QUANTIFICATION OF DROUGHT TOLERANCE LEVELS OF SUNFLOWER INBRED LINES BY MEANS OF CHLOROPHYLL-*A* FLUORESCENCE

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ABSTRACT

Plants are often exposed to various environmental stresses such as drought. One of the major objectives in plant breeding programs for crops grown in arid/semiarid areas is selection of crop cultivars with remarkable resistance to drought stress. To select drought tolerant lines, chlorophyll *a* fluorescence (ChlF) measurements has used in addition to morphological and physiological analysis in recent years. Some sunflower (*Helianthus annuus* L.) inbred lines developed by Trakya Agriculture Research Institute (TARI) with National Sunflower Project were grown in Bahri Dagdas International Agricultural Research Institution in order to determine the drought tolerance levels using fast ChlF techniques. Fluorescence signals were recorded and analyzed using JIP-test. V_J, V_I, ABS/RC, ET_0/TR_0, DI_0/RC, RE_0/ET_0 and PI_{total} originated from JIP-test parameters were evaluated. Besides, drought factor index (DFI) was calculated using data of PI_{total} and lines were classified according to their drought tolerance levels. Results obtained from present study indicated that lines were markedly affected depending on the duration and severity of the drought. Additionally, sunflower inbred lines could be separated into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A) based on the DFI values.

Key words: Sunflower, Drought tolerance, Inbred lines, Chlorophyll *a* fluorescence kinetics

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important agricultural crops in the world and the main source of unsaturated vegetable oil (Baloğlu et al., 2012; Gholinezhad et al., 2015). While the world sunflower production has varied from 2010, production of Turkey has been increased (FAOSTAT, 2016). Turkey has among the top 10 in sunflower producer countries according to National Sunflower Association data. On the contrary of well-studied model plants, different water regimes adaptable plants could be valuable genetic sources to understand links between stresses and stress responses (Raineri et al., 2015). Due

to its drought tolerance and deep root system compared to other crops, sunflower has been an attractive alternative genetic source (Howell et al., 2015).

Among the various abiotic stresses, drought is the most significant environmental stress in agriculture worldwide and under the limited water conditions improving yield and yield capacity is major goal of plant breeder. Due to urbanisation, industrialisation, depletion of ground-water and global warming, the amount of available water is decresing day by day. dorught triggered by these conditions cause major constraints on the physiology, biochemistry, growth, development and productivity of plants (Bechtold et al., 2016; Szechyńska-Hebda et al., 2016) depending on the stres intensity and duration (Raineri et al., 2015). More than 80 years of breeding activities have caused some yield increase for crop plants grown in areas affected by drought (Cattivelli et al., 2008) and this situation would be the most economical approach to improving agricultural productivity and reducing agricultural use of substantial water resources (Sperdouli and Moustakas, 2012). Drought stress primarily influences photosynthesis, by multidimensional ways just as reduce in leaves expansion, decreased CO_2 diffusion to the chloroplast, impared photosynthetic apparatus with enzymes and expedite of leaf senecence (Farooq et al., 2009; Pinheiro and Chaves, 2011; Hasanuzzaman et al., 2014). Based upon limitation of CO_2 uptake and imbalance between absorbing and using of sunlight, the possibility of overexcitation of photosystem II (PSII) increases. This case induces a decrease of photosynthetic rate and an increase in the dissipation of absorbed energy through non-radiative processes (Faraloni et al., 2011). Under drought conditions, photosystem II (PSII) is more sensitive than photosystem I (PSI) (Deng et al., 2003), therefore PSII has a key role to analyze changes that occur in photosynthesis (Baker, 1991). ChlF is a non-invasive measurements of PSII activity and is a commonly used technique (Murchie and Lawson, 2013; Schansker et al. 2014). To determine the photosynthetic performance, ChlF kinetics can be considered as a biosensor tool. All oxygenic photosynthetic samples investigated so far using ChlF techniques show the characteristic polyphasic rise from the ground state value (F_0, 20μs) at the O step to its maximum value (F_M, approx. 300-500 ms) at the P step with J (F_J, 2 ms) and I (F_I, 30 ms) intermediate steps (Strasser et al. 2004). An analysis of the fast OJIP fluorescence kinetics, called JIP test, quantifies the in vivo energy fluxes passing through the reaction centres and photosystems (Strasser and Strasser, 1995; Strasser et al., 2000). An analysis of the fast OJIP fluorescence kinetics, called JIP test, links different steps and phases of the transient with the redox states of PSII, also correlates the phases with the efficiencies of electron transfer in the intersystem chain between PSII and PSI and to the end electron acceptors at the PSI acceptor side (Strasser et al., 2004).

