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Analysis of drought response of sunflower inbred lines by chlorophyll *a* fluorescence induction kinetics

Ö. ARSLAN[*], A.S. BALKAN NALÇAİYİ[**,***], Ş. ÇULHA ERDAL[**], V. PEKCAN[#], Y. KAYA[##], N. ÇİÇEK[**], and Y. EKMEKÇİ[**,+]

Department of Food Processing, University College of Espiye, University of Giresun, 28600 Giresun, Turkey[*]
Hacettepe University, Faculty of Science, Department of Biology, 06800 Beytepe, Ankara, Turkey[**]
Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, 42090 Meram, Konya, Turkey[***]
Trakya Agricultural Research Institute, PO Box 16, 22100 Edirne, Turkey[#]
Trakya University, Faculty of Engineering, Genetics and Bioengineering, Ahmet Karadeniz Campus, Edirne, Turkey[##]

Abstract

The aim of the study was to screen nine inbred lines of sunflower by inducing drought for 10 d and subsequent rewatering for 5 d. Impact of drought was determined by chlorophyll fluorescence and some physiological parameters. Drought led to a decrease in the photosynthetic performance, the quantum yield, and efficiency of electron transport in sunflower lines, while it caused an increase in the absorption flux per reaction centre, dissipation of an active reaction centre, and K-band as well as L-band. Drought also decreased the total chlorophyll contents and water status of the lines, which contributed to photoinhibition. Our results suggested that drought may restrict light harvesting and electron transport in the sunflower lines at various levels. Drought did not cause irreversible membrane damage, since the lines recovered after rewatering. Considering all results, the inbred lines TT317-R and 2478-A were adversely affected by drought when compared to other lines, while 9753-2R exhibited better photosynthetic performance under drought and might be considered as the most tolerant among the lines.

Additional key words: *Helianthus annuus* L.; JIP-test; OJIP transient; water deficit.

Introduction

Limited water is among the most commonly encountered problems under field conditions that reduce the growth and productivity of crops. Thereby, the major goal of the plant breeders is to improve crop productivity and to select the drought-tolerant genotypes (Rauf *et al*. 2016). Plant breeders screen germplasm on the basis of multiplicity of traits and selection criteria in various crops species including sunflower (Hussain *et al*. 2019).

Sunflower (*Helianthus annuus* L.) is one of the most important and oldest edible oilseed crops in the world, especially in Turkey. As a summer crop, sunflower frequently suffers from drought stress leading to crop yield losses (Rauf 2008). Selection for drought-tolerant genotypes under targeted local environment has been proposed as breeding strategies (Rauf *et al*. 2016). A tolerant genotype might be able to maintain vital plant

metabolism as well as the rapid recovery of water status after rehydration (Falqueto *et al.* 2017). Due to lack of information about the physiological characters reflecting the full genetic potential and their relationship with yield and morphological traits, the development of drought tolerant genotypes of field crops is complicated (Zakhidov *et al.* 2016). Furthermore, physiological responses of plants to limited water are notably variable and depend on plant genotype/species, along with the severity and duration of stress (Guo *et al.* 2018).

Responses to drought are multiple and interconnected. Drought stress impairs numerous metabolic and physiological processes in plants (Macar and Ekmekçi 2008, Guo *et al.* 2018, Zheng *et al.* 2019). It leads to a growth reduction, reduction in the content of chlorophyll (Chl) pigments and water, and changes in photosynthetic activity. It has deteriorating impacts on photosynthesis such as decreased PSII photochemical activity and damaged PSII reaction centres. The damage to PSII was caused by the formation of reactive oxygen species, which can lead to membrane damage and deterioration of membrane integrity (Nishiyama *et al.* 2006). Chl *a* fluorescence (ChlF) measurements have been widely used to detect response of photosynthetic apparatus under stress conditions in wide range of species due to its simple, reliable, nondestructive, and rapid testing technique (Boureima *et al.* 2012, Çiçek *et al.* 2015, 2018; Kaya *et al.* 2016, Umar *et al.* 2019). ChlF measurements allow determining the physiological status of plants based on the detection of changes in PSII components, electron transport chain, and light-dependent photochemical reactions (Lotfi *et al.* 2018). The recorded signals from ChlF can be analyzed by the JIP (OJIP) test to calculate specific biophysical parameters, quantum yields, and probabilities characterizing structure and function of PSII (Strasser *et al.* 2004, 2010; Kalaji *et al.* 2014a,b). The JIP-test was potentially valuable tool for estimating the effects of drought on photosynthetic electron transport (Goltsev *et al.* 2012). Numerous studies have shown that determination of the photosynthetic activity was very informative to find out the drought tolerance of plants (Oukarroum *et al.* 2007, Saglam *et al.* 2011, Boureima *et al.* 2012, Ceppi *et al.* 2012, Goltsev *et al.* 2012, Brestič and Živčák 2013, Živčák *et al.* 2013, 2014; Çiçek *et al.* 2015, Kalaji *et al.* 2016, 2018a,b; Kaya *et al.* 2016, Falqueto *et al.* 2017, Semerci *et al.* 2017, Stirbet *et al.* 2018, Khatri and Rathore 2019).

This study was a part of breeding efforts to develop drought-tolerant hybrid varieties in Turkey. As a first step, 50 maintainer and restorer lines were screened to detect tolerant lines under field conditions (Pekcan *et al.* 2015, Kaya *et al.* 2016). Screened material was used to develop 26 sunflower hybrids that were tested under drought conditions at three different growth stages under field conditions (Çiçek *et al.* 2019). Finally, nine inbred lines were selected to perform detailed analyses under controlled conditions. The objectives of this study were to screen nine inbred lines and to elucidate the drought tolerance and recovery of these lines in terms of the structural and functional integrity of photosynthetic apparatus with ChlF measurements under controlled conditions.

