (photon) m^{-2} s^{-1}, and 12 h in the dark, representing a light/dark cycle of 12 h/12 h. Temperature was 22°C, and relative humidity was between 50 and 70%.

Plants were grown for either three (*H. annuus*) or four weeks (*A. hierochuntica*). After this initial period, drought was applied to half of the plants, for one week, while the other half of the plants continued to be irrigated in order to maintain soil water content above 50%. In the drought-treated plants, the soil water content was between 15 and 30% (data not shown). Measurements of the leaf relative water content (RWC_{leaf}) were performed by cutting and weighing fresh leaves (FM), incubating them for 12 h in double-distilled water, and reweighing the leaves (TM). Then, the leaves were oven dried for 48 h at 60°C, after which the leaves were again weighed (DM). We used the following formula to calculate the relative water content: RWC_{leaf} [%] = 100 × (FM – DM)/(TM – DM).

Measurements of the short-term response to different CO_2 concentrations were made using an *LI-COR 6400-XT* infrared gas analyzer (IRGA) (portable photosynthesis system *LI-6400*, *LI-COR Inc.*, Lincoln, NE, USA). Measurements were performed as follows: the well-irrigated plants were dark-adapted for 2 h, and then the intact leaves of these plants were exposed to 10 min of 1,200 μmol(photon) m^{-2} s^{-1} and CO_2 concentrations (80, 400, and 1,600 ppm). NPQ was calculated according to Bilger and Björkman (1990), and ETR was calculated according to Krall and Edwards (1992). In the drought experiment, the plants were illuminated for 2–4 h at 600 μmol(photon) m^{-2} s^{-1} in a growth chamber (see growth conditions above), and then leaves were clamped inside the *LI-COR 6400-XT* IRGA. Conditions during measurements in the drought experiment were: actinic light intensity of 600 μmol(photon) m^{-2} s^{-1}, relative humidity of 40–50%, and leaf temperature of 22°C. The readings were taken when values were stabilized, usually 3–4 min after the leaves were clamped into the device. In order to calculate NPQ in the drought experiment, the F_m values were taken from measurements of the leaves at dawn.

NPQ and ETR were measured in the dark-adapted, well-irrigated, 30-day-old *A. hierochuntica* plants and 21-day-old *H. annuus* plants. After a short period of 10 min in the light, ETR was similar in *A. hierochuntica* and in *H. annus* under high and medium CO_2 concentrations (Fig. 1*A*,*B*); however, under low CO_2 concentrations, ETR was 70% higher in *A. hierochuntica* than that in *H. annuus*. NPQ, after 10 min at low CO_2 concentrations, was 40% lower in *A. hierochuntica* than that in *H. annuus*; at medium and high CO_2, NPQ was only 20% lower in *A. hierochuntica* than that in *H. annuus*. These results affirmed previous observations regarding the low induction of NPQ and high ETR during the short-term exposure to low CO_2 concentrations in *A. hierochuntica*.

Drought inhibits P_N by reducing CO_2 availability. In order to examine whether the short-term response of *A. hierochuntica* to low CO_2 concentrations is related to reduced CO_2 availability under drought, we carried out a drought experiment with *A. hierochuntica* and *H. annuus* plants.

Thirty-day-old *A. hierochuntica* plants and 21-day-old *H. annuus* plants were subjected to either one week of drought or to continuous irrigation. Measurements of the plants were conducted between the fourth and the seventh day of that week; the measurements were taken between 2 and 4 h from the beginning of the light period of each day, without any dark adaptation. At the single leaf level, under well-irrigated conditions, RWC_{leaf} and stomatal conductance (g_s) were high. Under drought conditions, a decrease in RWC_{leaf} to levels below 80%, caused a sharp decrease in g_s in both species (Fig. 2*A*).