The aim of this study was to evaluate the effects of drought on ChlF kinetics in sunflower female inbred lines developed in National Sunflower project (TÜBİTAK-113O926) conducted by TARI under controlled conditions in Konya, Turkey.

MATERIALS AND METHODS

The study was conducted in Bahri Dagdas International Agricultural Research Institution's research fields in 2014. A total of fifty female inbred lines, originated different genetic sources, were initially sown, however the measurements of 38 lines were used to analyse the drought tolerance, and the rest of lines were excluded. Trials were conducted in controlled environmental conditions with randomized complete block design with one row and three replications. In each row, there were 5 plants and the distance between rows 70 cm and in rows were 30 cm. Trials were planted by hand in 31 May and drip irrigation was

applied and as covering rain shelters. Chlorophyll fluorescence measurements were three times like below in the experiments.

Control: All plant water need were supplied by drip irrigation.

Stress group 1 (S_1): 65-day-old sunflower lines under natural condition without irrigation (R3 stage).

Stress group 2 (S_2): 75-day-old sunflower lines under natural condition without irrigation (R5-1 stage).

Stress group 3 (S_3): 85-day-old sunflower lines under natural condition without irrigation (R6 stage).

At the end of the treatments, polyphasic ChlF measurements were carried out from leaves. The polyphasic OJIP fluorescence transient was measured with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) fluorimeter. Samples dark-adapted for at least 30 min were illuminated with continuous light (650 nm peak wavelength, 3000 μmol m^{-2} s^{-1} maximum light intensity, for 1 s) provided by 3 LEDs, and the Chl a fluorescence signals were recorded according to Strasser and Strasser (1995). The OJIP transient was analysed by JIP test, based on the energy flux theory for biomembranes in a photosynthetic sample, which leads to the equations and calculations for the efficiencies of the whole energy cascade from absorption to the reduction of end electron acceptors at the PSI acceptor side and the performance indexes (Tsimilli-Michael et al., 2000; Strasser et al., 2004; Strasser et al., 2010). The fluorescence parameters (Table 1) were calculated using the Biolyzer software package.

Drought factor index (DFI) was calculated from using data of performance index (PI_{total}) and sunflower lines were ranked. DFI was calculated according to Strauss et al. (2006) and Oukarroum et al. (2007) with minor modification and calculated with the formula: DFI = $logA + 2\ logB + 4\ logC$, where C is the average relative performance index (PI) during the first treatment of drought, B is the average relative PI_{total} during the second treatment, and A is the average relative PI_{total} of the third treatment. The relative PI_{total} was calculated as $PI_{drought}/PI_{control}$.

Experimental data were subjected to Analysis of Variance (ANOVA) using the statistical software SPSS Statistics. Means were compared with least significant differences (LSD) at 5% level ($P<0.05$).

RESULTS AND DISCUSSION

Photosynthesis, the key process of plant metabolism, is strongly influenced by environmental conditions (Kalaji et al., 2014). Measurements of photosynthetic efficiencies are an important component of agricultural, environmental, and ecological studies. ChlF measurements represent a simple, non-destructive, inexpensive and rapid tool allowing scientists to get information on the photosynthetic process without destroying the tested samples. The ChlF parameters are potentially useful for screening genotypes for drought tolerance (Oukarroum et al., 2007; Strasser et al., 2010; Boureima et al., 2012; Çiçek et al., 2015; Kalaji et al., 2016).