Materials and methods

Plant material and treatments: The experiment was performed using seeds of four male (R) (9753-2R, 9702-R, TT317-R, and 8129-R) and five female (A) (9725-A, 9718-A, 9814-A, 9209-A, and 2478-A) sunflower (*Helianthus annuus* L.) inbred lines which were developed by Trakya Agricultural Research Institute. Three seeds of each line were planted in 1-L pots holding air-dried soil (108 pots were used for three replicates of four treatments of nine lines) and some characteristics of the soil were as follows: texture – clay; water-holding capacity of 19.5%; pH 8; electrical conductivity of 0.54 dS m^{-1}; total nitrogen (N): 0.06%; phosphorus (P): 7.6 ppm and potassium (K): 315 ppm. Since N and P contents of the soil were not sufficient, 100 µg(NH$_4$NO$_3$) g^{-1} and 50 µg(KH$_2$PO$_4$) g^{-1} were added to the soil. Plants were thinned to twin seedling per pot after 7 d from sowing (DAS). Plants were grown in a controlled growth chamber for 40 DAS under well-watered conditions at $25 \pm 1°C$ with a 16-h photoperiod at $40 \pm 5\%$ humidity, and light intensity of 250 µmol(photon) m^{-2} s^{-1}. Drought treatment imposed *via* withholding water for 10 d was initiated at 40 DAS, while 10-d stressed plants at 50 DAS were rewatered for 5 d (55 DAS). Control and rewatered plants were irrigated with tap water every other day and the soil was kept at water-holding capacity until harvesting. Measurements were made at the end of each treatment and the representative leaves, which were at the middle of plants, were sampled for ChlF measurements and the same leaves were used in the physiological analyses.

Chl fluorescence: ChlF measurements were performed using the *Handy PEA (Hansatech Instruments Ltd.*, Norfolk, UK) fluorimeter on selected fully expanded leaves. Following a 30-min dark adaptation, samples were illuminated with continuous light [650 nm peak wavelength; maximum light intensity of 3,000 µmol(photon) m^{-2} s^{-1} for 1 s] provided by three LEDs. The fast fluorescence kinetics (F_0 to F_M) was recorded from 10 µs to 1 s. The fluorescence intensity at 20 µs (F_0), 100 µs, 300 µs, 2 ms (F_J), 30 ms (F_I), and maximum fluorescence (F_M) were recorded (Strasser *et al.* 2004). Photoinduced Chl fluorescence transients (OJIP), flux ratios or quantum yields (per absorption flux, ABS or antenna Chls), and specific energy fluxes (per reaction centre, RC), parameters, and photosynthetic performance indexes were calculated and specific characteristics of the light phase of photosynthesis were analyzed according to the JIP-test described in detail by Strasser *et al.* (2004, 2010). The differences between lines in response to drought and rewatering were elucidated by the relative fluorescence between the steps O and K [20 and 300 µs, respectively; $V_{OK} = (F_K - F_0)/(F_M - F_0)$], O and J [20 µs and 2 ms, respectively; $V_{OJ} = (F_J - F_0)/(F_M - F_0)$], and I and P [30 ms and 300 ms, respectively; $V_{IP} = (F_M - F_I)/(F_M - F_0)$] were calculated (Strasser *et al.* 2004, Oukarroum *et al.* 2015). The driving force (DF = logPI$_{ABS}$) of photosynthesis and its three partial components log(10 RC/ABS), log[$\varphi_{P0}/(1 - \varphi_{P0})$], and log[$\Psi_0/(1 - \Psi_0)$] were calculated according to Boureima *et al.* (2012). The relative deviation of PI$_{Total}$ (ΔPI$_{Total}$) values, calculated as ΔPI$_{Total\ Relative} = [(PI_{Total\ Control} -$

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PI$_{\text{Total Treatment}}$)/PI$_{\text{Total Control}}$] × 100 (Boureima *et al.* 2012 with minor modifications).

Chl and carotenoid (Car) contents and relative leakage ratio (RLR): Content of Chl (*a+b*) and Car (*x+c*) was calculated using adjusted extinction coefficients (Lichtenthaler 1987). For measuring the leakage, discs of leaves (0.5 cm in diameter) were measured at 80 nm using a UV-vis spectrophotometer (*Mini-1240*, *Shimadzu*, Japan), 24 h before and after incubation in liquid nitrogen for 20 min (Redmann *et al.* 1986). Relative leakage ratio (RLR) was calculated as A$_{280}$/A$_{280}$', where A$_{280}$ and A$_{280}$' are first and second measurements, respectively.

Leaf water potential (Ψ_{WL}) and soil water potential (Ψ_{WS}): Ψ_{WL} and Ψ_{WS} were measured using the *WP4* (*Decagon Devices Inc.*, Pullman, WA, USA) water potential meter.

Statistical analysis: The experiment was performed in a completely randomized design with three replicates for each line and treatment and differences in measured variables between lines and treatments were analyzed by analysis of variance (*ANOVA*) and according to the least significant difference (LSD) at the 5% level. Principal component analysis (PCA) was used to analyze the data pattern and classification of inbred lines. The PCA was performed by eigenvalue decomposition of a data correlation matrix. All the analyses were performed using the *IBM SPSS Statistics* for *Windows, v. 20* (IBM Corp., Armonk, USA).

Results

The shape of the OJIP fluorescence transients in drought-treated lines differed from those of their controls and rewatered plants (Fig. 1*A*). Lines 9209-A, 9702-R, and 2478-A were adversely affected by drought than the other lines (Fig. 1*B–D*). These sunflower lines showed much lower I–P amplitude compared to control that exhibited typical OJIP curve. Exposure to drought resulted in enhancement of both V$_{OK}$ and V$_{OJ}$ values of all lines (Fig. 1*B,C*). The highest increase for V$_{OK}$ and V$_{OJ}$ parameters was determined in TT317-R (90.9%) and 9702-R (56.8%), while the lowest enhancement was found in 9753-2R for both parameters (14.3 and 8.6%, respectively) (Table 1S, *supplement*). Drought adversely influenced the I–P amplitude of polyphasic fluorescence rise in all lines (Fig. 1*D*). The highest and the lowest decrease of V$_{IP}$ value was observed in TT317-R (52.2%) and 9753-2R (15%), respectively (Table 1S). Rewatering treatment resulted in recovery almost up to control values of lines.

