

FLORESCENCE ASSESSMENT OF SUNFLOWER GENOTYPES AGAINST DROUGHT STRESS ENVIRONMENT

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Abstract

Florescence assessments of Sunflower (*Helianthus annuus* L.) genotypes in field under drought stress environment were studied. Climate change and low precipitation represents the main constraints in sunflower production in Pakistan. In present investigation two sunflower genotypes, viz S.28111 and SF0049 were selected to study their photosynthetic performance through non-destructive technique in field. Three treatments were applied to impose drought in plants i.e. irrigation after 6 days (D1), irrigation after 8 days (D2) and irrigation after 16 days (D3) with control irrigation after 4 days. Results revealed that drought stresses altered the electron transport rate through PSII (Fm/Fo), photochemical quenching (qP), efficiency of photosystem II (ΦPSII), linear electron transport rate (rETR), non-photochemical quenching (NPQ), size and number of active reaction centre of photosynthetic apparatus (Fv/Fo), driving forces (DF_{ABS} and DF_{CS}) and performance indexes (PI_{ABS} and PI_{CS}) parameters that depends on the efficiency and yield of energy transfer and primary photochemistry. However, S.28111 maintained substantial quantum yield and enhanced photosynthetic performance under drought stress environment compared to SF0049. It is suggested that the assessment of photochemistry of PSII offers a practical and sensitive field screening regarding test for drought stress tolerance in sunflower. Results are discussed in relation to photosynthetic performance and florescence responses of sunflower genotypes in the drought stress environment.

Key words: Chlorophyll 'a' fluorescence; Drought stress; JIP-test; Photosynthesis; Sunflower.

Abbreviations

ABS/RC:	Apparent antenna size of active PSII RC
Area:	Area over the fluorescence curve between Fo and Fm
DIo/RC:	Effective dissipation of energy in active reaction centre
DF _{ABS} :	Driving Force on absorption basis
DF _{CS} :	Driving Force on cross section basis
ETo/RC:	Electron transport per reaction centre (Electron flux transferred from reduced Q _A to PQ per active PSII)
Fo/Fv:	Efficiency of water splitting complex
Fo/Fm:	Physiological state of the photosynthetic apparatus
Fm:	Maximum fluorescence
Fm/Fo:	Electron transport rate through PSII
Fo:	Minimum fluorescence
Fv/Fo:	The ratio of photochemical to nonphotochemical quantum efficiencies
Fv/Fm:	Maximum quantum yield of photosystem II
rETR:	Linear electron transport rate (J)
NPQ:	Non-photochemical quenching
PI _{ABS} :	Photosynthetic performance index on absorption basis
PI _{CS} :	Photosynthetic performance index based on cross section of leaf
ΦPSII:	Effective quantum yield of PSII Photochemistry
qP:	Photochemical quenching
RC/ABS	Density of reaction centres on chlorophyll basis
RC/CSm:	Amount of active reaction center per excited cross section
TRo/RC:	Maximal trapping rate of absorbed photons in RC
[Φ _{Po} / (1 - Φ _{Po})]:	The efficiency of light reaction
[Ψ _o / (1 - Ψ _o)]:	The efficiency of biochemical reaction
Φ _{EO} :	Quantum yield of electron transport
Φ _{PO} :	Quantum yield for primary photochemistry
Ψ _o = ETo/TRo = 1-Vj;	Yield of electron transport per trapped excitation or probability with which a PSII trapped electron is transferred from reduced Q _A to Q _B

Introduction

In nature, plant growth and production depend on environmental features, including availability of water. Plants continuously face random environmental stresses that affect the plant growth in field. Among these stresses, drought is a largest factor that restricts the plants growth and production in natural environments. Drought may be the result of greater transpiration and supply of water is reduced through roots. Plants growing in arid and semi-arid regions often face this condition. Lower water supply adversely disturbs the plant physiology and thus affects morphological features (Iqbal *et al.*, 2008). Physiological mechanisms that provide drought stress tolerance are crucial for the developing and selection and breeding tactics. Further, there are few

reports that provide physiological or photosynthetic understanding in field environments to identify those traits that limits the yield under drought stress. These traits can be used in successful crop improvement programs to enhance agricultural production.

Worldwide, Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oilseed crop. The seeds contain 30-40% oil and is used for cooking, lightening paints, lubricants and biofuel. Environmental extremes decrease the growth of sunflower plants and leads to the lower seed production and yield. Sunflower is tolerant to the short period of drought stress due to its deep-rooted nature (Karam *et al.*, 2007). Drought stress caused oxidative damages in plants cell (Blokina *et al.*, 2003; Hussain *et al.*, 2018). To overcome the drought stress, plants close their stomata (Ozkur *et al.*, 2009). Closed stomata decrease in CO₂ uptake which leads to

over-excitation of the photosynthetic apparatus. In this regard, the drought stress limit various physiological processes in sunflower, including photosynthesis (Umar & Siddiqui, 2018; Umar *et al.*, 2019). The screening protocol based on the photosynthetic efficiency of sunflower cultivars could be useful for the field assessment under water limited environments. The drought sensitivity of photosynthetic apparatus is dependent on both plant varieties and duration of drought stress exposure. Recently many researchers are focusing on the early detection of stresses using non-destructive technique (Siddiqui *et al.*, 2014; Kalaji *et al.*, 2018; Iqbal *et al.*, 2019).

