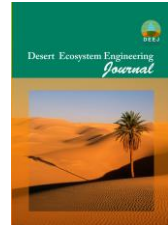




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## Comparative Functioning of Photosynthetic Apparatus and Leaf Water Potential in *Zygophyllum eurypterum* (Boiss & Bushe) During Phenological Phases and Summer Drought

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### Abstrac

**Background:** In arid regions, seasons are often marked by differences in rainfall, with life-history events, along with phenological stages. **Materials and Methods:** Three phenological phases were distinguished as vegetative phase (VP), flowering phase (FP) and seeding phase (SP). Chlorophyll fluorescence parameters (Chl. FPs) such as maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ), photochemical efficiency of photosystem II ( $\Phi_{PSII}$ ), effective quantum yield ( $F_v'/F_m'$ ), photochemical dissipation of absorbed energy (qP) and non-photochemical dissipation of the absorbed energy (NPQ) along with pigment contents and predawn leaf water potential ( $\Psi_L$ ) were determined. **Results:** All Chl. FPs changed along drought stress gradient and phenological phases, with significant changes at SP. **Discussion:** A significant change in the mentioned parameters explains the happening of severe photoinhibition because of photo-inactivation of the PSII reaction centers, or expresses thermal dispersion from the antenna pigment-protein compound. A remarkable alteration in pigment content was noticed at the SP. Decrease in the chlorophyll content under drought stress can be due to a reduction in synthesis of pigment complexes encoded by the *cab* gene family or destruction of light harvesting chlorophyll 'a' or 'b' pigment protein systems. **Conclusions:** we can say that *Z. eurypterum* can protect the PSII reaction center from damage at the middle stage of drought stress (end of July) and can be qualified as a drought tolerant species.

**Keywords:** phenophase, photoinhibition, photosystem, pigment, quenching, water deficit.

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## 1. Background

Arid regions are characterized by low annual precipitation, abundant sunshine, and high potential of evaporation rate. In these regions, plants are exposed to a wide variety of abiotic stresses, including excessive light, extreme temperatures, drought stress, and atmospheric pollutants, which can directly or indirectly affect photosynthetic function (31). In arid regions, seasons are often marked by differences in rainfall, with life-history events occurring in response to water availability (21) and these events come into view as phenological stages.

Water deficit affects many morphological features and physiological processes associated with plant growth and development. These changes include reduction of water content, diminished leaf water potential ( $\Psi_L$ ) along with turgor loss (5), closure of stomata and a decrease in cell enlargement as well as plant growth.

Several studies have demonstrated that drought stress remarkably decreases chlorophylls *a* and *b*, the total chlorophyll and carotenoids content of different crops (12). Plants with drought stress exhibit a reduction of the photochemical chlorophyll fluorescence quenching, photosystem II quantum yield and electron transport rate (ETR) and have more heat dissipation as compared to non-stressed plants (4). Declining the values of maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ) would indicate stress. Dark adapted values of  $F_v/F_m$  represent the potential quantum efficiency of the PSII and are used as sensitive indicators of plant photosynthetic performance (2). Non-photochemical quenching (NPQ) can represent the energy which cannot be applied to carry photosynthetic electrons but be scattered safely as heat energy from the PSII antennae (16).

*Zygophyllum eurypterum*, a perennial nutritive fodder shrub that colonizes arid areas in central Asia i.e., Iran, Iraq, Pakistan, Syria and Turkmenistan, is a  $C_3$  xerophyte with wonderful adaptability to antagonistic arid environments. This species has a strong ability for sand-fixing, soil and water conservation in desert regions, and possesses medicinal value (22, 9).

## 2. Objective

Very little information is available about the functioning of the photosynthetic apparatus in *Z. eurypterum* plants during their phenological phases, particularly under field conditions. Therefore, the object of this study was to evaluate the functioning of the photosynthetic apparatus and leaf water potential in *Z. eurypterum* during summer drought. For this purpose, the water relations, photosynthetic pigments and chlorophyll fluorescence yields were investigated. Furthermore, our study was focused on comparing the changes in the mentioned characteristics, as triggered by drought.