All JIP parameters, which are represented by spider plot graphics (Fig. 2), are relative values of the fully hydrated leaf. Water deficit had a significant effect on all ChlF parameters as quantum yields (flux ratio) and specific energy fluxes (Fig. 2). The maximum quantum yield of primary photochemistry of PSII ($\varphi_{P0} = TR_0/ABS = F_V/F_M$) of lines decreased significantly after exposure to drought, except 9753-2R (Fig. 2*A*). The highest decrease was determined in 9702-R and 9725-A as 6% of related controls (Table 1S). After rewatering, φ_{P0} values of most of the lines reached
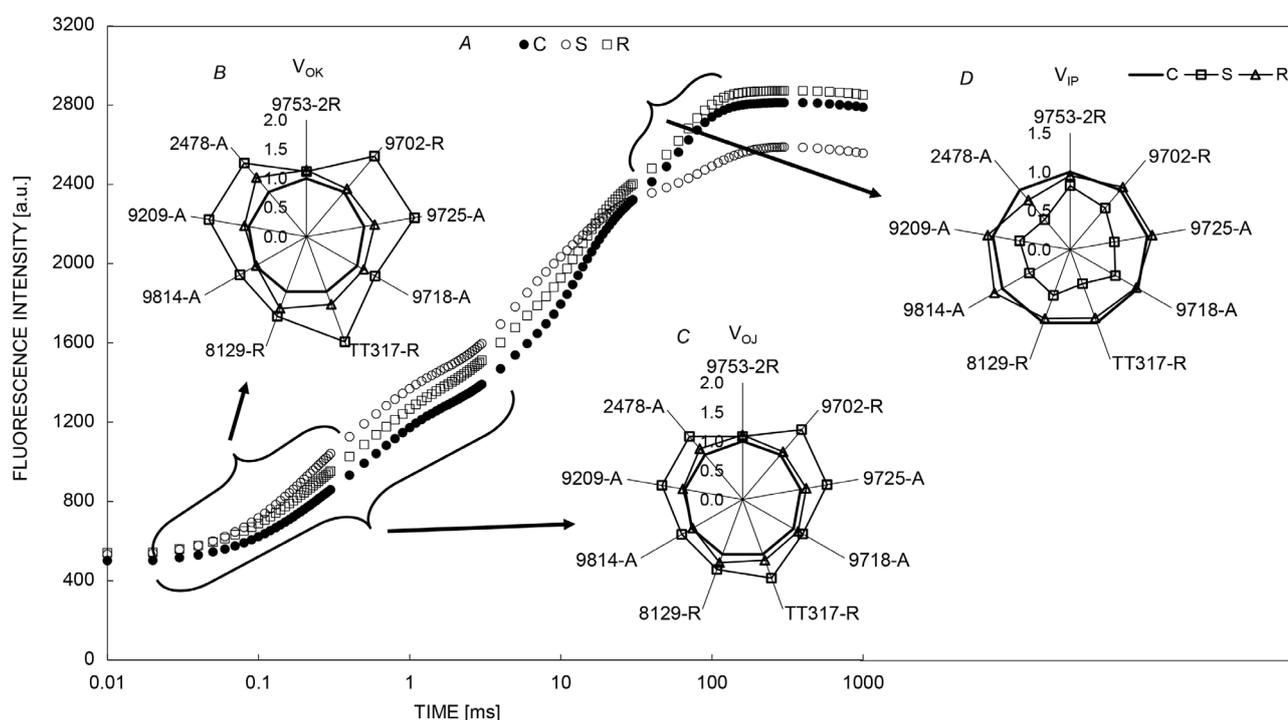
Fig. 1. The OJIP-transients of ChlF in sunflower lines exposed to drought (S) and rewatering (R) compared to control (C) (*A*). The radar plot presentation of relative fluorescence between the steps O (20 µs) and K (300 µs) (V$_{OK}$, *B*), relative fluorescence between the steps O (20 µs) and J (2 ms) (V$_{OJ}$, *C*), and relative fluorescence between the steps I (30 ms) and P (300 ms) (V$_{IP}$, *D*) for drought-stressed (S) and rewatered (R) lines of sunflower. Mean values of parameters were plotted relative to their respective controls (C) for two plants from each pot (*n* = 6).