Plants responses to drought stress are determined by many factors. Chlorophyll 'a' fluorescence is an innovative and very informative tool for studying the effects of various environmental stresses on photosynthesis (Kalaji *et al.*, 2011; Stirbet & Govindjee, 2011). Among the different tactics to analysis of chlorophyll fluorescence, the 'JIP-test' is often used in plant biology to understand the photosynthetic responses under suboptimal environments (Yusuf *et al.*, 2010). This test allows to understand the relationship between PSII, fluorescence signals of a leaf, and their analytical expressions (Strasser *et al.*, 2000; Bussotti *et al.*, 2007). This offers the simple explanation of equilibrium between the inflow and outflow of the entire energy flux within PSII. Further, chlorophyll fluorescence analysis provides relevant information regarding the fate of absorbed energy (Kalaji *et al.*, 2011). The parameters through JIP test are related to energy fluxes for light absorption, trapping, of excitation energy and electron transport (ETR) per cross section (sample area) or per reaction centre. The main advantages of this test are that non-invasive, accurate, much sensitive, and most importantly it is fast method.

Many areas of Pakistan are frequently exposed to the cyclic and unpredictable drought. In many areas, irrigation resources are decreasing during summer. Hence the crops of this season face drought stress. Moreover, there are few reports regarding the common drought stress tolerant mechanism in sunflower plants and which physiological parameter is related to the functioning of photosynthetic apparatus. The objective of the present study was to examine the photosynthetic responses through chlorophyll 'a' fluorescence in sunflower cultivars to advance our information under drought stress environments. In this study, an attempt was made to find the changes in selected chlorophyll 'a' fluorescence parameters and to find a method for early detection of plants stress resulting from the drought stress in field. The current investigation was based on the belief that intra-cultivar differences in the responses of PSII against drought stress would contribute the understanding of the physiological basis of tolerance. Furthermore, such information is needed to increase production through breeding and genetic transformation for drought stress tolerance.

Materials and Methods

Study location: This research was conducted at experimental

field adjacent to the Stress Physiology Phenomic Centre, Department of Botany, University of Karachi, Pakistan, in 2016. Experiment was conducted on a 1:3 clay loam soil with pH 7.8.

Treatments and agronomic operations: Each experiment was conducted as a split plot based on randomized complete block design. Irrigation regimes were considered as main plots and cultivars as sub-plots. Main plots were control (irrigation after 4 days = C), mild drought stress (irrigation after 6 days = D1), moderate drought stress (irrigation after 8 days = D2) and severe drought stress (irrigation after 16 days D3). Sub-plots were two sunflower cultivars including S.28111 and SF0049. Seeds of each sunflower cultivars were sown in the month of July 2016. Each plot consisted of 3 rows, 1.5 m length, 35 cm distance between rows and 30 cm distance between plants. The plants were watered well, and all plants received irrigation uniformly until the initiation of stress treatments. Drought stress were imposed at the stem elongation. At the time of physiological analysis, the water contents as field capacity (FC) was recorded, control 95% FC, mild drought stress (D1) 80% FC, moderate drought stress (D2) 60% FC and severe drought stress (D3) 25% field capacity. During the experimental period weeds were controlled continuously by hand. Bioclimatic and physicochemical properties of soil presented in Table 1.

Chlorophyll 'a' fluorescence: The chlorophyll a fluorescence was recorded between 9:00 AM – 11:00 on intact youngest fully expanded leaves with chlorophyll fluorescence meter (OS-30p+, Opti-Science, USA). Leaves were dark adapted using leaf clips, after dark adaption, the chlorophyll fluorescence parameters were measured. Leaves were exposed to strong light pulse 3500 ($\mu\text{mol photons m}^{-2} \text{S}^{-1}$). The recorded data were transferred to the computer by connecting instrument. Chlorophyll fluorescence were done on 6 plants from each treatment and three replicates for each plot (n=18). The experimental protocol of Genty *et al.*, (1989), Strasser *et al.*, (2004) and Stirbet & Govindjee (2011) was basically used for this investigation. The following calculations were made by using fluorescence parameters determined by the leaves:

$$\begin{aligned} qP &= (F_m' - F) / (F_m' - F_o') \\ NPQ &= (F_m - F_m') / F_m' \\ \Phi_{PSII} &= (F_m' - F_t) / F_m' \\ \Phi_{E_o} &= \{1 - (F_o / F_m)\} \cdot \Psi_o \\ PI_{ABS} &= (RC / ABS) \cdot \{\phi_{P_o} / (1 - \phi_{P_o})\} \cdot \{\Psi_o / (1 - \Psi_o)\} \\ PI_{CS} &= (RC / CS) \cdot \{\phi_{P_o} / (1 - \phi_{P_o})\} \cdot \{\Psi_o / (1 - \Psi_o)\} \\ DF_{ABS} &= \log (PI_{ABS}) \\ DF_{CS} &= \log (PI_{CS}) \\ J &= \Phi_{PSII} \times PFD \times (0.5) \end{aligned}$$

where PFDa is absorbed light ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$)

Table 1. Physicochemical and bioclimatic condition of study area.

