Physiotypic Plasticity and Survival of Arido-active Euphorbia triaculeata (Euphorbiaceae) in its Natural Habitat

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Abstract. Leafless stem succulent *Euphorbia triaculeata* Forssk. is abundant in arid regions southwest of the Arabian Peninsula. These regions are characterized by short wet season with erratic rainfall and long dry season with high temperature and high irradiance. Field observations indicated that the plant survives the long dry season and acquires a red stem colour under high irradiance and protracted drought. Our work aimed at investigating survival of this arido-active species in its natural habitat by studying diurnal and seasonal changes in stomatal conductance, cell sap acidity, and chlorophyll fluorescence. Results showed that *E. triaculeata* is obligate crassulacean acid metabolism (CAM) plant. Under protracted drought, low stomatal conductance and dampening of CAM denoted a shift to CAM-idling. Observed stress-induced reduction of Photosystem II activity occurred in concomitance with increased non-photochemical quenching of chlorophyll fluorescence and increased anthocyanin content. These results reflected operation of a photoprotective mechanism involving interplay of non-photochemical energy dissipation via xanthophyll cycle and alleviation of oxidative stress by anthocyanin. It was concluded that *E. triaculeata* survives in its arid habitat by performing obligate CAM, shifting to CAM-idling under protracted drought, increasing non-photochemical excess energy dissipation, and accumulating anthocyanin pigment for its antioxidant attributes.

Key words: chlorophyll fluorescence, Crassulacean Acid Metabolism (CAM), CAM-idling, *Euphorbia triaculeata*, photoprotection.

Introduction

Arid regions southwest of Arabian Peninsula are characterized by high temperature, high irradiance, scarce water, and erratic rainfall (FISHER & MEMBERY, 1998). In these regions, leafless stem succulents rely on large water storage capacity and physiological adaptations including CAM pathway to improves plant water economy (SAYED, 2001a; SAYED, 2001b; LÜTTGE, 2010; MASRAHI et al. 2011; MASRAHI et al., 2012a). Although several reports indicated that stem succulent *Euphorbia* species exhibit CAM (FEAKINS & SESSIONS, 2010; AL-TURKI et al. 2014), scarce information exists on ecophysiology of *E. triaculeata* (MASRAHI, 2004). Our field observations indicated that *E. triaculeata* survives long dry seasons and acquires a red stem colour under protracted drought in arid regions southwest of Saudi Arabia. The present paper aimed at investigating existence of CAM in *E. triaculeata* by studying diurnal and seasonal changes in stomatal conductance and chlorenchyma cell sap acidity. Phase III of CAM takes place under closed stomata and high irradiance, and PSII can become over-energized

(NIEWIADOMSKA & BORLAND, 2008; LÜTTGE, 2010). Under such conditions, plants evoke photoprotection by non-radiative excess energy dissipation via xanthophyll cycle (HORTON & RUBAN, 2005; MURCHIE & NIYOGI, 2011). Therefore, our work also involved studying chlorophyll fluorescence quenching, and accumulation of anthocyanin pigment to envisage possible photoprotective mechanisms involved in mitigating harmful effects of high irradiance and drought.

Materials and methods

Study Site: Study site in Jazan Province (17°02′N - 42°54′E, and 125 m.a.s.l.) Southwest of Saudi Arabia is characterized by sand-loam soil, high temperature, high irradiance, scarce water, erratic rainfall, and climate influenced by tropical maritime air mass (MASRAHI, 2012). The dry season is nine-months-long, and the short wet season (June–August) is associated with strong sand storms (MIDDLETON, 1986).

Climatic Conditions. Climatic records (1970-2010) of mean monthly air temperature, and precipitation were obtained curtsey of Ministry of Electricity and Water (Riyadh, Saudi Arabia). During dry season, air temperature and photosynthetic photon flux density (PPFD) were monitored using field digital thermometer (Kestrel 2000, Philadelphia, USA), and PAR/LAI Ceptometer (AcuPar LP-80, Decagon, Pullman, USA), respectively.

Plant Material. Mature *E. triaculeata* Forssk. (Euphorbiaceae) plants were used as plant material.

