Comparative Ecophysiology and Photoprotection in Two Stem Succulent Euphorbia Species in Arid Habitat

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Abstract. Stem succulents Euphorbia fractiflexa and Euphorbia triaculeata (Euphorbiaceae) are common arido-active perennials in arid regions of Arabian Peninsula. Work presented in this paper aimed at investigating Crassulacean Acid Metabolism (CAM) in these two species by studying diurnal and seasonal changes in stomatal conductance and cell sap acidity. Chlorophyll fluorescence was measured to elucidate effect of drought and high irradiance on photosynthetic apparatus. Pigment profile analysis was carried out to provide comparative view of photoprotective mechanisms in these two species. Results indicated that these two Euphorbia species are obligate CAM plants that shift to CAM-idling in response to protracted drought. Results also revealed stress induced changes in activity and antenna size of Photosystem II. Changes in Photosystem II associated with increased non-photochemical quenching of chlorophyll fluorescence reflecting operation of photoprotective excess energy dissipation mechanisms. Pigment profile analysis revealed that photoprotection in E. fractiflexa relies on operation of xanthophyll cycle while that in E. triaculeata relies on anthocyanin accumulation.

Key words: Chlorophyll Fluorescence, Crassulacean Acid Metabolism (CAM), Euphorbia, Photoprotection.

Introduction

Arid regions southwest of Arabian Peninsula are among the most inhospitable habitats where climate is a composite of harsh environmental conditions. The dry season is conspicuously long with severe drought and high irradiance, the short wet season features high temperature and high evaporation, and summer erratic rainfall is associated with strong sand storms (FISHER & MEMBERY, 1998; VINCENT, 2008). In such habitats, stem succulents rely for survival on water storage and physiological adaptations of which CAM is prevalent due to improved water economy (SAYED, 2001a; b; LÜTTGE, 2010). In these regions Euphorbia fractiflexa and Euphorbia triaculeata (Euphorbiaceae) are abundant arido-active stem succulents (MASRAHI, 2012). We studied CAM in these two species by investigating diurnal and seasonal changes in stomatal conductance and cell sap acidity. Seasonal changes in chlorophyll fluorescence and pigment profile were studies to elucidate effects of drought and high irradiance on photosynthetic machinery. Moreover, our filed observations indicated that while E. fractiflexa retains its green stem colour, E. triaculeata stem acquires an antocyanin-like red colour under protracted drought. Photoprotective properties of carotenoids and anthocyanin
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have repeatedly been reported (GOULD et al., 2010; ADAMS et al., 2004; MURCHEI & NIYOGI, 2011; JAHNS & HOLZWARTH, 2012). We studied seasonal changes in carotenoid and anthocyanin contents and chlorophyll fluorescence quenching to provide a comparative view of photoprotective mechanisms in these two species.

Materials and Methods

Study area. The study site (17°19’N – 42°48’E) in lowland arid region southwest of Saudi Arabia is characterized by nine-months-long dry season and a short wet season (June–August). Mean monthly records of air temperature, precipitation, and evaporation were obtained from Ministry of Electricity and Water (Riyadh, Saudi Arabia). During dry season, daytime changes in air temperature and photosynthetic photon flux density (PPFD) were monitored using digital thermometer (“Kestrel 2000”, Philadelphia, USA) and PAR/LAI Ceptometer (AcuPar LP-80, Decagon, Pullman, USA), respectively. Mature plants of Euphorbia fractiflexa S.Carter & J.R.I.Wood and Euphorbia triaculeata Forssk. (Euphorbiaceae) were used as plant material.

Climatic records (Curtsey of Saudi Meteorological Office, Riyadh, Saudi Arabia) indicated aridity of study site with nine-months-long dry season, short wet season (June-August), and total annual rainfall of less than 100 mm (Table 1).