National Sunflower Project was conducted by TARI in Edirne. Many inbred female lines and F_1 hybrids producted within this project and registered for Turkey. To determine of the level of drought tolerance these lines were grown under field conditions in Konya,

Turkey. In this study, the effects of different drought stress on photosynthetic efficiency were examined by using the changes in some chlorophyll a fluorescence parameters (JIP-test parameters), such as V_J, V_I, ABS/RC, ET_0/TR_0, DI_0/RC, RE_0/ET_0 and PI_{total}. The means of these parameters were calculated across each treatment of all the sunflower hybrids and the values of stress groups were normalized by the values of the control plants (control value: 1) for each hybrid. In general, the changes in these parameters were observed compared to their controls.

The relative variable fluorescence at the J-step (V_J) increased almost all stress duration compared to control (Figure 1). V_J values of sunflower lines were prominently increased depending on stress duration. The highest increases were observed in 8959A, 9728A, 9907A (more than 50% or almost 60% increase) under severe drought stress (S3). It has been suggests that the fluorescence yield at the J-step is strongly determined by the redox state of the electron carriers in the electron transport chain (Haldimann and Strasser, 1999). Drought stress might blocked or inhibited the re-oxidation of the electron carriers in the sunflower lines.

The relative variable fluorescence at the I-step (V_I) was significantly increased compared to control for all stress treatments (Figure 2). Depending on the drought intensity, the highest increase was observed in 2478 A, 9728 A, 9412 A and 97181 A hybrids (44-46 %) for S3. It has been suggested that V_I values is as an approximate estimation of the fraction of QB-non-reducing PS II (Hsu and Lee, 1991). Drought stress significantly increased the fraction of Q_B-non-reducing PSII centers in almost all inbred sunflower lines. Electron transfer between Q_A^- and Q_B does not function in the Q_B-non-reducing reaction centers (Cao and Govindjee, 1990). This situation might affect the electron transport towards PS I. Moreover, an increased V_I was used as a probe for the inhibition of electron transport at the acceptor side of PSII under stress condition (Chen et al., 2004).

It has been suggested that Drought Factor Index (DFI) represents the relative drought induced changes of Performance Index (PI) during a freely time of drought stress (Kalaji et al., 2016). Sunflower inbred lines investigated in present study were ranked according to their DFI values given on Table 2. Drought-tolerant genotypes with the lowest reduction in the PI_{total} under drought stress had the largest (less negative) DFI values. Based on the DFI values, thirty eight sunflower inbred lines could be separated into four groups: 9814 A is the first group (highly drought tolerant; DFI: -1.19), TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A are the second group (drought tolerant; DFI: -1.58 - -1.90), 8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A are the third group (less drought tolerant; DFI: -2.01 - -2.48) and 6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A are the fourth group (drought sensitive; DFI: -2.53 - -3.01).

DFI is calculated from PI values, so it is closely related to PI as seen above. At all stages of development PI_{total} was decreased significantly compared to control and depending on the drought periods (S1, S2 and S3) the highest decreases were in 6626 A and 9942 A (approx.35%), 8435 A and 8543 A (approx. 55%), 8959 A, 6545 A and 9728 A (approx. 75%) respectively (Figure 3). The performance index (PI_{total}) are used to utilize the effects of the environmental constraints on the plant. It has been proved that these parameters are more sensitive to the environmental changes than other fluorescence parameters, such as F_V/F_M and they correlates well with plant vitality (Oukarroum et al., 2007; Tsimilli-Michael and Strasser,

2008, Kalaji et al., 2016; Siddiqui et al., 2016). PI_{total} is predicating the performance up to the PSI end electron acceptors.

ABS/RC is reciprocal of RC/ABS utilized to calculate PI. ABS/RC known as average antenna size, expresses the total absorption of PSII antenna chlorophylls divided by the number of active (in the sense of QA reducing) reaction centers (Strasser et al., 2000). ABS/RC parameter was decreased in S1 and S2 (except for 9942A, 6626 A and 8543A) stages, the parameter values was increased approximately half of the hybrids in the S3 stage and the other half was close to control value or higher than it (Figure 4). Under stress conditions ABS/RC was increased by the reason of inactivation of the PSII reaction centers (van Heerden et al., 2007; Mladenov et al., 2015).