Measurements. Stomatal conductance was measured using Porometer (AP4, Delta-T, Cambridge, UK). Pulse amplitude modulated chlorophyll fluorescence was measured using fluorometer (FMS2, Hansatech, Norfolk, UK). Measured chlorophyll fluorescence parameters included efficiency of PSII antenna (Fv/Fm), and quantum yield of PSII (ΦPSII) (BAKER, 2008). Non-photochemical quenching of chlorophyll fluorescence (qNP) was calculated using standard fluorescence

nomenclature (SAYED, 2003; BAKER, 2008) and the equation:

$$qNP = (Fm-Fm') / (Fm-Fo)$$

where: *Fo* - minimal fluorescence emitted by antenna chlorophyll molecules, *Fm* - maximal fluorescence emitted when PSII traps become closed, and Fm′: light-adapted fluorescence maximum.

Oscillation of CAM acidification was studied in chlorenchyma cell sap extracted in the field by grinding a known weight of tissue in distilled water, expressing cell sap through two layers of muslin, and determining cell sap acidity by titration against 0.01N NaOH (OSMOND *et al.*, 1991). Laboratory determination of anthocyanin pigment content involved grinding a known weight of stem chlorenchyma in ice-cold methanolic HCl (0.1% HCl v/v), determining absorbance at 532,653 nm, and calculating anthocyanin content as A532 – 0.24A653 using extinction coefficient 30000 L mol^{-1}cm^{-1} (MURRAY *et al.*, 1991). All experiments were routinely repeated in samples taken from ten different individual plants and standard error was calculated.

Results

Climatic records indicated extreme aridity of the study site reflected by a long dry season, a short wet season (June-August), and total annual rainfall of about 100 mm (Fig. 1). Monitoring daytime air temperature and PPFD during dry season indicated that these parameters can attain high midday values of 43°C, and 2025 μmol m^{-2}s^{-1}, respectively (Table 1).

During wet season, nighttime values of stomatal conductance were higher than those recorded during the day (Fig. 2). During dry season, measurements of stomatal conductance indicated that *E. triacultata* exhibited markedly low values of stomatal conductance in the range of 2-4 mmol m^{-2}s^{-1} during both day and night (Fig. 2).

Determination of chlorenchyma cell sap titratable acidity indicated that *E. triaculeata* showed evident diurnal acidity changes

during wet season, and that these changes were considerably reduced during dry season (Fig. 3).

Chlorophyll fluorescence measurements during dry season showed reduction in values of Fv/Fm, and ΦPSII compared to those measured during wet season (Fig. 4). This reduction of chlorophyll fluorescence parameters during dry season coincided with increased value of qNP and chlorenchyma anthocyanin pigment content (Table 2).

Table 1. Daytime changes in air temperature, soil temperature, and PPFD at the study site during the dry season in mid-May.

Parameters	Time of Day						
	0600	0800	1000	1200	1400	1600	1800
Air Temperature (ºC)	34	36	37	43	42	40	38
PPFD (μmol m^{-2} s^{-1})	500	1500	2000	2025	2250	2125	200

Table 2. Seasonal changes in non-photochemical quenching of chlorophyll fluorescence (qNP), and anthocyanin content of *E. triaculeata* (± se, n =10).