Measurements. Stomatal conductance was measured using Porometer (AP4, Delta-T, Cambridge, UK). Chlorophyll fluorescence was measured using fluorescence monitoring system (FM52, Hansatech, Norfolk, UK). Measured chlorophyll fluorescence parameters were Fv/Fm and ΦPSII denoting PSII antenna efficiency and quantum yield, respectively (BAKER, 2008; KALAJI et al., 2012). Fluorescence non-photochemical quenching (NPQ) was calculated using standard nomenclature (SAYED, 2003; BAKER, 2008; KALAJI et al., 2014) and the equation:

$$NPQ = \frac{(Fm - Fm')}{(Fm - Fo)}$$

where: (Fo) minimal fluorescence emitted by antenna chlorophyll molecules, (Fm) maximal fluorescence emitted when PSII traps are closed, and (Fm') light-adapted fluorescence maximum.

Acidification-deacidification of CAM were studied in stem chlorenchyma cell sap extracted by grinding known weight of tissue in distilled water, expressing cell sap through muslin, and determining acidity using 0.01N NaOH and phenolphthalein (ÖSMOND et al., 1991). Chlorophyll and carotenoid contents were determined using spectroscopy (METZNER et al., 1965). Anthocyanin was determined by extraction in ice-cold 10% methanolic HCl (0.1% HCl v/v), determining absorbance at 535, 653 nm, and calculating anthocyanin content as (A535 – 0.24A653) using extinction coefficient 30000 L mol⁻¹cm⁻¹ (MURRAY & HACKETT, 1991). Experiments were routinely repeated in samples from five individual plants and standard error was calculated.

Results

Monitoring daytime changes in air temperature and PPFD during dry season indicated that these parameters attained high midday values of 43°C and 2250 μmol m⁻²s⁻¹, respectively (Table 2). Measurements of stomatal conductance during wet season indicated that E. fractiflexa and E. triaculeata exhibited high nighttime values and low daytime values (Fig. 1). Measurements during dry season indicated that both species exhibited very low values of stomatal conductance of 2-5 mmol m⁻²s⁻¹ during day and night (Fig. 1). In addition, both species exhibited diurnal acidity changes during wet season and these acidity changes were markedly dampened during dry season (Fig. 2).

Chlorophyll fluorescence measurements during the dry season showed reduction in values of Fv/Fm and ΦPSII compared to those measured during wet season in both E. fractiflexa and E. triaculeata (Table 3).

Reduction of chlorophyll fluorescence during dry season associated with markedly increased NPQ in both species (Table 3). Pigment profile analysis indicated that both species showed increased Chl a/b ratio and increased carotenoid and anthocyanin contents during the dry season (Table 4). It
was also noticeable that while carotenoid content markedly increased in *E. fractiflexa*, anthocyanin content markedly increased in *E. triaculeata* (Table 4).

**Table 1.** Mean monthly climatic norms at the study site (1970-2010).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>J</th>
<th>F</th>
<th>M</th>
<th>A</th>
<th>M</th>
<th>J</th>
<th>A</th>
<th>S</th>
<th>O</th>
<th>N</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temperature (°C)</td>
<td>30.8</td>
<td>34.7</td>
<td>35.4</td>
<td>35.5</td>
<td>38.5</td>
<td>39.0</td>
<td>40.0</td>
<td>40.3</td>
<td>38.3</td>
<td>37.3</td>
<td>36.0</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
<td>5.0</td>
<td>4.5</td>
<td>5.0</td>
<td>4.5</td>
<td>5.0</td>
<td>4.4</td>
<td>20.0</td>
<td>8.0</td>
<td>9.0</td>
<td>8.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Evaporation (mm)</td>
<td>4.9</td>
<td>5.6</td>
<td>7.5</td>
<td>8.9</td>
<td>10.2</td>
<td>9.6</td>
<td>10.0</td>
<td>8.6</td>
<td>8.8</td>
<td>9.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

**Table 2.** Daytime changes in air temperature and PPFD during dry season.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>0600</th>
<th>0800</th>
<th>1000</th>
<th>1200</th>
<th>1400</th>
<th>1600</th>
<th>1800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temperature (°C)</td>
<td>34</td>
<td>36</td>
<td>37</td>
<td>43</td>
<td>42</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>PPFD (μmol m⁻² s⁻¹)</td>
<td>500</td>
<td>1500</td>
<td>2000</td>
<td>2025</td>
<td>2250</td>
<td>2125</td>
<td>200</td>
</tr>
</tbody>
</table>

**Fig. 1.** Diurnal and seasonal changes in stomatal conductance of *E. fractiflexa* (a) and *E. triculeata* (b), during wet season (○) and dry season (●). (± se, n = 5).