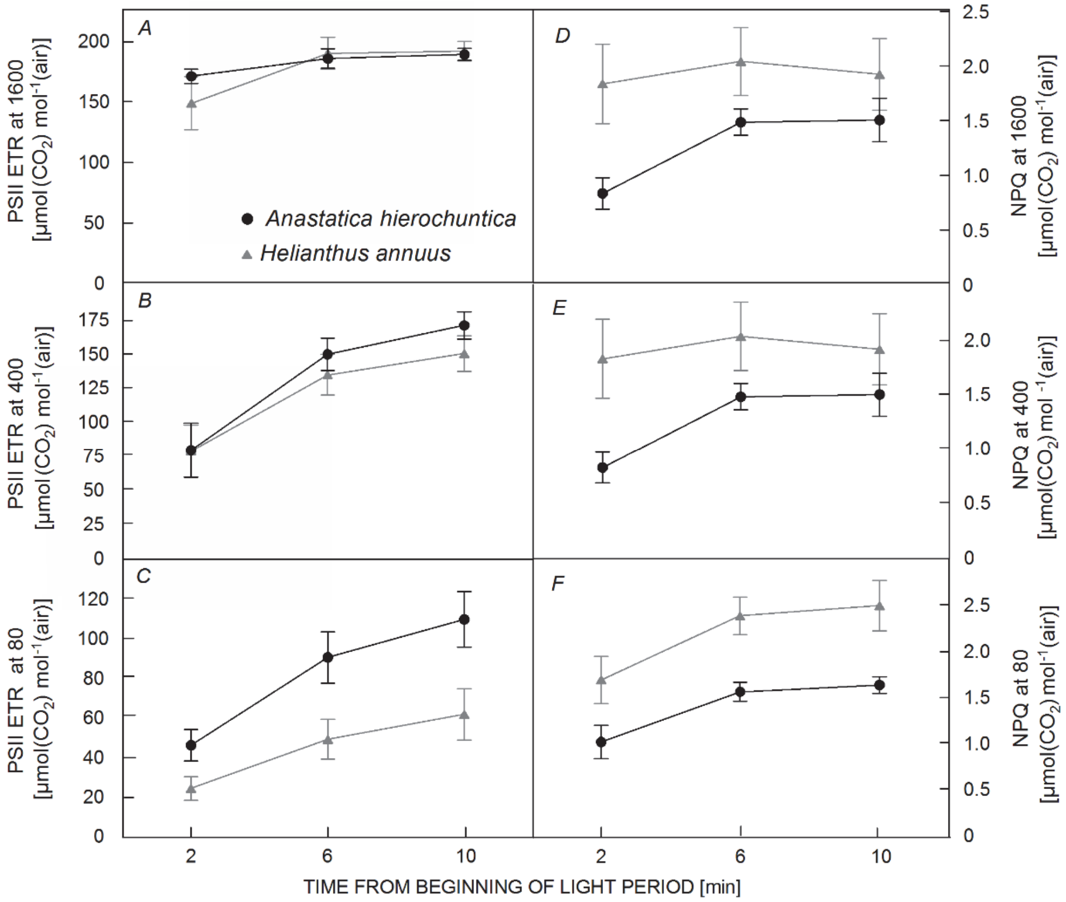

Fig. 1. Photosystem II electron transport rate (ETR) and nonphotochemical quenching (NPQ) response to short period of exposure to different CO_2 concentrations in *Anastatica hierochuntica* and *Helianthus annuus*. Measurements were performed in leaves of dark-adapted plants. Light intensity was 1,200 µmol(photon) m^{-2} s^{-1} during the measurement, leaf temperature was 22°C, O_2 concentration during growth and measurement was atmospheric (21%). *A*–*C*: ETR at 1,600 (*A*), 400 (*B*), and 80 (*C*) [µmol(CO_2) mol^{-1}(air)]. *D*–*F*: NPQ at 1,600 (*D*), 400 (*E*), and 80 (*F*) [µmol(CO_2) mol^{-1}(air)].