Fig. 2. The radar plot presentation of maximum quantum yield of PSII photochemistry (φ_{P0}, *A*), quantum yield for electron transport at t = 0 (φ_{E0}, *B*), quantum yield of electron transport from Q_A^- to the PSI end electron acceptors (φ_{R0}, *C*), absorption flux per reaction center (ABS/RC, *D*), dissipation energy flux per RC (DI_0/RC, *E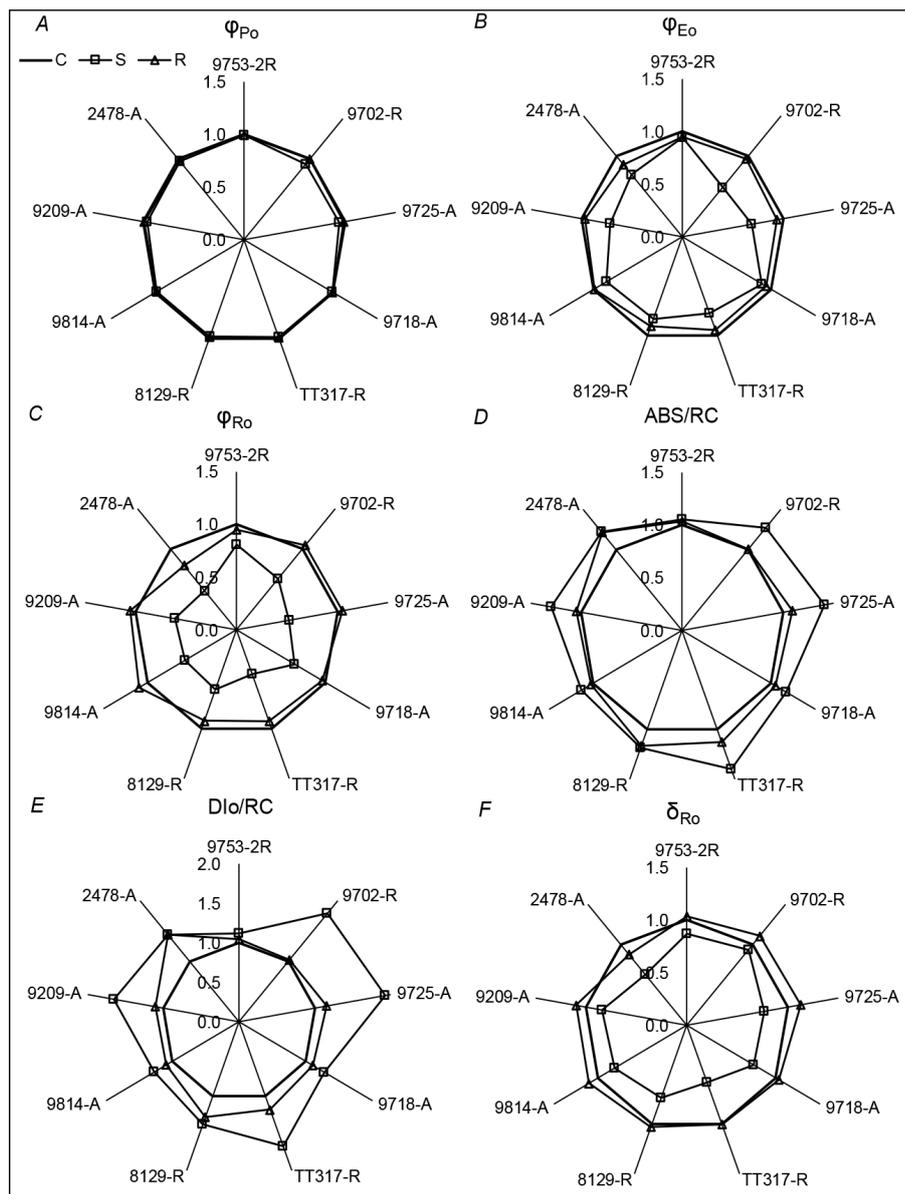*), and probability with which an electron from the intersystem electron carriers move to reduce end electron acceptors at the PSI acceptor side (δ_{R0}, *F*) of lines of sunflower exposed to drought (S) and rewatering (R). Mean values of parameters were plotted relative to their respective controls (C).

to control values, except for 2478-A (3.6%) (Fig. 2*A*, Table 1S). The φ_{E0} value, which represents the quantum yield efficiency with which a trapped exciton can move an electron to the electron transport chain ($\varphi_{E0} = ET_0/ABS$), declined markedly in all lines due to drought treatment, especially in 9702-R (39%), and the highest decline of φ_{E0} was determined in 9209-A (13.7%) and 2478-A (9%) during rewatering treatment (Fig. 2*B*) (Table 1S). Drought reduced the quantum yield with which electrons reduce the PSI end-electron acceptors ($\varphi_{R0} = RE_0/ABS$) in all lines to a remarkably greater extent; the highest decline was in TT317-R (52.6%) and 2478-A (52.2%), while the lowest in 9753-2R (18.8%) (Table 2S, *supplement*). On the other hand, only one of the lines, 2478-A, could not recover its φ_{R0} value to control level after rewatering treatment (22.7% decline) (Fig. 2*C*) (Table 2S). Drought treatment led to an increase of both absorption flux per reaction centre

(ABS/RC) and dissipation energy flux per reaction centre (DI_0/RC) values in all lines, except 9753-2R (Fig. 2*D*,*E*). The line 9725-A exhibited the highest enhancement of ABS/RC and DI_0/RC as 42.9 and 92.9% of related controls at drought treatment, respectively. Rewatering treatment increased the ABS/RC of several lines; the lines 8129-R and 2478-A had the highest values (20% for both) (Table 2S). The parameter δ_{R0} ($\delta_{R0} = RE_0/ET_0$), which refers to probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side, decreased by drought in all lines, except 9702-R, and the remarkable decrease was determined in TT317-R (42%) (Fig. 2*F*). Rewatering affected δ_{R0} differently; δ_{R0} values of 2478-A decreased significantly (14.3%), whereas δ_{R0} of 9725-A increased (12.9%) (Fig. 2*F*) (Table 2S).

Drought stress adversely effected the driving force

(DF = $logPI_{ABS}$) of photosynthesis and its three partial components $log(10RC/ABS)$, $log[\varphi_{P0}/(1 - \varphi_{P0})]$, and $log[\Psi_0/(1 - \Psi_0)]$ of nine sunflower lines (Fig. 3). $logPI_{ABS}$ declined significantly for all lines, except 9753-2R and the remarkable decrease was determined in both 9702-R (40%) and 9725-A (43.8%) (Table 3S, *supplement*). The density of active photosystems (10RC/ABS) in all lines, except 9753-2R, decreased significantly and mostly in 9725-A (20.9%). The efficiency of primary photochemistry or trapping $[\varphi_{P0}/(1 - \varphi_{P0})]$ in all lines declined significantly at drought except in 9753-2R; the highest decrease was in 9702-R and 9725-A (29.5% for both). The decline in PI_{ABS} was related to a significant decrease in electron transport component activity $[\Psi_0/(1 - \Psi_0)]$ of all lines, except 9753-2R. Marked decreases were determined in 9702-R (165%), 9725-A (103.7%), and 9209-A (108%) at drought treatment (Fig. 3, Table 3S).

Total performance index (PI_{Total}) indicates energy conservation from exciton to the reduction of PSI end acceptors $[PI_{Total} = PI_{ABS} \varphi_{R0}/(1 - \varphi_{R0})]$. PI_{Total} of all lines declined significantly at drought and the lowest decline was found in 9753-2R (38.9%), while the highest was observed in TT317-R (84.4%) (Table 4S, *supplement*).