## 3. Materials and Methods

### 3.1. Representation of the study area

The study was conducted in a typical habitat of *Z. eurypterum* in Abouzeid-abad region, Kashan, Iran (33°50–33°60 N, 51°40–51°52 E, 900–950 m.a.s.l.). The annual average precipitation, based on 30-year data is 110 mm, which shows the uneven distribution in the form of storms. The region lies between 2400 and 2600 mm is a potential evaporation line and according to the ombrothermic diagram, the study area has eight to nine dry months annually. The region is mostly placed between 15 and 17 °C isotherm lines. Also, the climate of this region is extremely warm with dry summers and vegetation experiencing drought stress during most of the phenological phases.

To ease performance and precision, the habitat area was divided into three sections. Then, within each section, four quadrates (each of 20×20) were selected for sampling. The sampling points were recorded to be tested in phenological phases. Observations on phenological phases were carried out during the year 2016, from March to mid-August, where monthly observations were taken. Three phenological phases were distinguished; vegetative phase (from leaf emergence to first flowering (VP)), flowering phase (from the commencement of flowering to first capsule at full paper wings (FP)) and seeding phase (from appearance of the capsules to full physiological maturity of them (SP)) (Table 1).

**Table 1: Phenological phases of *Z. eurypterum* and its relation with the main meteorological parameters. Growth initiation (GI); vegetative phase (VP); flowering phase (FP); seeding phase (SP); hibernation (Hn). Monthly mean values of maximum ( $T_{max}$ ), minimum ( $T_{min}$ ), average ( $T_{ave}$ ) temperature, and average precipitation (P) are based on a 30-year data.**

GI												
VP												
FP												
SP												
Hn												
months	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
$P_{ave}$ (mm)	13	19	20	19	11	5	0	0	0	4	8	11
$T_{max}$ (°C)	10	11	17	22	29	37	41	41	36	30	21	14
$T_{min}$ (°C)	-1	0	5	10	15	20	24	24	20	14	9	2
$T_{ave}$ (°C)	5	6	10	16	21	28	32	32	28	20	14	8

### 3.2. Chlorophyll fluorescence analysis

Chlorophyll fluorescence yields were measured using a portable fluorometer *PAM-2500* (*H. Walz*, Effeltrich, Germany). Measurements were carried out on the uppermost quite developed leaves. Before measuring chlorophyll fluorescence yields (Chl. FYs), leaves were put in a dark-adapted state for 30 min (11) (*Genty et al.*, 1989) using light exclusion clips. The dark-adapted state situation is essential for rapid fluorescence induction kinetics and for recording Chl. fluorescence parameters.

The following Chl. FYs were measured: minimum Chl. FY in the dark and light-adapted states ( $F_0$  and  $F_0'$ ), maximum Chl. FY in the dark and light-adapted states ( $F_m$  and  $F_m'$ ), and steady state Chl. FY in the light-adapted state ( $F_s$ ) (*Zhang et al.*, 2011) (29). Some basic and mutually independent chlorophyll fluorescence parameters (Chl. FPs), such as  $F_v/F_m$ ; photochemical efficiency of photosystem II, ( $\Phi_{PSII}$ ) =  $(F_m' - F_s) / F_m'$ ; effective quantum yield ( $F_v'/F_m'$ ); photochemical dissipation of absorbed energy,  $qP$  =  $(F_m - F_m') / (F_m' - F_0)$  and non-photochemical dissipation of absorbed energy,  $NPQ$  =  $(F_m - F_m') / F_m'$  can be calculated from Chl. FYs that give insight into the photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research (26).

### 3.3. Pigment contents

Measurements of pigment content in leaves of *Z. eurypterum* plants were performed during the experimental period, from VP until SP. Analyses were done in samples collected from the same leaves upon which the chlorophyll fluorescence parameters were determined. Chlorophyll concentrations were determined according to the methodology described by José Francisco *et al.*

(15). The optical density of the extracted chlorophyll was measured at 645, 663, and 480 nm, using a spectrophotometer (U-2001-Hitachi) against 80% acetone as blank. Carotenoid content was estimated using the formula of Kirk and Allen (17). Pigment contents were calculated and expressed in milligram per gram fresh weight ( $mg\ g^{-1}\ FW$ ).

#### 3.4. Leaf water potential

Measurement of predawn leaf water potential was carried out within one hour before dawn. At that time, the plants are in balance with the soil water potential. Leaves were removed with a scalpel, put in plastic bags and transferred in a cool-box to the laboratory for further measurements. The leaf water potential ( $\Psi_L$ ) was examined using a potentiometer (Decagon, Devices, Inc., USA). Values were determined in four leaves in each quadrat on the same sprout that was utilized for finding Chl. FYs and determining chlorophyll pigments.