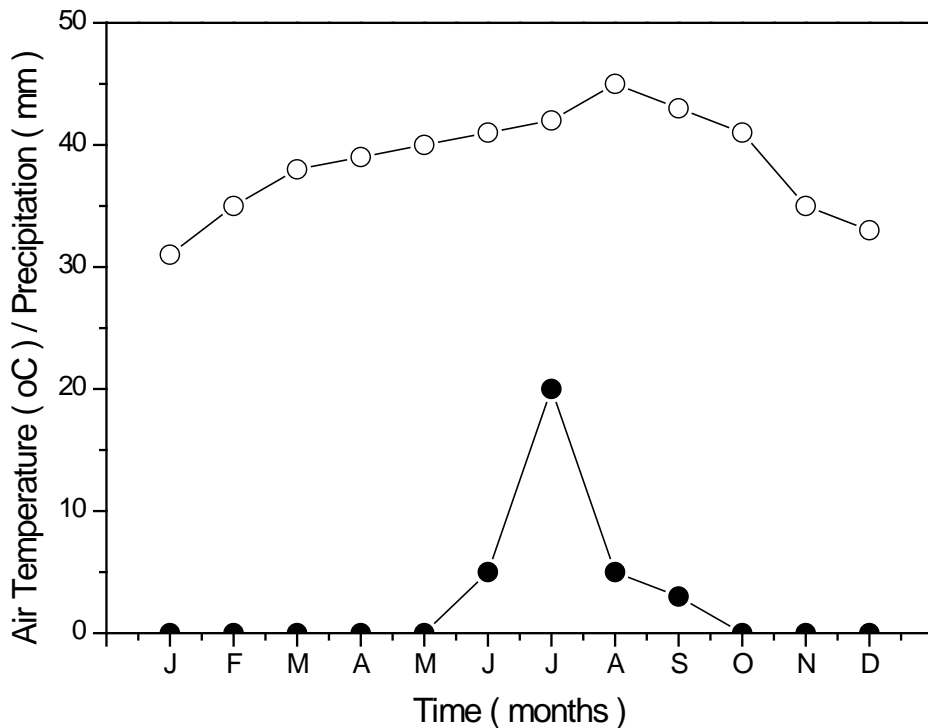| Parameter	Season	
	Wet	Dry
qNP (relative units)	0.02 ± 0.003	0.60 ± 0.005
Anthocyanin (μg g^{-1} dry weight)	150 ± 5.5	850 ± 3.8

Fig. 1. Climatic records of air temperature and precipitation at the study site.
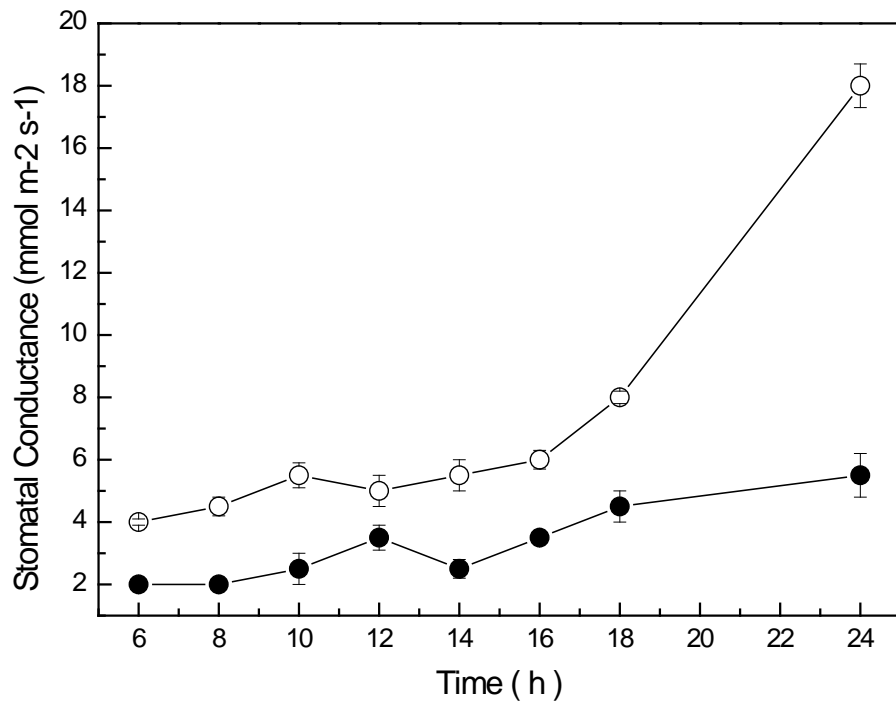
Fig. 2. Diurnal changes in stomatal conductance of *E. triaculeata* during wet season (○) and dry season (●), (± se, n = 10).
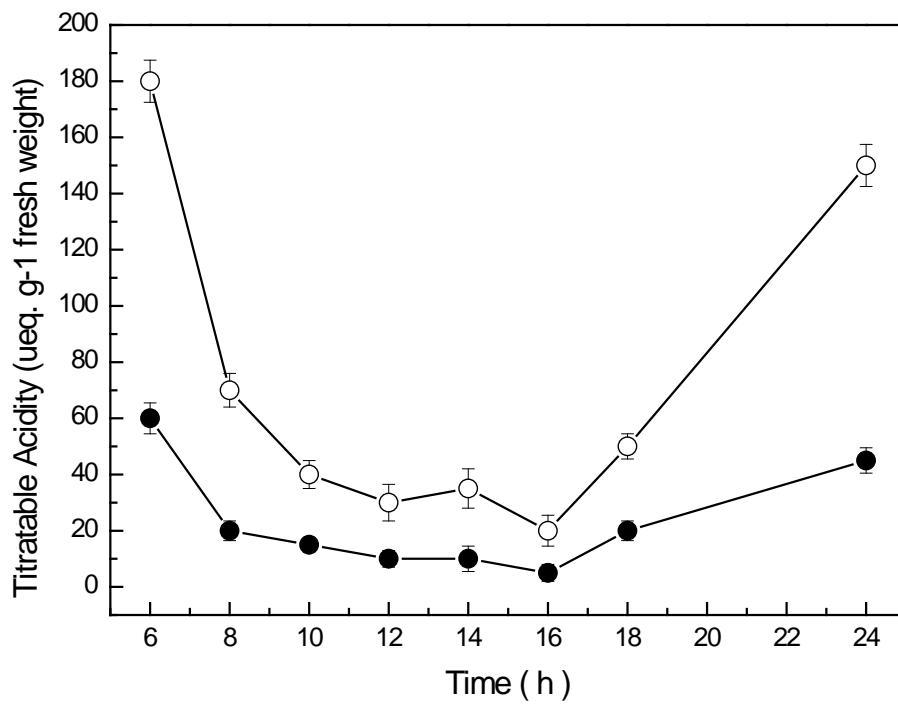