Physicochemical property	Result	Physicochemical property	Result
Soil texture	Clay loam	Calcium (mg/kg of dry soil)	62.72
Sand (%)	20	NH ₄ -N (mg/kg dry soil)	0.12
Silt (%)	15	Available phosphorus (mg/kg of dry soil)	3.3
Clay (%)	65	Potassium (mg/kg of dry soil)	235
Saturation (%)	46	Relative humidity %	55-78
Precipitation (mm)	0.0	ECe (dS m ⁻¹)	1.42
Day/night temperature	36°C / 27°C ± 3	pH	7.8
Day/night hours	15/9	Humus Contents (%)	3.17

Statistical analysis

Statistical analysis: Statistical analysis of the collected data was computed using Duncan's multiple range test ($p \leq 0.05$) and analysis of variance with the help of the personal computer software packages IBM SPSS Statistics (version 20). To test the differences among mean value Duncan's test were expressed on bar graph as alphabets.

Results

Experiment was conducted using three drought stress levels. When sunflower plants were exposed to mild drought and moderate stresses it increased the photosynthetic activities in general. We could see only small differences in a couple of parameters between S.28111 and SF0049. When the drought stress effect was severe and obvious the SF0049 showed remarkably a greater reduction in almost all the PSII parameters and in photosynthetic electron transport, compared to S.28111.

There was significant difference among the genotypes in terms of performance indexes (PI_{ABS} and PI_{CS}) and driving forces (DF_{ABS} and DF_{CS}). The drought stresses showed that S.28111 is more tolerant compared to the SF0049. The substantial decline upon severe drought stress treatment was noted in performance indexes (Fig. 1). PI_{ABS} is relatively complex parameter that is related to three other parameters like; RC/ABS , ϕ_{P_0} and ϕ_{E_0} . Effects of severe drought stress were more than moderate and severe drought treatments (Fig. 1). Our results revealed that the PI_{ABS} and PI_{CS} were very sensitive parameters and indicated a minor change in photosynthetic machinery under drought stress environments. Higher decline in PI_{ABS} was found in SF0049 compared to S.28111. performance indexes of S.28111 was greater as compared to SF0049 under drought stress (D3). Reduction in PI_{ABS} due to the severe drought stress were 38% and 88% in S.28111 and SF0049 respectively.

Under normal conditions the F_v/F_m ratio vary between 0.70 to 0.85 and its value decreased below 0.7 under environmental extremes. Lower F_v/F_m ratio indicates the reduction in photosynthetic potential of plants. In this experiment F_v/F_m ratio decreased upto 48% in both sunflower cultivars. This indicated that there was a chronic phot-inhibition in sunflower genotypes under severe drought stress environment. Plants often show a marked photo-inhibitory effects, characterized by a significant decreased in the quantum yield potential. In field condition high irradiance level in combination with drought stress significantly reduced the photosynthetic efficiency (dos Santos *et al.*, 2013). The physiological state of the photosynthetic apparatus or quantum yield baseline (F_0/F_m), The ratio of photochemical to nonphotochemical quantum efficiencies (F_v/F_0), RC/CS_m (number of active reaction centre), and Electron transport rate through PS II (F_m/F_0) increased non-significantly in mild stress whereas decreased under moderate and severe drought stress compared to control (Fig. 2). $\psi_0/(1-\psi_0)$, $\phi_{P_0}/(1-\phi_{P_0})$ and ϕ_{E_0} are the sensitive parameters that showed substantial decline under mild and severe drought stress. The value of the area over the fluorescence induction curve between F_0 and F_m parameter (Area) of SF0049 decreased to 45% of the value determined in the control; in contrast the genotype S.28111 had a very low decline (18%). Likewise, F_v/F_0 values were reduced upon drought stress; SF0049 had a value that was 24% of

control whereas this value was greater upto 36% of control, in S.28111 changes in F_0 was non-significant whereas F_m decreased under drought stress compared to control in both cultivars. However, there is a substantial increase in number of inactive reaction centres. Substantial changes were observed in the 'radar plot' figure in many of the estimated parameters of both the genotypes compared to control. Fig. 2 showed that ϕ_{E_0} (quantum yield of electron transport), $\phi_{P_0}/(1-\phi_{P_0})$ (the efficiency of light reaction), $\psi_0/(1-\psi_0)$ (the efficiency of biochemical reaction) were lower and the values of V_j (relative variable fluorescence at time J) were decreased in severe drought stress compared to control environment. Further, greater and dramatical alterations in the PSII parameters were observed in SF0049 under severe drought stress environments.