Probability that a trapped exciton moves an electron into the electron transport chain beyond QA^- (Ψ_0, ET_0/TR_0) was significantly decreased in all stress groups compared to controls (Figure 5). In relation to the stress duration, the highest decrease in the Ψ_0 was observed in S3 treatment. Also, increase in V_I value supports results of Ψ_0 obtained from this study. Lower Ψ_0 values might exhibit that the activity of electron transport beyond Q_A was considerably inhibited. Jiang et al. (2006) and Oukarroum et al. (2015) reported similar relationship between these parameters.

RE_0/ET_0, efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (δR_0), was significantly affected from drought stress durations dependent manner compared to control (Figure 6). As for ET_0/TR_0 and PI_{total}, RE_0/ET_0 values of sunflower inbred lines were decreased depending on duration and the severity of drought stresses. Schansker et al. (2005) stated that a lower RE_0/ET_0 level indicated a decrease of a traffic jam of electrons at the acceptor side of PSI caused by an inactivation of ferredoxin-$NADP^+$-reductase.

Chlorophyll fluorescence (ChlF) transients allow the evaluation of the physiological condition of photosystem II (PSII) and energy fluxes of thylakoid membranes. It also gives information on the cooperation of photochemical and nonphotochemical reactions. DI_0/RC which expresses the ratio of dissipation to the amount of active reaction center (Strasser et al., 2000), was decreased in S1 and S2 (except for 9942 A, 6626 A and 8543 A) stages. In addition, for 2453 A, 2517 A, 62001 A, 6545 A, 6626 A, 8435 A, 8454 A, 8543 A, 8959 A, 9209 A, 9444 A, 9907 A, 9942 A and CL078 A hybrids DI_0/RC was significantly higher in drought treated leaves than in controls (Figure 7). Dissipation of light energy per active reaction centers (DI_0/RC) can be thought of as the absorption of photons in excess of what can be trapped by the reaction centers as heat (Mathur et al., 2011). Reduction in energy dissipation would explain increase in the fluorescence emission by the excited antenna chlorophyll *a* molecules before the migration of excitation to the reaction centers (Falqueto et al., 2012). DI_0/RC might be increased to avoid photo-oxidative damage of photosynthetic apparatus.

CONCLUSION

Drought stress affected adversely the photosynthetic efficiency of examined sunflower hybrids. The tolerance levels of sunflower inbred lines were determined using ChlF techniques. Sunflower lines could be classified into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107

A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A). The drought factor index calculated from PI parameter can be used to classify the level of stress tolerances. The use of supplementary parameters like PI can be more useful than complex biophysical parameters to understand the photochemical processes, also to interpret the data correctly.

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Table 1. ABSTRACT of the JIP test formulae using data extracted from the polyphasic chlorophyll a fluorescence (OJIP) transient in this study (Han et al., 2009; Strasser et al., 2010).

Data extracted from the recorded fluorescence transient OJIP	
$F_0 = F_{20\mu s}$	Initial fluorescence intensity, when all PSII RCs are open
$F_K = F_{300\mu s}$	Fluorescence intensity at 300 ms
$F_J = F_{2ms}$	Fluorescence intensity at the J-step (at 2 ms)
$F_I = F_{30ms}$	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity, when all PSII RCs are closed
V_J $(F_{2ms} - F_0)/(F_M - F_0)$	Relative variable fluorescence at the J-step (2 ms)
V_I $(F_{30ms} - F_0)/(F_M - F_0)$	Relative variable fluorescence at the I-step (30 ms)
$M_0 = 4(F_{300\mu s} - F_0)/(F_M - F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V
Specific energy fluxes or activities expressed per reaction center (RC)	
$ABS/RC = M_0 (1/V_J)(1/\varphi_{Po})$	Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size)
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy flux per RC at t = 0
Quantum yields and efficiencies/probabilities	
$F_V/F_M = \varphi_{Po} = TR_0/ABS = [1 - (F_0/F_M)$	Maximum quantum yield for primary photochemistry