The relative deviation of PI_{Total} (ΔPI_{Total}) values showed that PI_{Total} was destructively affected by drought treatment in all lines, especially in lines which had $\Delta PI_{Total} \geq 50\%$ (Fig. 4). However, ΔPI_{Total} value of 9753-2R was lower than 50% at drought treatment. Rewatering led to the significant decrease of PI_{Total} in 9753-2R (14%) and 9702-R (23.5%) and increase in 9718-A (16.7%) and TT317-R (18.5%) (Table 4S). $\Delta PI_{Total} < 0$ values were determined in 9753-2R and 9814-A at rewatering treatment (Fig. 4).

Drought significantly reduced Chl ($a+b$) content of TT317-R, 8129-R, and 2478-A (17, 7, and 30%, respectively) (Table 1). Similarly, the rewatering treatment resulted in the decline of Chl ($a+b$) content of 9725-A and TT317-R (20 and 33%, respectively). On the contrary to Chl ($a+b$) content, Car contents of the lines were variable (Table 1). Car contents of 9753-2R (25%), 9702-R (57%), 9725-A (37.5%), 9718-A (25%), 8129-R (33%), and 9814-A (37.5%) increased significantly at drought, while the contents of TT317-R and 9209-A remained unchanged and 2478-A declined slightly (11%). At rewatering treatment, the Car content of most of the lines did not change significantly. The Car contents of 9725-A (12.5%),
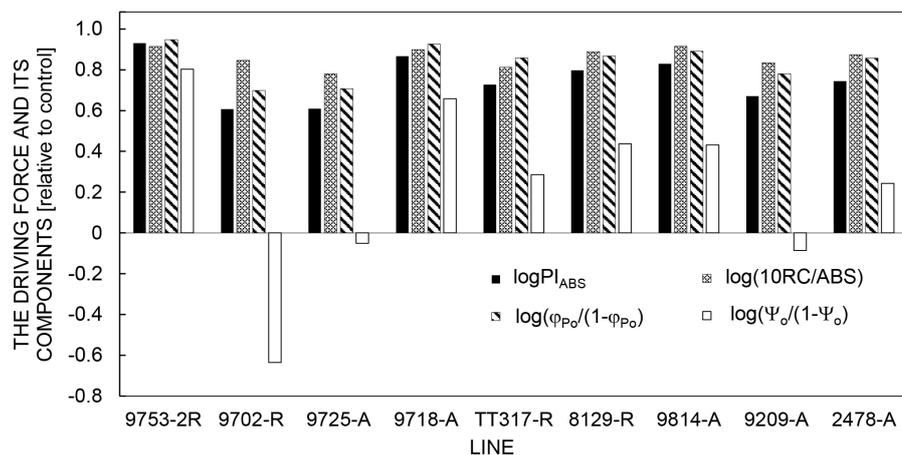
Fig. 3. The driving force (DF = $logPI_{ABS}$) of photosynthesis and its three partial components $log(10RC/ABS)$, $log[\varphi_{P0}/(1 - \varphi_{P0})]$, and $log[\Psi_0/(1 - \Psi_0)]$ of drought-stressed sunflower lines. Mean values of parameters were calculated relative to their respective controls.
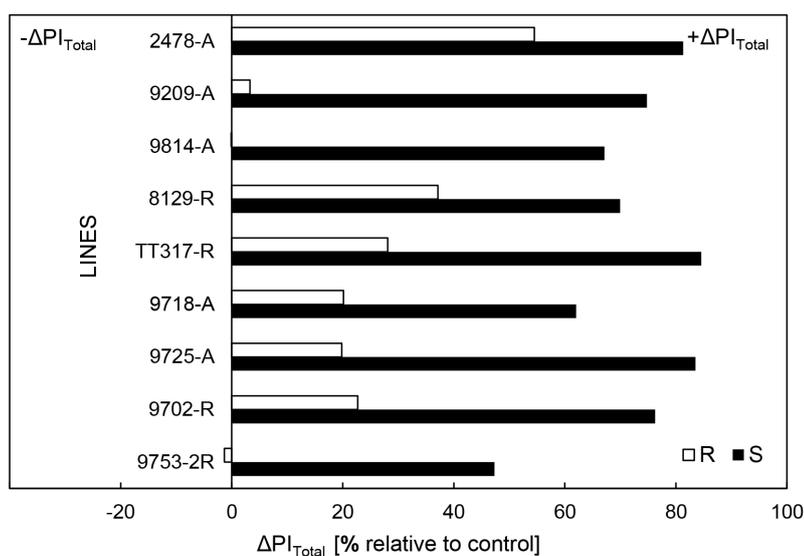
Fig. 4. Relative deviation of the total performance index (PI_{Total}) in percent of control of each sunflower line exposed to drought (S) and rewatering (R), calculated as $\Delta PI_{Total\ Relative} = [(PI_{Total\ Control} - PI_{Total\ Treatment})/PI_{Total\ Control}] \times 100$ according to Boureima *et al*. (2012) with minor modifications.

Table 1. The Chl ($a+b$) [mg cm^{-2}], Car [mg cm^{-2}], and RLR values of the leaves of sunflower genotypes exposed to drought (S) and rewatering (R). C_1 and C_2 represent 50- and 55-d-old control plants that are corresponding controls of S and R, respectively. *Different letters* indicate significant difference at $P<0.05$ according to LSD.