In sunflower cultivars, DI_0/CS (dissipation per cross section of leaf), DI_0/RC (dissipation energy flux per reaction centre), and ABS/RC (apparent antenna size of active PSII reaction centre) were higher under severe drought stress (Fig. 3). The values of ABS/RC , DI_0/RC were higher in severe drought whereas remain almost stable in mild and moderate drought stress. The values of various other parameters were lower compared to control plants. Sunflower genotypes had lower electron transport fluxes than the control plants and they had greater number of non-active reaction centres. The drought stressed (D3) SF0049 had much higher DI_0/ABS than the control plants (Fig. 4). The damages in photosynthetic apparatus of SF0049 were drastic. This is obvious from the substantial change in the size of DI_0/ABS , ET_0/ABS and increased inactive reaction centre.

The OJIP curve obtained from severe drought stress, S.28111 showed much slower fluorescence rise and reached a much lower "P" level (Fig. 5). On the other hand, salt treated fluorescence transient curve for SF0049 was nearly flat. These data indicate multiple and greater effects of severe drought stress on SF0049. A significant increase in chlorophyll transient (OJIP) curve was observed in SF0049 under mild stress and greatly reduced under severe drought stress whereas OJIP curve decreased consistently in S.28111 under mild, moderate and severe drought stress compared to their respective control.

Results revealed that NPQ, Φ_{PSII} , qP and $rETR$ were less affected under moderate drought stress compared to severe drought stress. S.28111 showed greater activity under stressful environments compared to SF0049. The down regulation of PSII efficiency was due to reduction in maximum quantum yield in drought. The proportion of reaction centres (qP), $rETR$ and yield of PSII, decreased significantly under severe drought stress. $rETR$ is sensitive to water stress and slow test that increases with moderate to severe drought stress. Lower $rETR$ indicated that plant is not converting the absorbed light energy into efficient photochemistry and increasing dissipation as shown by SF0049 (Fig. 6). S.28111 had better $rETR$ compared to SF0049 (in terms of percentage decline) indicated better photochemistry with less dissipation under severe drought stress. Higher NPQ values to some extent protect the photosynthetic apparatus during photodamage under stressed environments. It is suggested that S.28111 performing better by using absorbed light to drive better photochemistry and protecting photodamage by slightly increased their NPQ. SF0049 do not converting absorbed light into efficient photochemistry and dissipating more energy.

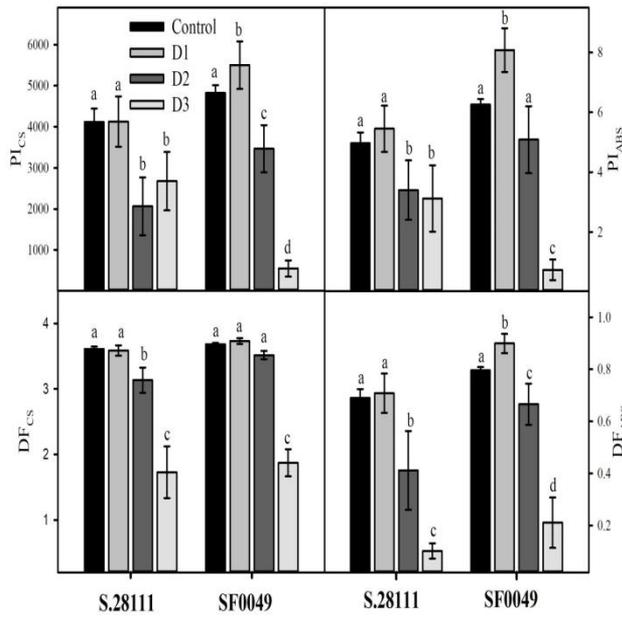


Fig. 1. Effects of well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments on Performances (PI_{ABS} and PI_{CS}) and driving forces (DF_{ABS} and DF_{CS}) of two sunflower genotypes. Vertical line on each bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at p<0.05.