Under well-irrigated conditions, g_s and P_N were similar in *A. hierochuntica* and *H. annus* (Fig. 2*B*). In the drought-treated plants, P_N dropped with the decrease in g_s; the trend of decrease was similar in both plants (Fig. 2*B*). In order to examine whether assimilation under drought decreased due to low CO_2 diffusion into the leaf, the plants were exposed to high concentrations of CO_2. Under these conditions, the difference in P_N between the drought-treated and well-irrigated plants was much lower and was much less dependent on g_s (Fig. 2*C*), in comparison to the differences in P_N under average CO_2.

Our results suggest that in both *A. hierochuntica* and *H. annuus*, the inhibition of CO_2 assimilation, under low g_s (drought), could be mostly attributed to the low availability of CO_2.

In response to low g_s and drought, ETR decreased in a similar trend and to similar values in both *A. hierochuntica and H. annuus* (Fig. 2*D*); the decrease was up to 50% in comparison with the irrigated plants. NPQ increased under drought similarly in both plant species and was inversely related to g_s (Fig. 2*E*). NPQ was ~3.5 times higher in the drought-treated plants than that in the irrigated plants. In both plant species, the decrease in NPQ was greater than the decrease in ETR. In both plants, the ratio of ETR/NPQ decreased similarly and linearly with the decrease in g_s (not shown).

The initial NPQ and ETR responses of *A. hierochuntica* and *H. annuus* to different CO_2 concentrations supported previous observations of low NPQ and high ETR at low CO_2.

In the drought experiment conducted in these plants, inhibition of P_N, at low g_s, was almost completely reversed when the high CO_2 concentration was applied to the plants under drought. This result confirmed previous reports (Wise *et al.* 1990, Cornic and Fresneau 2002, Flexas *et al.* 2004) and also supported the hypothesis that P_N, under drought, is mainly inhibited due to a lack of CO_2 in the leaves (diffusion limitation), and not because of metabolic impairment (metabolic limitation), as it was suggested (Tezara *et al.*1999).

A similar decrease in ETR and an increase in NPQ were recorded under drought in both *A. hierochuntica* and