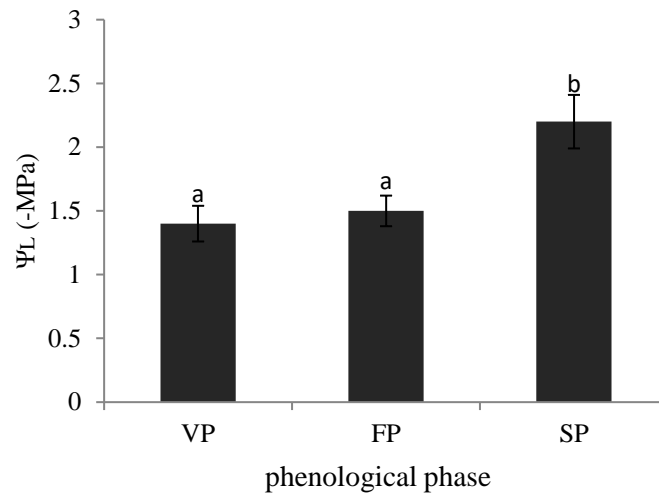
#### 3.5. Statistical analysis

The relationships among the  $\Psi_L$ , chlorophyll content and Chl. FPs were determined with linear regression analysis. Analysis of variance and Tukey's test were carried out to assay the mean differences among phenological phases.

## 4. Results

Figure 1 shows alteration in the range of  $\Psi_L$  in the study area in each phenophase. As the figure shows,  $\Psi_L$  is significantly lower in SP than VP and FP water potentials ( $\Psi_L$ ). A significant variability of  $\Psi_L$  between VP and FP was not observed. In general, the values of  $\Psi_L$  at VP and FP indicated no to weak water deficit but in SP the  $\Psi_L$  value depicted severe water deficit (Figure 1). Chl. FPs changed along the drought stress gradient and

phenological phases, and  $F_0$  increased gradually from VP to FP, with a significant increase at SP.



**Fig 1. Phenological variation in leaf water potential (-MPa) of *Z. eurypterum* plants. Values are means ± SE. Different letters in the figure indicate significant differences between treatments ( $P < 0.05$ ).**

Results of the effects of water deficit and phenological phases on the fluorescence parameters and chlorophyll content of *Z. eurypterum* plants are presented in tables 3 and 4. Also, the effects of development of the phenological phases on  $\Psi_L$  are depicted in Figure 1.

As Table 2 showed, Chl. FPs changed along drought stress gradient and phenological phases.  $F_0$  increased gradually from VP to FP, with a significant increase at SP (Table 2). A gradual reduction in  $F_m$  was observed with increasing summer drought with significantly lower  $F_m$  in SP. An increase in the summer drought that was

concomitant with the progress of phenological phases provided the decline in  $F_v$  which was the lowest in SP. The progressing of phenological stages provided the decline in the  $F_v/F_m$  ratio. Plants in VP and FP did not show a significant difference in  $F_v/F_m$ . However, extending phenological phases together with increasing summer drought resulted in significant reduction in  $F_v/F_m$  at SP.  $F_v/F_m'$ ,  $\Phi PSII$  and  $qP$  were the highest at VP and the lowest at SP. A gradual decreasing trend was observed in NPQ from VP to FP, and then a significant ascending trend emerged till SP (Table 2).

**Table 2: Parameters of chlorophyll *a* fluorescence (Chl. FPs) in *Z. eurypterum* plants measured in different phenological phases and summer water deficit. Different letters in each column represent statistically significant at  $P \leq 0.01$  (DMRT);  $n = 4$**

Chl. FPs	$F_0$	$F_m$	$F_v$	$F_v/F_m$	$\Phi PSII$	$F_v'/F_m'$	$qP$	NPQ
<b>Phenophase</b>								
VP	330 <sup>a</sup>	1750 <sup>a</sup>	1420 <sup>a</sup>	0.810 <sup>a</sup>	0.46 <sup>a</sup>	0.77 <sup>a</sup>	0.64 <sup>a</sup>	0.9 <sup>a</sup>
FP	342 <sup>a</sup>	1736 <sup>a</sup>	1394 <sup>a</sup>	0.803 <sup>a</sup>	0.44 <sup>a</sup>	0.76 <sup>a</sup>	0.67 <sup>a</sup>	0.8 <sup>a</sup>
SP	420 <sup>b</sup>	1418 <sup>b</sup>	998 <sup>b</sup>	0.702 <sup>b</sup>	0.33 <sup>b</sup>	0.66 <sup>b</sup>	0.43 <sup>b</sup>	1.3 <sup>b</sup>
<b>ANOVA</b>								
<i>F</i>	30.961	291.814	386.963	101.060	34.889	22.800	34.889	18.129
<i>MS</i>	12281.67	211792	0.019	0.019	0.021	0.018	0.068	0.280
<i>P</i>	0.000 <sup>***</sup>	0.001 <sup>**</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>