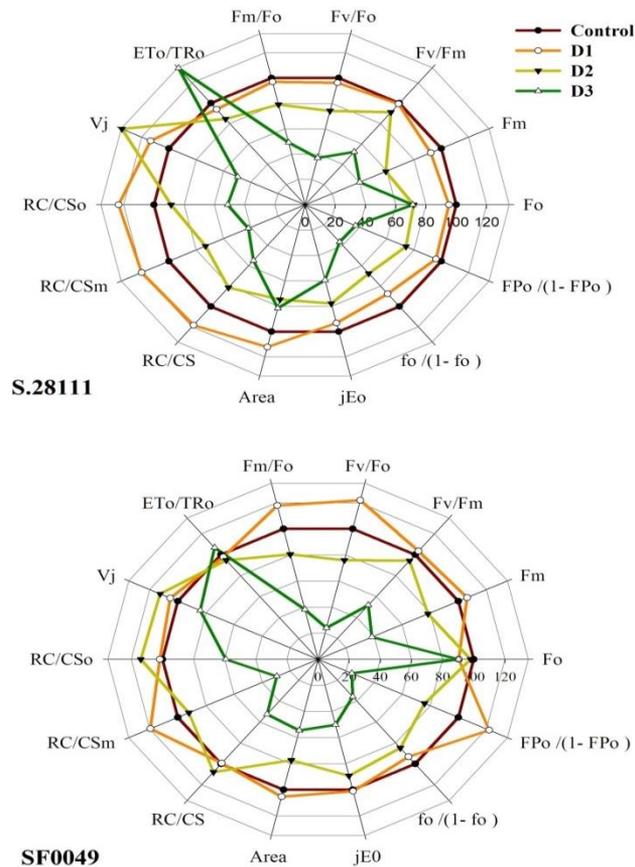


Fig. 2. A radar plot for the changes in selected parameters of PSII of sunflower genotypes under well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments. Control plants considered as 100% and all stress values were shown as percent over their respective control. Some abbreviations are as: $fo/(1-fo) = \psi_o / (1-\psi_o)$; $FPo/(1-FPo) = \phi_{Po} / (1-\phi_{Po})$; $jEo = \phi_{E0}$.

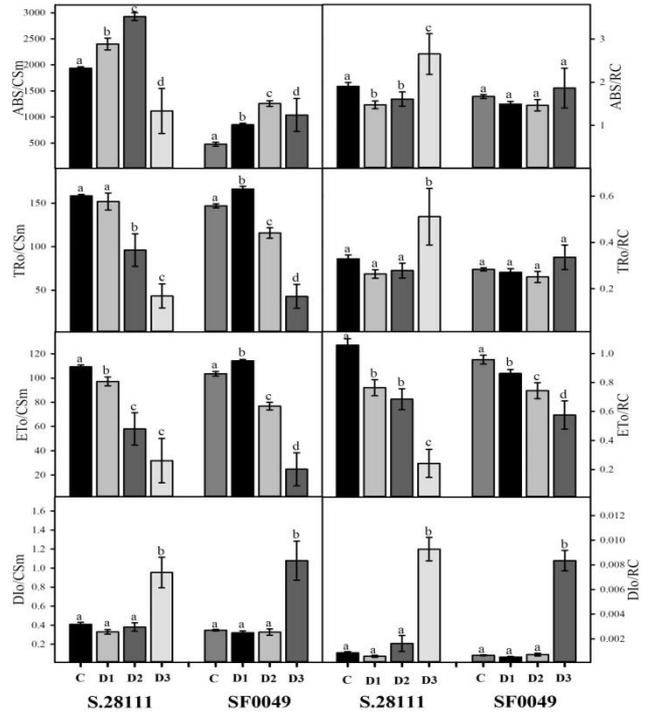


Fig. 3. Effects of well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments on energy fluxes, calculated per reaction centre (RC) and maximum cross section of leaf (CSm); ABS/CSm, TRo/CSm, ETo/CSm and DIo/CSm of two sunflower genotypes. Vertical line on each bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at p<0.05.

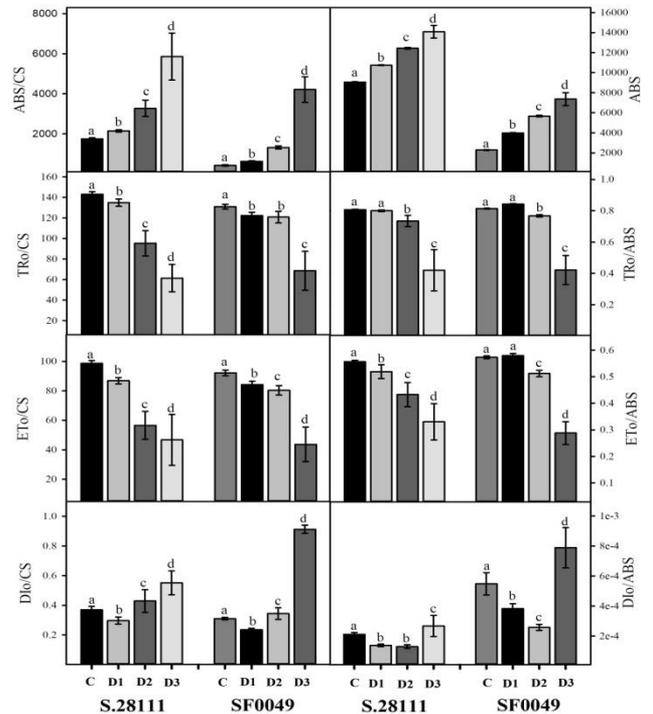


Fig. 4. Effects of well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments on phenomenological energy fluxes within a leaf and calculated per equal absorption; ABS, TRo/ABS, ETo/ABS and DIo/ABS & phenomenological activities calculated per cross section; ABS/CS, TRo/CS, ETo/CS and DIo/CS of two sunflower genotypes. Vertical line on each bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at p<0.05.