| Parameter | Treatment | Genotype | | | | | | | | | LSD 5% |
		9753-2R	9702-R	9725-A	9718-A	TT317-R	8129-R	9814-A	9209-A	2478-A	
Chl ($a+b$)	C_1	0.046^{a}	0.040^{bf}	0.047^{aj}	0.064^{cg}	0.042^{b}	0.061^{n}	0.040^{bf}	0.050^{e}	0.046^{a}	0.003
	S	0.047^{aj}	0.039^{fl}	0.047^{aj}	0.065^{g}	0.035^{h}	0.057^{i}	0.039^{fl}	0.049^{ej}	0.032^{k}	
	C_2	0.047^{aj}	0.040^{bf}	0.046^{a}	0.066^{g}	0.042^{b}	0.061^{n}	0.041^{bf}	0.050^{e}	0.046^{a}	
	R	0.048^{ae}	0.042^{b}	0.037^{hl}	0.065^{g}	0.028^{m}	0.060^{n}	0.041^{bf}	0.048^{ae}	0.047^{aj}	
Car	C_1	0.008^{a}	0.007^{b}	0.008^{a}	0.012^{c}	0.008^{a}	0.009^{d}	0.008^{a}	0.010^{e}	0.009^{d}	0.001
	S	0.010^{e}	0.011^{f}	0.011^{f}	0.015^{g}	0.008^{a}	0.012^{h}	0.011^{f}	0.010^{e}	0.008^{a}	
	C_2	0.008^{a}	0.008^{a}	0.008^{a}	0.012^{h}	0.007^{b}	0.009^{d}	0.008^{a}	0.010^{e}	0.009^{d}	
	R	0.008^{a}	0.008^{a}	0.007^{b}	0.015^{g}	0.005^{i}	0.010^{e}	0.007^{b}	0.010^{e}	0.009^{d}	
RLR	C_1	0.19^{ag}	0.22^{ae}	0.25^{ae}	0.19^{ag}	0.21^{a}	0.22^{ae}	0.20^{a}	0.23^{ae}	0.22^{ae}	0.07
	S	0.44^{b}	0.51^{c}	0.65^{d}	0.28^{e}	0.84^{f}	0.51^{c}	0.61^{d}	0.59^{d}	0.84^{f}	
	C_2	0.24^{ae}	0.25^{ae}	0.21^{a}	0.19^{ag}	0.20^{a}	0.19^{ag}	0.19^{ag}	0.24^{ae}	0.22^{ae}	
	R	0.19^{ag}	0.24^{ae}	0.22^{ae}	0.13^{g}	0.20^{a}	0.19^{ag}	0.19^{ag}	0.21^{a}	0.19^{ag}	

TT317-R (28.6%), and 9814-A (12.5%) decreased, while in 9718-A (25%) and 8129-R (11%) increased significantly. RLR was higher than that of corresponding controls at drought treatment in all lines (Table 1). The highest RLR was determined in TT317-R and 2478-A approximately with similar extent (4- and 3.8-fold of control), whereas rewatering did not lead to any significant changes of RLR values in all lines.

Ψ_{WL} of leaves declined sharply at drought treatment in all lines (Fig. 5A). The highest decline of Ψ_{WL} was found in 2478-A (17.5-fold of control) (Table 4S). Rewatering resulted in statistically significant decrease in Ψ_{WL} values of 9702-R (2.45-fold of control) and 8129-R (1.92-fold of control). Similarly to Ψ_{WL} results, Ψ_{WS} decreased in all lines at drought treatment (Fig. 5B), mostly in TT317-R (61-fold), while the lowest decline was found in 9753-2R (9-fold) compared to controls (Table 4S). Rewatering led to the significant decrease in Ψ_{WS} values of TT317-R (4-fold) in contrast to control.

A detailed analysis of the effects of drought and rewatering on photosynthetic performance and some physiological parameters of sunflower inbred lines was performed (Fig. 6). PCA suggested that OJIP parameters, water status (Ψ_{WL} and Ψ_{WS}) and membrane integrity (RLR) results were mainly affected by the relationship between treatments and lines. The first two components (PC1 and PC2) explained 82% of the variation in the data. PC2 explained the photosynthetic pigments variation (10%), while PC1 mainly accounted for variation in the physiological state of lines (72%) (Fig. 6A). The diversity of nine lines was plotted with the contribution of each parameter and all treatments (Fig. 6B). Differences between drought and well-watered treatments were evident and lines were distributed according to PC1. Drought-treated lines were placed on the left (negative) side of the PC1 axes, whereas both rewatering treated lines and all controls were placed on the right (positive) side of the axes. Special energy fluxes (per RC), K-band, L-band, and RLR data

had main contribution on distribution of drought treated lines, while performance indexes and quantum efficiency data contributed to distribution of rewatering treatments. The PCA data related to 9753-2R under all treatments were distributed very close, reflecting 9753-2R being the most tolerant to drought when compared to other lines (Fig. 6B).

Discussion

Environmental restrictions such as drought potentially generate detrimental effects in plants (Rauf *et al*. 2016). The results of study showed that drought treatment adversely affected the lines and rewatering ameliorated the detrimental effects of drought on the lines. As an intrinsic probe for monitoring the successive steps of excitation energy transformation (Goltsev *et al*. 2012), we used ChlF signals to obtain the drought tolerance levels of the lines. The OJIP curves include valuable information about the structure and function of the photosynthetic apparatus (Schreiber *et al*. 1995, Goltsev *et al*. 2016, Stirbet *et al*. 2018). The O–J represents the reduction of the acceptor side of PSII (and also gives information on the connectivity between the PSII photosynthetic units), the J–I is associated with the partial reduction of the PQ pool and the I–P reflects the reduction of the acceptor side of PSI (Yusuf *et al*. 2010, Ripoll *et al*. 2016). Polyphasic Chl a fluorescence transient of sunflower lines subjected to drought followed by rewatering was plotted on a logarithmic time scale (Fig. 1) which provided key information about the impact of treatments and photochemical behaviour of lines. OJIP transients in Fig. 1A demonstrated that the drought had cumulative effect over the photosynthetic electron transport on thylakoid membranes. V_{OK} and V_{OJ} values indicated changes in L- and K-bands of lines, respectively, and the pattern of the changes of L- and K-bands were similar across the lines (Fig. 1B,C). The L- and K-bands increased at drought that pointed out disturbed energy transfer at light harvesting level and inactivation of oxygen-
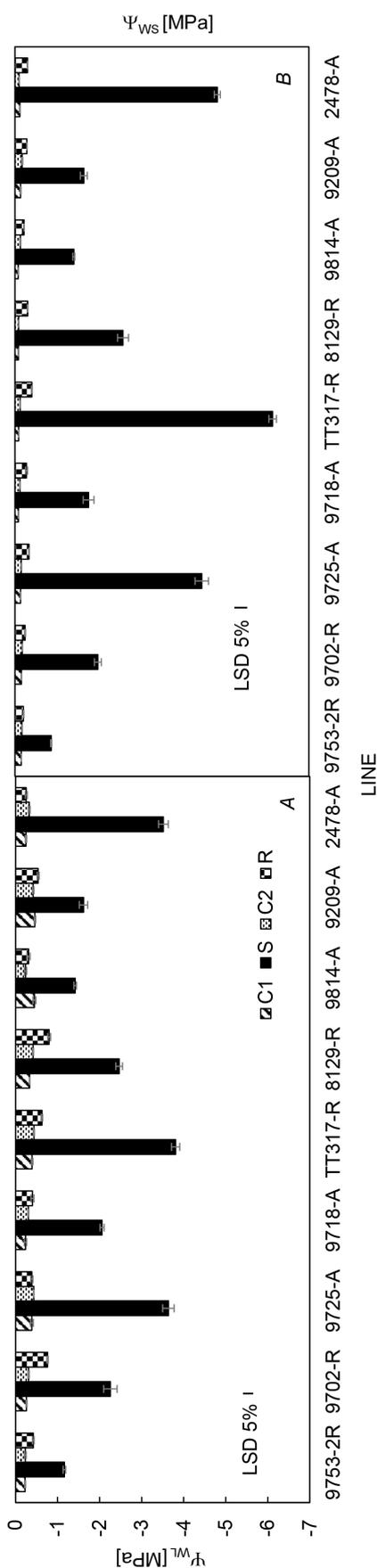
Fig. 5. Leaf water potential (Ψ_{WL}, *A*) and soil water potential (Ψ_{WS}, *B*) of lines of sunflower exposed to drought (S) and rewatering (R). C_1 and C_2 represent 50- and 55-d-old control plants that are corresponding controls of S and R, respectively. The error bars represent the standard error (± SE) for three replicates.