$RC/ABS = \varphi_{Po} \times (V_J/M_0)$	The concentration of reaction centres per chlorophyll
$\Psi_0 = ET_0/TR_0 = (1-V_J)$	Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta R_0 = RE_0/ET_0 = (1 - V_I) / (1 - V_J)$,	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
Performance index (products of terms expressing partial potentials at steps of energy bifurcations)	
$PI_{total} = (RC/ABS) \times [\varphi_{Po} /(1 - \varphi_{Po})] \times [\Psi_0 /(1 - \Psi_0)] \times [\delta R_0/(1 - \delta R_0)]$	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors

Table 2. Drought factor index (DFI) values of 38 sunflower inbred lines grown under drought stress. The inbred lines were ranked according to their DFI values.

	Lines	**DFI Values**
1	9814 A	-1,19
2	TT 179 A	-1,58
3	0046 A	-1,80
4	CL 068 A	-1,84
5	9725 A	-1,85
6	2517 A	-1,90
7	8454 A	-2,01
8	9209 A	-2,01
9	9178 A	-2,09
10	9661 A	-2,15
11	8255 A	-2,18
12	CL 078 A	-2,23
13	9444 A	-2,26
14	TT 176 A	-2,26
15	96172107 A	-2,31
16	TT 188 A	-2,36
17	2478 A	-2,38
18	9718 A	-2,44
19	6388 A	-2,45
20	9942 A	-2,47
21	62001 A	-2,48
22	6626 A	-2,53
23	6163 A	-2,57
24	97181 A	-2,58
25	TT 187 A	-2,58
26	9907 A	-2,58
27	7751 A	-2,58
28	917574 A	-2,68

29	6545 A	-2,68
30	9726 A	-2,69
31	TT 198 A	-2,71
32	8428 A	-2,74
33	8435 A	-2,80
34	8543 A	-2,82
35	9412 A	-2,84
36	8959 A	-2,86
37	2453 A	-2,92
38	9728 A	-3,01

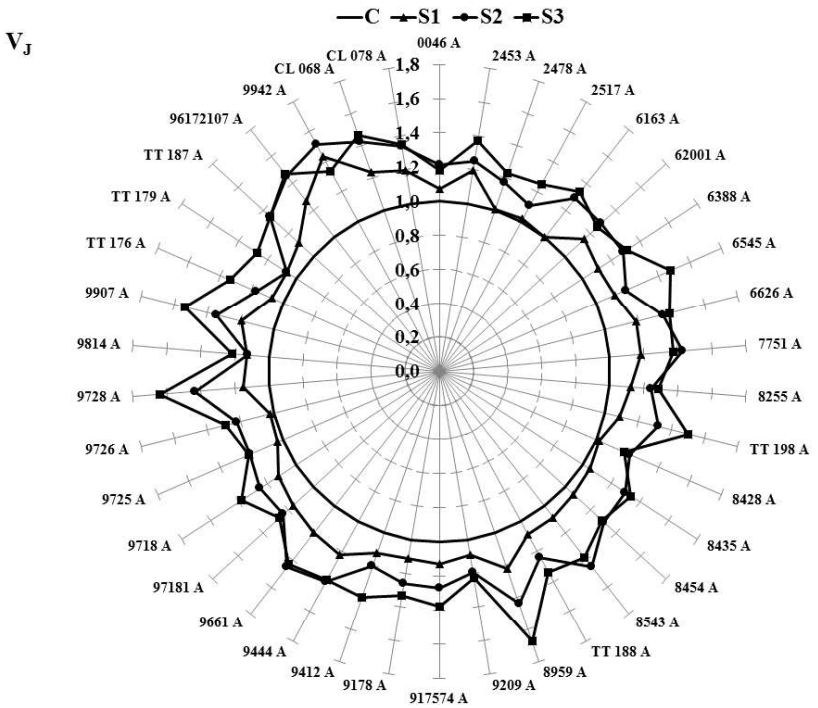

Figure 1. A radar-plot presentation of the changes in the relative variable fluorescence at the J-step (V_J) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