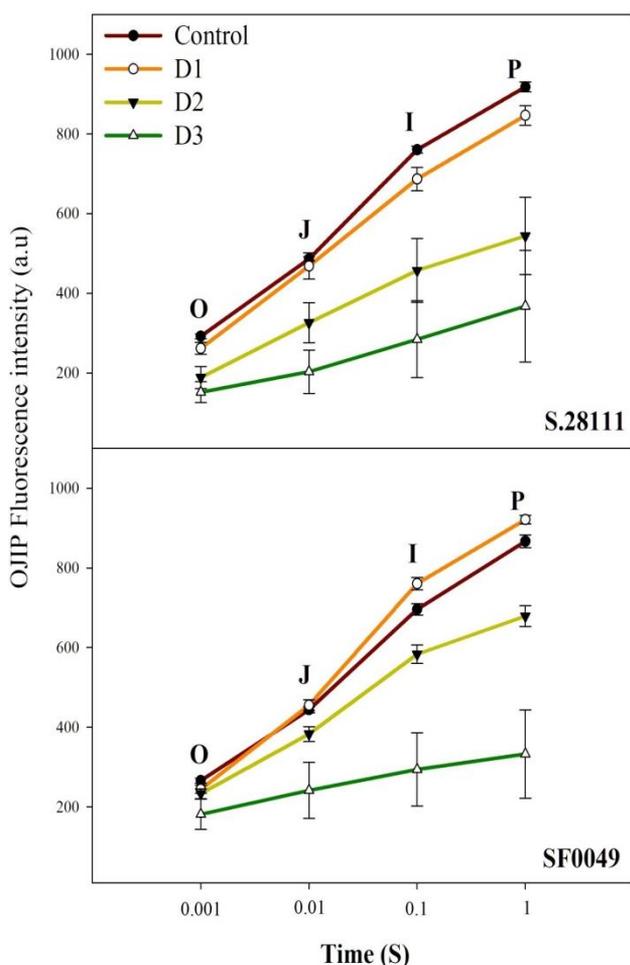


Fig. 5. Effects of well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments on chlorophyll a fluorescence induction curve of two sunflower genotypes. Vertical line on each bar represents mean standard error (\pm).

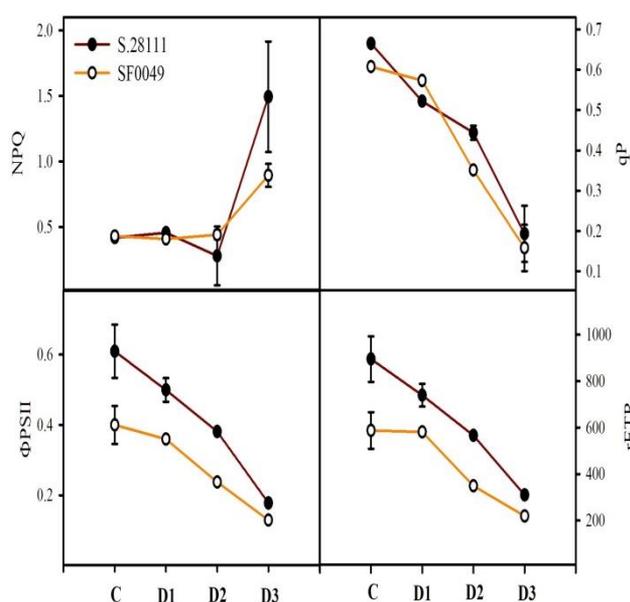


Fig. 6. Effects of well-watered, mild (D1), moderate (D2) and severe (D3) drought stress treatments on quenching parameters; NPQ, PSII, qP, and rETR of two sunflower genotypes. Vertical line on each bar represents mean standard error (\pm). Similar alphabet on the error showed t-test non-significant at $p < 0.05$.

Discussions

In modern photosynthetic research, the chlorophyll ‘a’ fluorescence becomes a valuable technique to study the performance of PSII. Current research includes the analysis of chlorophyll ‘a’ fluorescence transient through JIP-test of two sunflower genotypes to translate the drought-induced damage in photosynthetic machinery. Parameters related to chlorophyll ‘a’ fluorescence also give the picture of electron journey within the thylakoid membrane. Sunflower genotypes were grown under three drought stress levels along with well-watered (control) plants. The photosynthetic responses to desertification are complex in terms of light dependent reaction. In this research, an attempt was made to abridge the interpretation of various chlorophyll ‘a’ fluorescence parameters in crop plants.

Severe drought stress markedly decreased the PI_{ABS} , PI_{CS} , DF_{ABS} and DF_{CS} in both sunflower genotypes. Performance indexes are more sensitive parameters for drought stress tolerance and can be used to detect early drought stress. Umar and Siddiqui (2017) reported that PI was more sensitive compared to Fv/Fm ratio in sunflower cultivars under osmotic stress. Due to the alteration in certain fractions of reaction centres the greater decrement observed in SF0049 compared to S.28111 under severe drought stress. PI_{ABS} described the overall performance linked with density of reaction centres on chlorophyll basis (RC/ABS), efficiency of conversion of excited energy to electron transport (ETo/TRo-ETo) and ratio of trapping and dissipation fluxes (TRo/DIo). These three parameters are the functional characteristics of PSII. Based on results, SF0049 had drastic changes in these parameters and hence showed lower performance of PSII under severe drought stress.