evolving complex (Yusuf *et al.* 2010, Mlinarić *et al.* 2017). Oukarroum *et al.* (2007) suggested that higher inactivation of oxygen-evolving complex caused an incompatibility between acceptor and donor side of PSII. Drought may limit the electron transport of the PSI acceptor side, which was determined by smaller I and P amplitudes (Çiçek *et al.* 2015). V_{IP} decreased, which indicated a loss of the structure and function of PSI (Ceppi *et al.* 2012) (Fig. 1*D*). The changes in V_{OK}, V_{OJ}, and V_{IP} values of 9753-2R were lower when compared to other lines.

The JIP-test parameters, derived from ChlF signals, might be used as indicators for drought tolerance and physiological disorders before the appearance of visible signs of drought (Kalaji *et al.* 2016). Among the quantum yields of photoinduced electron transfer from P_{680} to Q_A (φ_{P0}), from Q_A^- to PQ (φ_{E0}), and from PQ to the PSI electron acceptors (φ_{R0}) parameters, φ_{R0} exhibited the highest decrease when compared with controls (Fig. 2*A–C*). The limitation of the transfer of photoinduced electron from PQ to the PSI electron acceptors was the highest in TT317-R and 2478-A, while the lowest in 9753-2R. Our results are in accordance with Goltsev *et al.* (2012) who arranged the parameters of the quantum yields of the reaction close to the PSII reaction centre according to their decreased sensitivity to drought in the sequence $\varphi_{R0} > \varphi_{E0} > \varphi_{P0}$. Reduction of φ_{R0} may be a symptom either of decrease in PSI content (Ceppi *et al.* 2012) or decrease of PSI fraction involved in linear electron flow (Kalaji *et al.* 2014a, Živčák *et al.* 2014). The parameter ABS/RC defines the effective antenna size of active reaction centre. Significant enhancement of ABS/RC indicated a decrease in the antenna size and could result from PSII inactivation and excitation energy transfer from inactive PSII to active PSII units (Öz *et al.* 2014) (Fig. 2*D*). The energy dissipation per reaction center (DI_0/RC) increased by drought in all lines, reflecting that the lines might dissipate the excitation energy as heat and fluorescence more than photochemistry (Fig. 2*E*). ABS/RC and DI_0/RC values of 9753-2R did not change significantly. Reduced δ_{R0} indicated that drought had an adverse effect on electron flow at the PSI acceptor side caused by an inactivation of ferredoxin-NADP⁺-reductase (Schansker *et al.* 2005) (Fig. 2*F*). Among the lines, TT317-R exhibited a remarkable decrease of δ_{R0} (42%) at drought (Table 2S).

PI_{ABS} is the overall expression of the energy absorption, trapping, and conversion into the electron transport steps. Sensitivity of PI_{ABS} may be used as a marker for screening the genotypes against water stress. For instance, 24 sesame genotypes were screened on the basis of driving force for photosynthesis and relative deviation of PI_{ABS} (Boureima *et al.* 2012). In our experiment, the driving force (DF = $logPI_{ABS}$) of photosynthesis of drought treated lines declined due to decrease in the $\Psi_0/(1 - \Psi_0)$, implying a decrease in intersystem electron transport flux (Fig. 3). The driving force of photosynthesis and electron transport component activity values were the highest in 9753-2R when compared to other lines.