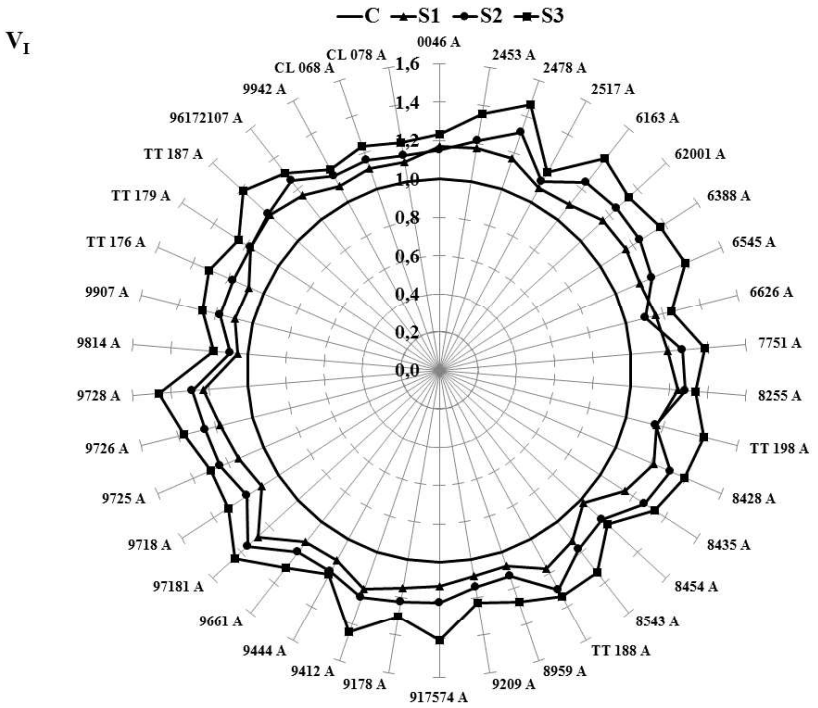

Figure 2. A radar-plot presentation of the changes in the relative variable fluorescence at the I-step (V_I) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

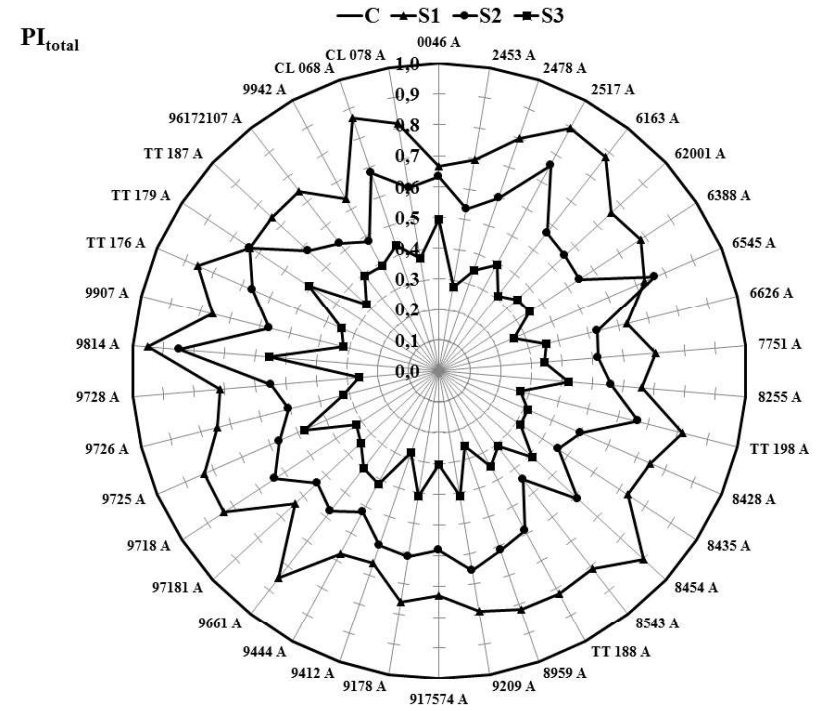

Figure 3. A radar-plot presentation of performance index (PI_{total}) parameter of dark-adapted sunflower leaves exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