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Fig. 2. Diurnal and seasonal changes in chlorenchyma cell sap acidity of *E. fractiflexa* (a) and *E. triculeata* (b), during wet season (○) and dry season (●). (± se, n = 5).

Table 3. Seasonal changes of chlorophyll fluorescence in *E. fractiflexa* and *E. triaculeata* (± se, n = 5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>E. fractiflexa</em> Wet Season</th>
<th><em>E. fractiflexa</em> Dry Season</th>
<th><em>E. triaculeata</em> Wet Season</th>
<th><em>E. triaculeata</em> Dry Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fv/Fm</td>
<td>0.84 ± 0.01</td>
<td>0.68 ± 0.007</td>
<td>0.81 ± 0.006</td>
<td>0.75 ± 0.01</td>
</tr>
<tr>
<td>ΦPSII</td>
<td>0.85 ± 0.003</td>
<td>0.64 ± 0.01</td>
<td>0.83 ± 0.01</td>
<td>0.65 ± 0.006</td>
</tr>
<tr>
<td>NPQ</td>
<td>0.02 ± 0.0003</td>
<td>0.07 ± 0.001</td>
<td>0.02 ± 0.004</td>
<td>0.06 ± 0.001</td>
</tr>
</tbody>
</table>

Table 4. Seasonal changes of chlorenchyma pigment profile in *E. fractiflexa* and *E. triaculeata* (± se, n = 5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>E. fractiflexa</em> Wet Season</th>
<th><em>E. fractiflexa</em> Dry Season</th>
<th><em>E. triaculeata</em> Wet Season</th>
<th><em>E. triaculeata</em> Dry Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a : Chl b</td>
<td>0.9 ± 0.04</td>
<td>1.5 ± 0.07</td>
<td>2.5 ± 0.05</td>
<td>3.5 ± 0.03</td>
</tr>
<tr>
<td>Carotenoides (µg g⁻¹ dry weight)</td>
<td>20 ± 1.2</td>
<td>256 ± 1.7</td>
<td>30 ± 2.3</td>
<td>50 ± 3.5</td>
</tr>
<tr>
<td>Anthocyanin (µg g⁻¹ dry weight)</td>
<td>150 ± 2.5</td>
<td>162 ± 5.5</td>
<td>150 ± 2.1</td>
<td>850 ± 5.4</td>
</tr>
</tbody>
</table>
Discussion

Extreme aridity of the study site was evident from prevailing harsh conditions of nine-month-long dry season and short wet season with scarce water, high temperature, and high evaporation (Table 1). Long dry season and low precipitation are typical features of arid regions of Arabian Peninsula (FISHER & MEMBERY, 1998; VINCENT, 2008). Daytime changes in air temperature and PPFD during dry season showed that these parameters can attain high midday values that add to the harshness of the environment (Table 2).

During the wet season, both E. fractiflexa and E. triaculeata exhibited stomatal conductance that was high during the night and low during the day (Fig. 1). Determination of chlorenchyma cell sap acidity during wet and dry seasons indicated presence of diurnal acidification-deacidification cycles in both species (Fig. 2). This stomatal behaviour and diurnal acidity oscillations are typical of CAM plants (LÜTTGE, 2010; WINTER et al., 2015). Occurrence of these features during both wet and dry seasons reflects operation of obligate CAM in both species. Obligate CAM has previously been reported in stem succulent Euphorbia species (PEARCY & TROUGHTON, 1975; WILLERT et al., 1985; MARTIN et al., 1990; VAN DAMME, 1991; MIES et al., 1996; ELHAAK et al., 1997; WINTER et al., 2005; AMEH, 2006). Daytime stomatal closure and nocturnal CO\textsubscript{2} uptake of CAM enhance plant water economy by preventing gas exchange when environmental conditions favor water loss (SAVED, 2001b; LÜTTGE, 2004; 2010; OWEN & GRIFFITHS, 2013). During the dry season, E. fractiflexa and E. triaculeata showed low stomatal conductance the entire day and night (Fig. 1) and markedly dampened diurnal oscillation of chlorenchyma cell sap acidity (Fig. 2). These results indicated that both species shifted from obligate CAM to CAM-idling under protracted drought. The CAM-idling mode is a CAM modification that further enhances plant water economy by day and night stomatal closure and sustaining diurnal acidity changes by nocturnal re-fixation of respiratory CO\textsubscript{2} (DODD et al., 2002; LÜTTGE, 2004; 2010; WINTER et al., 2015). Drought-induced CAM to CAM-idling shift has previously been reported in several stem succulent species in arid regions of Arabian Peninsula and appears to constitute an important survival strategy in such arid habitats (MASRAHI et al., 2011; 2012a; b; 2015).