The results on the effects of gradual water deficit on the pigment parameters in leaves of *Z. eurypterum* plants are presented in Table 3. A

remarkable alteration in pigment contents of the leaf was not noticed with increasing summer

drought stress up to FP, thereafter, it significantly declined in SP.

The effects of phenophase development along with the summer drought stress on chlorophyll content were evident only in SP, in which the plants showed 50%, 19% and 41% reductions in Chl. *a*, *b*, and *a + b* (Table 3), respectively, compared to plants in VP. In addition, the content of Chl. *b* was lower than Chl. *a*, but it achieved the same trend; being higher in VP than in SP. The Chl. (*a+b*) showed the same trend as chlorophyll *a*. FP showed higher chlorophyll *a + b* /Car ratio than

the SP.

The significant effects of phenophase development on carotenoid content were evident in SP, in which a 125% increase was observed in comparison to the plants in VP (Table 3). Phenophase development along with soil water depletion led to reduction in predawn  $\Psi_L$  in all the studied plants (Figure 1). A remarkable alteration in  $\Psi_L$  was not noticed with increasing summer drought stress up to FP and thereafter, it significantly declined in SP (-2.4 MPa).

**Table 3: Concentration of photosynthetic pigments (mg g<sup>-1</sup>) in leaves of *Z. eurypterum* plants measured in different phenological phases and summer water deficit.**

pigment content	Chl. <i>a</i>	Chl. <i>b</i>	Chl. ( <i>a+b</i> )	Chl. ( <i>a/b</i> )	Car	Chl. ( <i>a+b</i> ) / car
<b>phenophase</b>						
VP	31.60 <sup>a</sup>	12.60 <sup>a</sup>	44.20 <sup>a</sup>	2.51 <sup>a</sup>	23.60 <sup>a</sup>	1.88 <sup>a</sup>
FP	29.10 <sup>a</sup>	13.20 <sup>a</sup>	42.30 <sup>a</sup>	2.23 <sup>a</sup>	21.50 <sup>a</sup>	1.99 <sup>a</sup>
SP	15.90 <sup>b</sup>	10.20 <sup>b</sup>	26.10 <sup>b</sup>	1.56 <sup>b</sup>	29.40 <sup>b</sup>	0.91 <sup>b</sup>
<b>ANOVA</b>						
<i>F</i>	180.707	7.293	161.842	62.352	5.758	52.165
<i>MS</i>	678.253	10.080	846.973	3.492	50.40	2.213
<i>P</i>	0.000 <sup>***</sup>	0.013 <sup>**</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>

Different letters in each column is statistically significant at  $P \leq 0.01$  (DMRT);  $n=4$

All measurements of pigment content and fluorescence parameters were significantly correlated with leaf water potential (Figure 2). Total chlorophyll content (*a + b*), Chl. *a*,  $\Phi$ PSII and  $F_v/F_m$  were more highly and negatively correlated with  $\Psi_L$ . The values of NPQ were significantly and positively correlated with  $\Psi_L$  ( $R^2 = 0.665$ ;  $P < 0.01$ ). Among the mentioned parameters, the highest correlation was observed between  $\Psi_L$  and Chl. *a* and,  $\Phi$ PSII, respectively (Figure. 2d, b).

#### 4. Discussion

The inhibitory effects of water deficit stress on the photosynthetic apparatus function have widely been reported. Changes of the fluorescence parameters under adverse conditions can be attributed to at least two different phenomena. The first phenomenon brings about an increased  $F_0$  and the second phenomenon is responsible for quenching of  $F_m$ . In the present study, all plants showed significant increase in  $F_0$  and decrease in  $F_m$  at SP. An increase in  $F_0$ , possibly due to the

reduced plastoquinone acceptor ( $Q_A$ ), cannot be oxidized completely because of retardation in the electron flow through the PSII, or to damage protein complexes of the PII (29). In several reports, decrease in  $F_m$  has been attributed to the stagnation of the water splitting enzyme system, causing inhibition of photolysis of water and perhaps a concomitant cyclic electron flow around the PSII, or increased non-radiative energy dissipation (32; 30; 27).