Drought stress affects the various photosynthetic attributes (Umar *et al.*, 2019; Ali *et al.*, 2020). Severe drought stress greatly reduced the RC/CSm, Fm, Fo, Fv/Fm, Fv/Fo, Fm/Fo, Vj and area above the fluorescence curve in sunflower (Fig. 2). Decline in Fo was greater in S.28111 as compared to SF0049 under severe drought stress. Parameters in radar plot have shown that SF0049 is more tolerant in mild and moderate drought stresses as compared to S.28111. However, S.28111 found to be very much resistant under severe drought stress. It was observed that sunflower had inactive reaction centres under severe drought as indicated lower Fm values. In sunflower the higher quantity of inactive reaction centre and light harvesting complex of photosystem II transfer low energy to PSII core (Murkowski, 2000; Baker, 2008; Umar *et al.*, 2019). Plant often reduce its Fv/Fm ratio (maximum quantum yield) to minimize the ROS production. Our results showed that Fv/Fm ratio decreased under moderate drought stress whereas decline was more pronounced under severe drought stress. Under mild stress Fv/Fm ratio increased in SF0049 and did not change in S.28111. Greater decline in Fv/Fm ratio indicated that these plants are photochemically inactive and reaction centres are closed or damaged. Further, abiotic stress can damage the photosynthetic apparatus either by reduction in electron transport rate or photo-damage. This leads to the reduction in Fv/Fo that caused inhibition in PSI and PSII oxidation

sides (Zaghdoudi *et al.*, 2011). Fv/Fo is the same dark-adapted measurement and is more sensitive plant stress detector since it is normalized over minimum fluorescence. Fv/Fo found to be more sensitive factor in the electron transport chain (light reaction) of photosynthesis in sunflower. It was reported that disturbance in the stomatal regulation caused higher ROS production, reduction in chlorophyll contents, and lowering photosynthetic capacity under drought stress environment. This leads to the diminution in the PSII performance (Hossain *et al.*, 2013; Taibi *et al.*, 2016). Performance of PSII using OJIP test was carried out to study the effects of photosynthetic O₂ evolution under water deficit environments. Some parameters like; area PI_{ABS}, Ψ_o , ϕ_{P_o} , ϕ_{E_o} were decreased under moderate drought stress and effects were more pronounced under severe drought stress.

Area above the fluorescence curve between maximal and minimal fluorescence decreased under drought stress. Decline in area might be due to the inhibition of electron transport to plastoquinone pool (Strasser *et al.*, 2000). Fo/Fm can be used as stress indicator. Fo/Fm increased under severe drought stress indicated the initial rate of reduction of plastoquinone (Q_A) is greater than the rate of plastoquinone re-oxidation by Q_B. Fo/Fm showed that Q_B or PSI is not accepting the electron. This indicated that PSI is much more damaged compared to PSII which is more resistant. It was observed that Ψ_o , ϕ_{P_o} , ϕ_{E_o} , $\psi_o / (1 - \psi_o)$ and $\phi_{P_o} / (1 - \phi_{P_o})$ values were effected in drought stress compared to control. Results regarding the Ψ_o or ET_o/TR_o (yield of electron transport per trapped excitation) showed reduction in mild stress whereas increased under severe stress (Fig. 2). Decline in Ψ_o might be due to the reduction of electron flow from reduced Q_A to Q_B under mild and moderate drought stress. The greater increment in Ψ_o might be due to the higher dissipation in severe drought stress. ϕ_{E_o} was also decreased under drought stress compared to control plants. The efficiency of light reaction and the efficiency of biochemical reaction were greater in S.28111 as compared to SF0049 under severe drought stress. Initially the rate of biochemical and light reaction was increased in SF0049 and decreased suddenly under severe stress. This indicated that SF0049 has tolerance against moderate drought stress and cannot bear severe drought stress compared to S.28111. This confirms the greater decline in net photosynthetic rate in SF0049 under severe drought stress.

S.28111 was found to be tolerant cultivars in terms of better photosynthetic capacity under drought stress. To explain the fluorescence responses, four types of phenomenological (defined per CS and ABS) and specific energy fluxes (defined per RC) were studied (Figs. 3 & 4). These parameters referred to the reaction centre in the membrane and deals with the energy fluxes absorption, trapping, electron transport and dissipation per reaction centre and per excited cross section of leaf (Fig. 3) whereas phenomenological energy fluxes per equal absorption and phenomenological activities per cross section (Fig. 4). Fraction of reaction centre (ABS/RC and DI_o/RC) becomes dissipative centres to prevent the photo-oxidative damage of photosynthetic machinery. A significant difference was observed in ABS/RC and DI_o/RC in both sunflower genotypes under drought stress. ABS/RC referred to the

functional size of antenna complexes and give evidences about the average amount of chlorophyll absorption. Greater values of ABS/RC represent the reduced active reaction centre under severe drought. This is due to the disturbance in the proton gradient in thylakoid membrane during oxidative stress and a portion of active reaction center becomes as dissipative centre and change the violaxanthin into zeaxanthin to avoid the oxidative damage under higher absorption (Laisk & Oja 2000; Siddiqui *et al.*, 2014). Electron transport per RC was decreased significantly under drought stress compared to control. It is suggested that sunflower cultivar SF0049 maintain better electron transport rate under drought stress to avoid higher energy absorption flux.