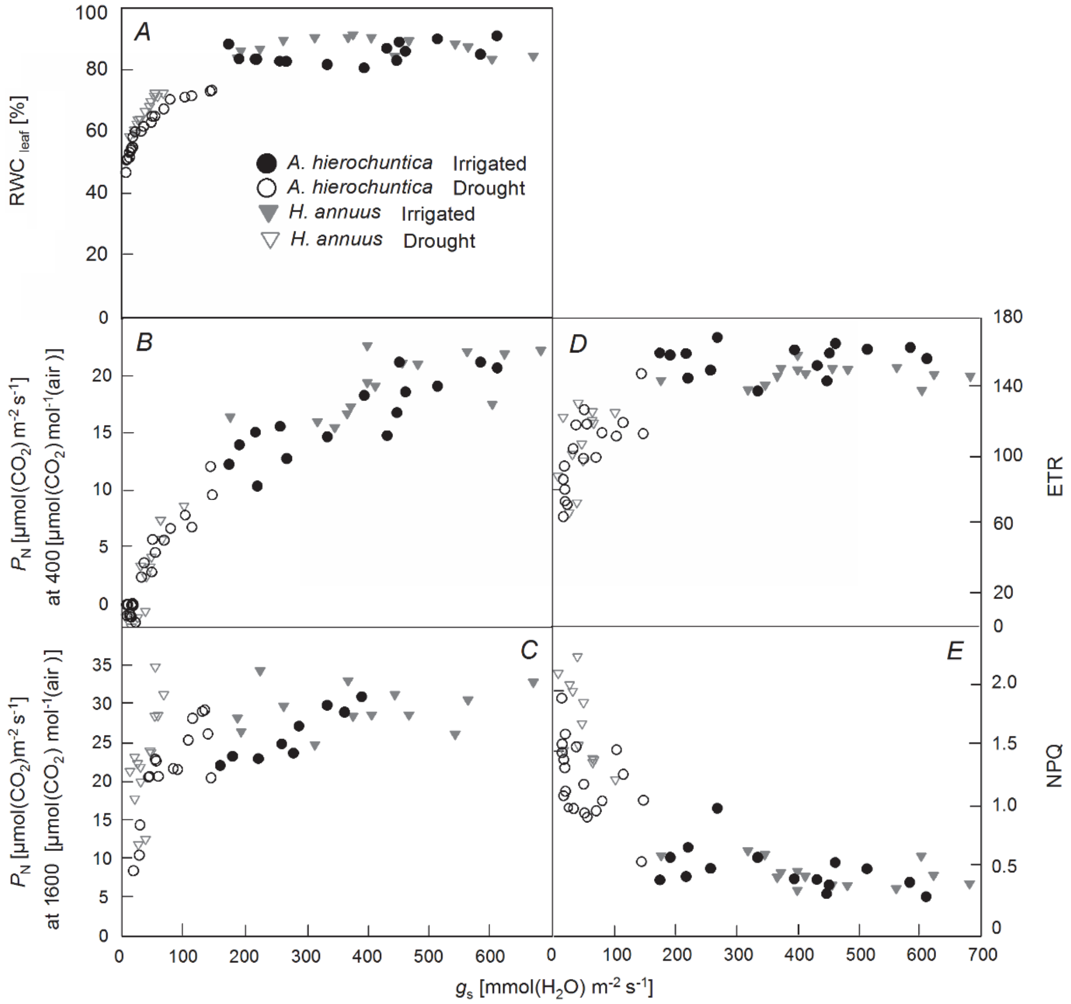

Fig. 2. The effect of drought on leaf physiology in *Anastatica hierochuntica* and *Helianthus annuus*. Measurements were performed in the growth chamber, between 10:00 and 12:00 h, while the light period begun at 8:00 h. Leaf relative water content (RWC$_{leaf}$) (*A*), net photosynthetic rate (P_N) at atmospheric CO$_2$ concentration (*B*), P_N at high CO$_2$ concentration (*C*), electron transport rate (ETR) (*D*), and nonphotochemical quenching (NPQ) (*E*).

H. annuus. These results suggest that the NPQ induction under drought occurred in *A. hierochuntica* in a similar manner to what is described for other plants, thus rejecting the hypothesis that *A. hierochuntica* is unusual in this sense. It is likely that in *A. hierochuntica*, the mechanism that is responsible for keeping NPQ low and ETR high during relatively short periods of low CO$_2$ concentrations has a smaller effect under conditions of the long-term inhibition of P_N, such as drought. The experiment presented in this study was held in controlled laboratory conditions; therefore, it is possible that under field conditions, the response to drought in *A. hierochuntica* would be different, and this is yet to be tested.

In an article published by Hegazi and Moser (1991), *A. hierochuntica* appeared as the first in transpiration rates and the second in photosynthetic rates, in a list comprised of 9 annual plants species and 19 perennial plants species from desert environment (Hegazi and Moser 1991). Recently, we have found that *A. hierochuntica* plants have unusually low NPQ and high ETR which allow them to divert more energy into productive processes such as photosynthesis (Eppel *et al.* 2014).

The results presented in this study suggest that the unusually low NPQ and high ETR in *A. hierochuntica*, are not related to drought conditions. Taken together, the photosynthetic and photoprotective functions in *A. hierochuntica* plants are adapted to utilize, relatively quickly, resources such as light and water during favorable growth conditions, which occur in the desert only within a short time period during the year. These physiological adaptations support the opportunistic life strategy of *A. hierochuntica*, a strategy that favors growth and reproduction during short periods of favorable growth conditions.

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