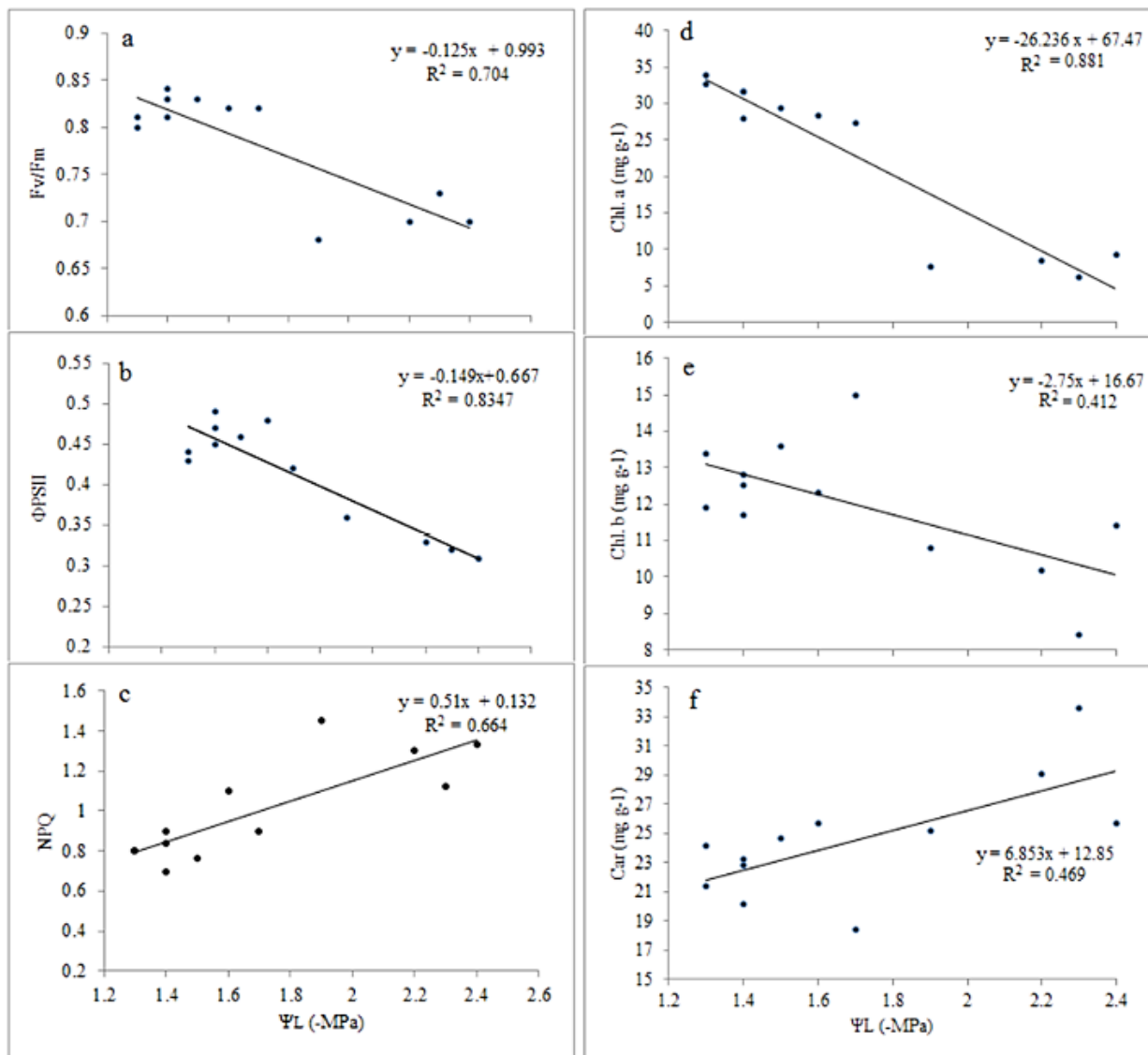
In our studied plants, the increase in  $F_0$  and decrease in  $F_m$  under summer drought stress was simultaneous with the decrease in  $F_v/F_0$  (Table 2). This phenomenon can be attributed to the occurrence of severe photoinhibition due to photo-inactivation of the PSII centers, possibly ascribable to D1 protein damage (25). Also, we found that there is a strong correlation (with a negative slope) between  $F_v/F_m$  ratio and  $\Psi_L$  ( $r^2=0.7$ ) (Figure 2a). Similar effects of drought stress on  $F_v/F_0$  ratio have been reported by Zlatev and Yordanove (32) on bean plants and Ranjbar (27) on *Smirnovia Iranica* seedlings. An increase in summer drought concomitant with the progress of phenological