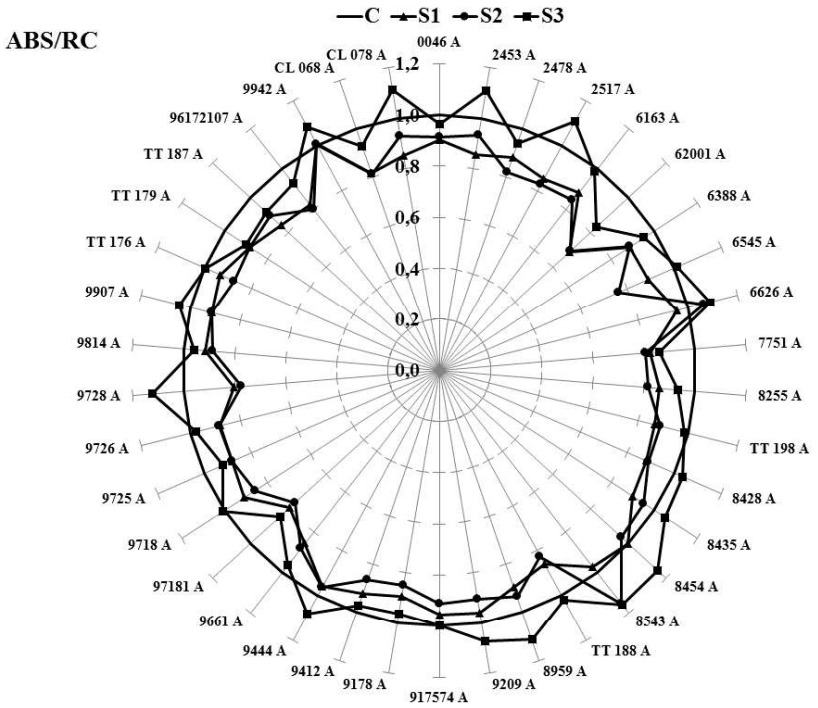

Figure 4. A radar-plot presentation of ABS/RC, effective antenna size of an active reaction centers, in the inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

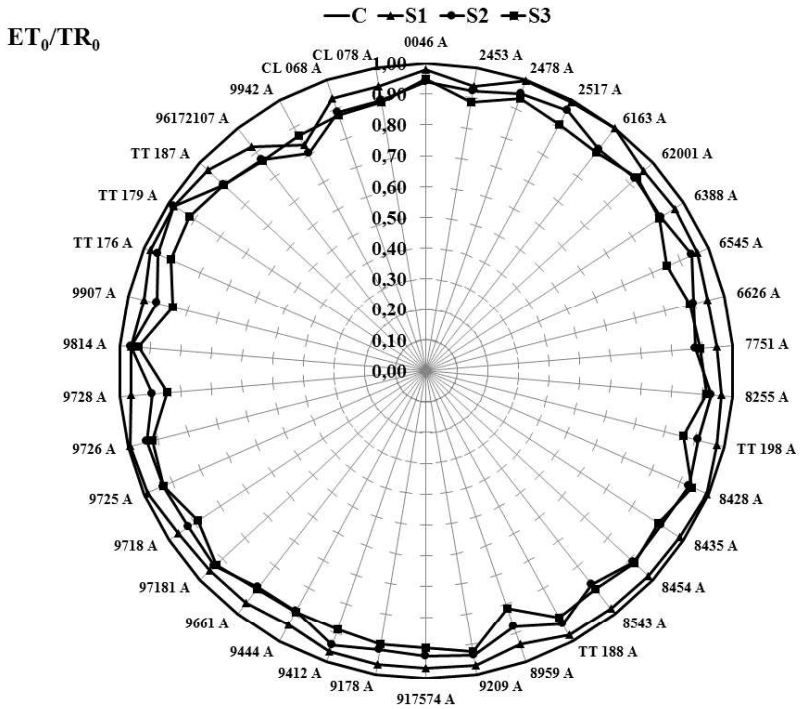

Figure 5. Drought stress effect on the efficiency with which the energy of a trapped exciton is converted into the electron transport beyond Q_A (ψ_o=ET_0/TR_0) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