Fig. 3. Diurnal changes in titratable acidity of *E. triaculeata* during wet season (○) and dry season (●), (± se, n = 10).
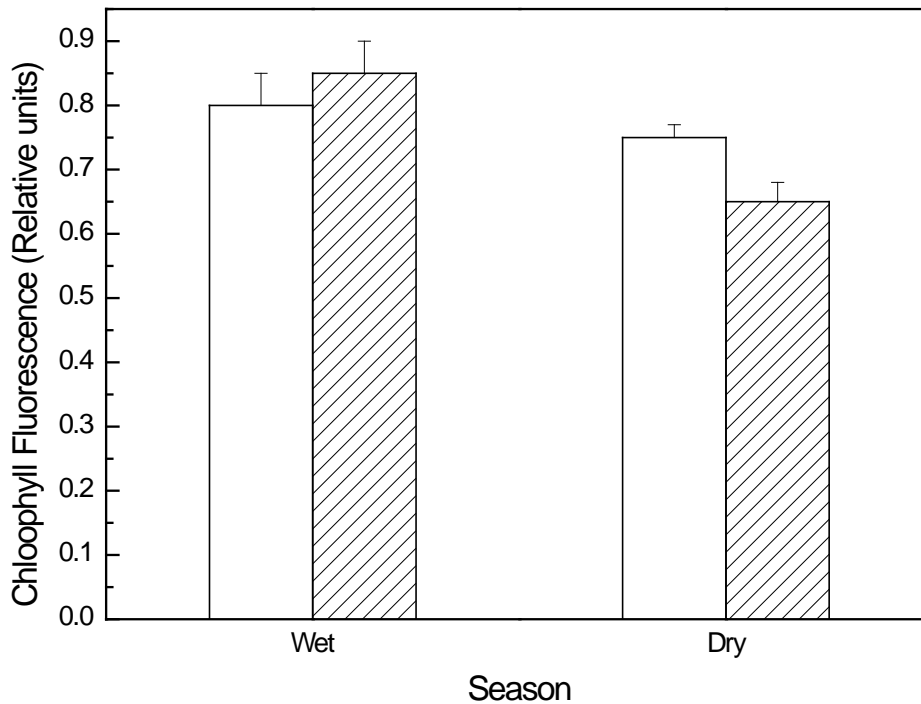
Fig. 4. Seasonal changes in chlorophyll fluorescence parameters F_V/F_m (□) and ΦPSII (■) in *E. triaculaeata.* (± se, n =10).