Measurement of chlorophyll fluorescence during dry season revealed 10-20% reduction in F\textsubscript{v}/F\textsubscript{m} and ΦPSII in both species (Table 3). Decreased F\textsubscript{v}/F\textsubscript{m} and ΦPSII denote reduction of PSII antenna efficiency and quantum yield, respectively (BAKER, 2008; KALAJI et al., 2014). Similar reduction of PSII activity has previously been reported in CAM-idling plants under protracted drought (LÜTTGE, 2004; MASRAHI et al., 2012b; 2015).

Pigment profile analysis indicated increased Chl a/b ratio in both species during dry season reflecting intrinsic changes in PSII (Table 4). The Chl a/b ratio is a measure of light harvesting complex chlorophyll relative to other Photosystem components (BAILEY et al., 2004). Increased Chl a/b ratio reflects changes in PSII antenna size (MURCHIE & HORTON, 1997; BISWAL et al., 2012; DINÇ et al., 2012) and is an integral part of acclimation to high irradiance (KITAJIMA & HOGAN, 2003; KOUŘIL et al., 2013). However, our observed reduction in PSII activity and increased Chl a/b ratio associated with increased NPQ (Table 3) denoting increased non-photochemical quenching and non-radiative excess energy dissipation (BAKER, 2008; PAPAGEORGIOU & GOVINDJEE, 2014; RUBAN & MULLINEAUX, 2014). Increased non-photochemical quenching was reported in CAM-idling plants and was related to increased zeaxanthin content (LÜTTGE, 2010). Our pigment profile analysis also showed increased carotenoid and anthocyanin contents in both Euphorbia species during the dry season (Table 4). These results reflect operation of photoprotective mechanisms in both species. Reports in the literature suggest that epoxidation-deepoxidation of xanthophyll...
cycle carotenoids play a major role in photoprotection under high irradiance (ADAMS et al., 2004; HORTON & RUBAN, 2006; MURCHEI & NIYOGI, 2011; JAHNS & HOLZWARTH, 2012). Anthocyanin is also thought to confer photoprotection by alleviation of oxidative stress via processing reactive oxygen species released when PSII becomes over-energized during CAM-idling (NAGATA et al., 2003; GOULD et al., 2010; ADAMS et al., 2004; NIEWIADOMSKA & BORLAND, 2008; BORLAND et al., 2011). It was also noticeable that while carotenoid content markedly increased in E. fractiflexa, anthocyanin content markedly increased in E. triaculeata (Table 4). These differences strongly suggest that while photoprotection in E. fractiflexa relied on operation of xanthophyll cycle, photoprotection in E. triaculeata relied more on anthocyanin accumulation. These views gain support from reports that anthocyanin accumulation under high irradiance compensates for reduced dependence of non-photochemical quenching on xanthophyll cycle pigment conversion (HATIER & GOULD, 2009; FONDOM et al., 2009).

Conclusions
It can be concluded that both E. fractiflexa and E. triaculeata are obligate CAM plants exhibiting stomatal behaviour and diurnal acidity oscillations typical of CAM plants. Low stomatal conductance both day and night and dampened acidity oscillations during dry season indicate that both species shift from obligate CAM to CAM-idling in response to protracted drought. Under severe drought, PSII activity was reduced in both species due to oxidative stress during CAM-idling with different photoprotective mechanisms operating in these two Euphorbia species. Photoprotection in E. fractiflexa relies on operation of xanthophyll cycle, while that in E. triaculeata relies more on anthocyanin accumulation.

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