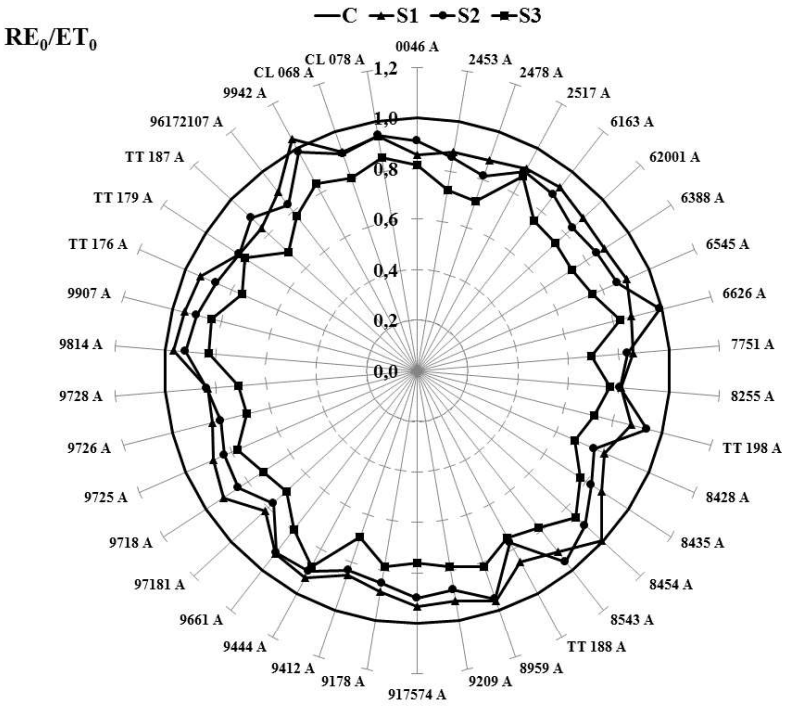

Figure 6. Drought stress effect on the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors ($\delta Ro = RE_0/ET_0$) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

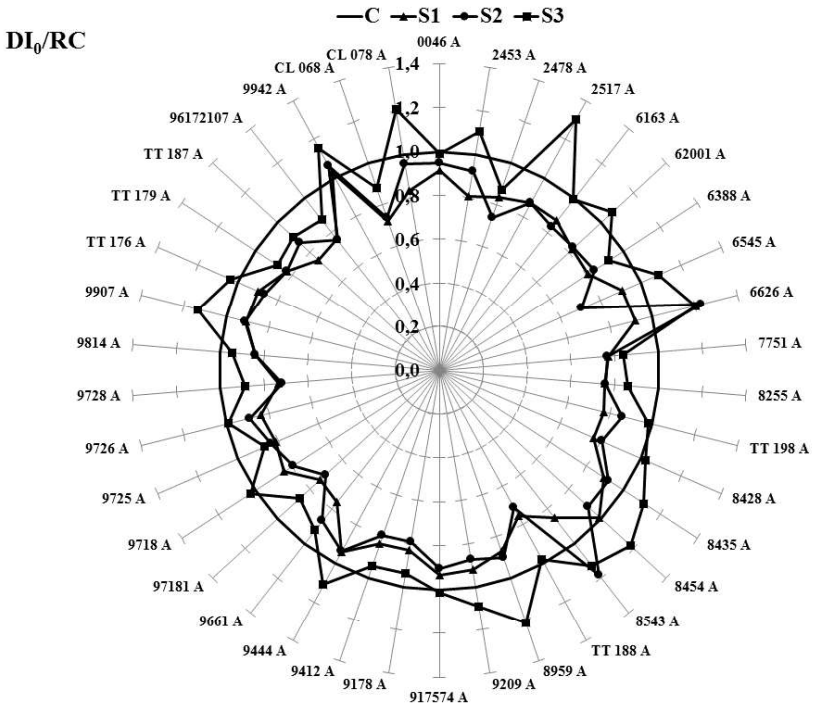

Figure 7. A radar-plot presentation of the flux of dissipated excitation energy per RC (DI_0/RC) of sunflower lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

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