Discussion

Long duration of dry season and low total annual rainfall are climatic features typical of arid regions (Fig. 1). These climatic features reflect extreme aridity of study site (FISHER & MEMBERY, 1998). Measurements of daytime changes in air temperatures and PPFD during dry season showed high midday values that add to the harshness of the environment (Table 1). Such harsh conditions represent formidable challenges for plant survival in arid regions. In these regions, arido-active stem succulents survive periods of protracted drought by physiological adaptations enabling them to tolerate harsh environmental conditions (SAYED, 2001a,b; MASRAHI *et al.*, 2011; MASRAHI *et al.*, 2012a,b). During wet season, *E. triaculeata* exhibited values of stomatal conductance that were high during the night and low during the day (Fig. 2) a stomatal behavior typical of CAM plants (LÜTTGE, 2010). Determination of chlorenchyma cell sap

titratable acidity during both wet and dry seasons revealed diurnal oscillation of acidification-deacidification cycles reflecting operation of obligate CAM (Fig. 3). Obligate CAM has previously been reported in stem succulent *Euphorbia* species (WINTER *et al.*, 2005; AMEH, 2006; FEAKINS & SESSIONS, 2010, AL-TURKI *et al.* 2014). Nocturnal CO_2 uptake and daytime stomatal closure of CAM implies avoidance of gas exchange when environmental conditions favour transpirational water loss and improved plant water economy (LÜTTGE, 2008). However, during dry season, *E. triaculeata* exhibited low values of stomatal conductance during both day and night (Fig. 2) and dampened diurnal oscillation of chlorenchyma cell sap acidity (Fig. 3). These results indicated that *E. triaculeata* shifted from obligate CAM to CAM-idling in response to protracted drought. CAM-idling is a physiotypic modification of CAM that greatly improves plant water economy by day and night stomatal closure, no net CO_2

39

uptake, and diurnal acidity changes sustained by nocturnal re-fixation of respiratory CO_2 (SAYED 2001b; LÜTTGE, 2010).

Comparison of chlorophyll fluorescence parameters measured in *E. triaculeata* during wet and dry seasons indicated reduction of Fv/Fm and ΦPSII (Fig. 4) denoting reduced efficiency of PSII antenna and PSII quantum yield, respectively (BAKER, 2008). This reduction of PSII activity during dry season coincided with high values of midday air temperature and PPFD (Table 1). Similar reduction of PSII activity manifested by reduction of Fv/Fm and ΦPSII was reported for other CAM plants under stress conditions (MATTOS *et al.*, 1999). This reduction of PSII activity was attributed to over-energized PSII during Phase III of CAM that takes place under closed stomata and high irradiance (NIEWIADOMSKA & BORLAND, 2008). Our observed reduction in PSII activity occurred in concomitance with increased qNP (Table 2) reflecting increased non-photochemical quenching of chlorophyll fluorescence and increased excess energy dissipation as heat (HORTON & RUBAN, 2005). Similar observation of increased qNP during CAM-idling was reported in CAM-performing *Clusia minor* and was attributed to excess energy dissipation as heat via xanthophyll cycle (ADAMS *et al.* 2004; LÜTTGE, 2007). Xanthophyll cycle pigment epoxidation/de-epoxidation is an important element in photoprotection under stress conditions (JAHNS & HOLZWARTH, 2012). Our results also showed marked rise in chlorenchyma anthocyanin content during dry season (Table 2). Anthocyanin is thought to result in alleviation of oxidative stress by processing reactive oxygen species released when PSII becomes over-energized during Phase III of CAM and hence confers photoprotection (GOULD, 2004).

Conclusions

It can be concluded that *E. triaculeata* is obligate CAM plant with ample physiotypic plasticity. This plasticity involves shifting to CAM-idling, operation of photoprotection by non-radiative excess energy dissipation via xanthophyll cycle, and mitigating oxidative stress by increased anthocyanin pigment content. This plasticity is of pivotal importance in alleviating multiple stress during CAM-Idling caused by drought, high irradiance, and high temperature prevailing in the long dry season.

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