Exposure to drought for 10 d led to a significant increase of ΔPI_{Total} of sunflower lines (Fig. 4). Enhanced ΔPI_{Total} explained the decline of the photosynthetic
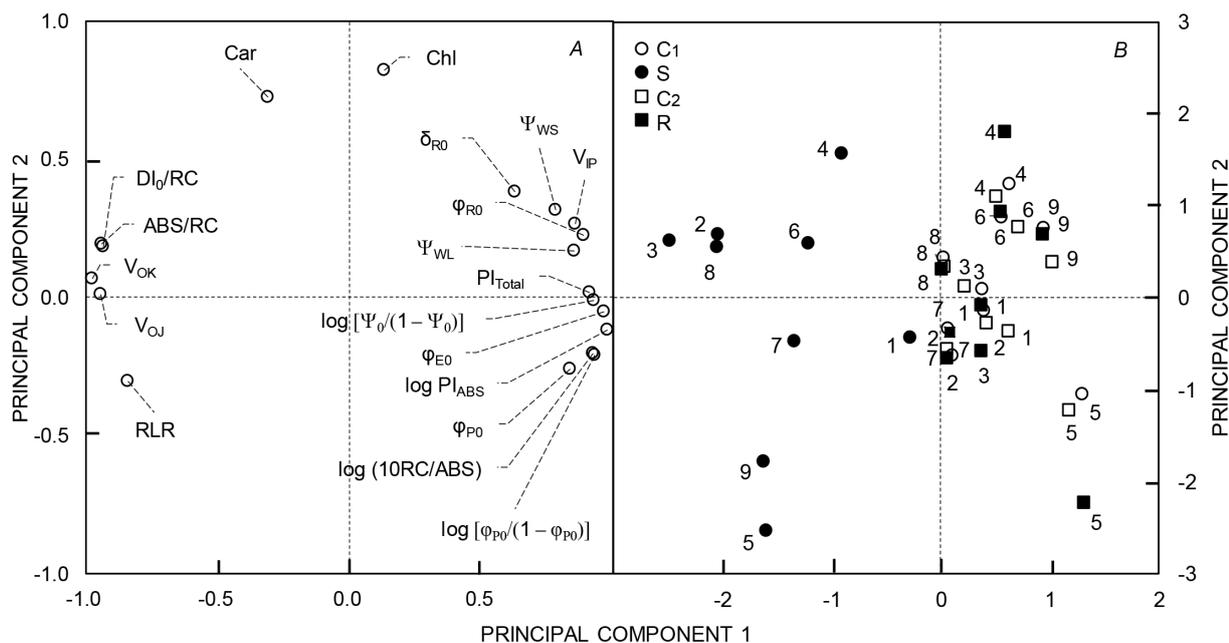
Fig. 6. Component plot in rotated space of principle component analysis (PCA) of variability of all tested parameters (*A*), and lines and treatments (drought, S; rewatering, R) (*B*). C_1 and C_2 represent 50- and 55-d-old control plants that are corresponding controls of S and R, respectively. Numbers represent lines in order: 9753-2R (1), 9702-R (2), 9725-A (3), 9718-A (4), TT317-R (5), 8129-R (6), 9814-A (7), 9209-A (8), and 2478-A (9). PC1 explained 72% and PC2 explained 10% of the variation in the data.

performance PI_{Total} that measured the performance up to the PSI end electron acceptors. Decreased PI_{Total} may be due to decreased electron transport chain which resulted from the loss of PSII activity and the damage of PSI function (Oukarroum *et al.* 2015). At the drought treatment, the highest decline of PI_{Total} was determined in TT317-R, whereas the lowest change in PI_{Total} was found in 9753-2R. In contrast to drought, rewatered plants exhibited better photosynthetic performance. Çiçek *et al.* (2015) screened 21 chickpea cultivars under drought and classified the cultivars according to the values of drought factor index and ΔPI_{Total}; the cultivars with the lowest drought factor index and the highest ΔPI_{Total} were considered as sensitive. Likewise, increased ΔPI_{Total} of lines was correlated with drought sensitivity in the present study.

The Chl (*a*+*b*) contents of the few lines declined with drought and rewatering treatments (Table 1). Contrarily, Car contents of most lines increased at drought (Table 1). It is well known that Car protect photosynthetic apparatus from photodamage under stress conditions. Despite mostly unchanged contents of Chl (*a*+*b*) and increased contents of Car, sunflower lines showed decline in PSII photo-chemical activity. Dwivedi *et al.* (2018) emphasized that determination of membrane stability is a widely used criterion to evaluate crop drought tolerance, since drought leads to water loss from plant tissues, which impairs both membrane structure and function. As water availability decreased, RLR increased in all lines, particularly in TT317-R and 2478-A (Table 1), indicating impaired mem-brane integrity. However, lines recovered their membrane integrity with rewatering treatment.

Ψ_{WL} and Ψ_{WS} values of all lines declined sharply at drought treatment which caused wilting (Fig. 5). By exposing to drought, plants are compelled to decrease their inner water potential to avoid dehydration and to uphold the water potential balance (Farooq *et al.* 2014, Liu *et al.* 2017). However, the water content of lines reached control levels after rewatering, suggesting successful recovery. Ψ_{WL} status of drought-treated lines was similarly correlated with Ψ_{WS}. Therefore, the criterion, which estimates the water status of plant, may also reflect the soil water content (Williams and Araujo 2002). Both Ψ_{WL} and Ψ_{WS} values of 9753-2R were close to control at drought, while 9725-A, TT317-R, and 2478-A showed the highest decline.

All the parameters, which we used in this study, were separated with PC1 and PC2 and evaluated according to their influence in drought response (Fig. 6*A*). In our experiment, the water status, membrane integrity, and PSII photochemical activities were more explanatory to understand drought effects than photosynthetic pigments. The application of PCA on JIP-test, RLR, and water status allowed describing and separating parameters according to their influence on sunflower inbred lines stress response (Fig. 6*B*).

Overall results of this study showed that drought adversely affected the photosynthetic activity of all lines. ChlF, water contents of leaves and soil, and RLR parameters played a primary role in determining drought tolerance or sensitivity. Nine sunflower lines were screened and these lines exhibited differences in responses to drought. Every step of photoinduced electron transfer from PSII reaction centre to PSI electron acceptors was affected by drought treatment. However, recovery of photosynthetic activity after rewatering indicated that the drought treatment

did not cause irreversible damage in the photosynthetic apparatus. Rewatering treatment helped us distinguish the line and/or lines that had low recovery capacity and thus had drought-sensitive response. TT317-R and 2478-A demonstrated drought-sensitive response, *e.g.*, high membrane damage, low photosynthetic performance, *etc*. These two lines might be considered as drought sensitive. TT317-R also exhibited poor performance during recovery, as well as drought. Breeding line 9753-2R maintained high membrane stability and photosynthetic functionality under drought stress; this line may be regarded as drought tolerant.

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