phases provided the decrease in  $F_v'/F_m'$  ( $P < 0.05$ ). This parameter is known as Genty parameter. It represents the conversion efficiency of the energy of electrons by open reaction centers of the PSII into chemical energy and affects the relative  $\Phi_{PSII}$  during steady-state photosynthesis (3). Our findings on  $F_v'/F_m'$  are consistent with those previously reported by Liu *et al.* (20) in two maize cultivars.

In the present study,  $\Phi_{PSII}$  decreased with increasing drought stress levels, and the decrease was significant in SP. This result implies that the photosynthetic apparatus PSII of *Z. eurypterum* plants leaves was damaged and lost its heat dissipation capability. Our findings are in agreement with those of Li *et al.* (19), who reported a decline in  $\Phi_{PSII}$ , which displays the efficiency of

light energy transformation in PSII; specially, it indicates the efficiency of primary light capture when the PSII reaction center is partially shut down. The relationship between  $\Phi_{PSII}$  and  $\Psi_L$  in SP was significant and had a negative slope (Figure 2b). This agreed with the findings of Alves *et al.* (1), who demonstrated that  $\Phi_{PSII}$  decreased significantly in drought stressed in grapevines compared to non-stressed ones (Figure 2b).

In the present study, a decrease of the qP was observed in response to the summer drought stress and development of phenological phases, indicating that a large section of the PSII reaction centers was damaged. It also indicated that the balance between excitation rate and electron transfer rate had collapsed (7).



**Fig 2: Correlation among  $F_v'/F_m'$ (a),  $\Phi_{PSII}$  (b), NPQ (c), Chl. a (a), Chl. b (b) and Car (c) on the  $\Psi_L$  at different phenological phases along with the development of summer drought**

A significant increase in non-photochemical fluorescence quenching (NPQ) was observed in SP (Table 2) which reflects expresses thermal dispersion from the antenna pigment-protein complex. Also, NPQ showed a significant relationship with  $\Psi_L$  (Figure 2c). The results agree with those of (13) Hailemichael *et al.* (2016), who exhibited a strong increase in NPQ as the drought developed.

Summer water deficit stress reduced chlorophyll contents in SP (Table 3). Drought not only causes a substantial loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (18). Decrease in the chlorophyll content under drought stress is a common phenomenon and has been reported in several studies (28; 27; 8). Our results are in agreement with those of Din *et al.* (6).

The decrease in chlorophyll under drought stress might be due to reduction in synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (24) or destruction of the light harvesting chlorophyll 'a' or 'b' pigment protein complexes which protect the photosynthetic apparatus (10), and oxidative damage of chloroplast lipids, pigments and proteins (23). The relationships of pigment contents with  $\Psi_L$  in SP were significant and had negative slope (Figure 2d, e), agreeing with Hailemichael *et al.* (13) findings on grapevine. On the other side, increase in carotenoids content was observed (Figure 2f) and this may be related to a defensive response to reduce the detrimental effects of drought stress (10). Further, the high level of carotenoids at SP can be attributed to drought tolerance of the species which has been reported by Hussein *et al.* (14).

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## 5. Conclusion

In this study, the chlorophyll fluorescence parameters and the pigments involved in the photosynthetic function of *Z. eurypterum* were quantified. The progressive soil water deficit concomitant with development of phenological phases resulted in lower  $\Psi_L$  value. All the fluorescence variables such as  $F_m$ ,  $F_v$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$  and  $qP$  showed an alteration trend when leaf water potential decreased. Progressive drought stress significantly reduced pigment content and increased Car contents. As a result, the  $Chl. (a + b)/Car$  ratio was  $> 1$  at VP and FP, while it was  $< 1$  at SP. Generally, *Zygophyllum eurypterum* can protect the PSII reaction center from damage in the middle stage of drought stress (end of July) and can be qualified as a drought tolerant species.

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## Conflict of Interest

The author has no conflict of interest.

## Author's Contribution

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