Electron transport per ABS and per CS was decreased under severe drought stress. Greater decline was observed in SF0049 compared to S.28111 under severe drought stress. Dissipation per CS and per ABS increased under stressful environments. Highest dissipation was observed in SF0049 in severe drought stress. These results indicated the action mechanism of drought stress, the inhibition of electron transport beyond Q_A- (ET_o/CS and ET_o/ABS) in SF0049. Small changes in the antenna size or chlorophyll density (ABS/RC, TR_o/RC) and dissipation (DI_o/RC, DI_o/CS_m, DI_o/ABS) were found in both sunflower genotypes under mild and moderate drought stress. It can be suggested that drought stress inhibit the electron flow from Q_A to Q_B on the acceptor side of PSII especially in SF0049 and not affect the donor side of PSII in all cultivars. The light harvesting of antenna pigment of PSII less reduced by drought stress in S.28111 compared to SF0049. A consistent decline in the trapping efficiency was observed in S.28111 whereas in SF0049 a sharp decline in severe drought stress was observed (Fig. 4). lower ET/ABS values in SF0049 expressed higher inactive reaction centres. It was proposed that higher number of inactive reaction centres was the reason for greater dissipation of absorbed light per RC, CS and CS_m. All these energy flux ratios suggested that the number of inactive centres have increased under severe drought stress in sunflower.

The PSII photochemistry can be quantified through OJIP test. The minimal fluorescence 'O' represents the state when Q_A is in oxidized state. Minimal fluorescence was not much effected in SF0049 whereas in S.28111 minimal fluorescence was decreased significantly. O to J rise represents the reduction of Q_A by PSII. A small deflection in this rise was observed in mild drought stress whereas in severe drought this rise was almost flat. This rise characterized the photochemical reduction of pheophytin and Q_A. Next step from 'J' to 'I' caused by photo-electro-chemical quenching in through which plastoquinone pool is reduced by the PSII. This rise represents the photochemical reduction of Q_B. the initial acceptor in the Q_A and Q_B- reaction can be represented by 'I'. It starts with the ratio 1:0 i.e. Q_B: Q_B- and this ends with reverse ratio (0:1). When electron transport from Q_A to Q_B was prevented by drought stress then reduction in QA occurs quickly and leads to a greater increase in 'J' stage due to photochemical quenching. This step evident for the down regulation of electron transport beyond Q_A in sunflower genotypes. Papageorgiou & Govindjee

(2004) reported that fluorescence rises to 'P' is due to traffic jam situation on PSI acceptor. This level 'I' to 'P' represents the values for Q_A , Q_B - and PQH_2 . This level is associated with the electric transthylacoid potential in PSI that is affected by the re-oxidation of PQH_2 to PQ and plastoquinone pool size. Decline at this level suggested that drought-induced damages caused reduction in the efficiency of PSI for the acceptance of electrons. Mehler's reaction has protective role in PSII and enable efficient cycling of water in the cell during drought stress (Lovelock & Winter, 1996). It was suggested that greater susceptibility of S.28111 to severe drought stress may be due to the reduced capacity of Mehler's reaction.

Stress-induced damage in PSII cause interruption in the Calvin cycle. Greater interruption may lead to irreversible restoration (Takahashi & Murata, 2005). The damages in SF0049 were greater under severe drought stress environment and PSII restoration is prevented. RC/CSm and RC/CSo of PSII increased initially and then decreased substantially under severe drought stress. Drought stress substantially affected the chlorophyll 'a' fluorescence and net photosynthetic rate in sunflower genotypes. This might be due to the reduction in leaf water potential under drought. However, PSII is rather resistant to drought stress (Shangguan *et al.*, 2000) as shown by sunflower genotype S.28111.

Drought stress damaged the macromolecules including thylakoid proteins and leads to the degradation. This degradation caused reduction in trapping, electron transport and CO_2 fixation (Abdalla & El-Koshiban, 2007). Lower chlorophyll a/b ratio indicated the higher PSII antenna complexes and permitting more absorption of photon that ultimately leads to excessive electrons in PSII. S.28111 showed highest tolerance in PSII activity compared to SF0049 under severe drought stress.

Lower intake of CO_2 under water deficit condition reduces the rubisco activity that leads to inhibition in net photosynthetic rate (Dulai *et al.*, 2006). Greater photosynthetic rate under stressful environments is needed for the plants tolerance. Sunflower genotype showed decline in their electron transport rate in thylakoid membrane and decreased effective quantum yield of PSII photochemistry. PSII thought to play important role in the photosynthetic response under water shortage environment (Baker, 2008). It was observed that NPQ, qP, Φ_{PSII} and rETR were less effected under mild and moderate drought stress (Fig. 6). However, PSII photochemistry of sunflower genotypes was more effected under drought stress. S.28111 was shown to be more tolerant in terms of NPQ, qP, Φ_{PSII} and rETR during sub-optimal conditions. Drought stress changes in photochemical quenching leads to alteration in fluorescence kinetics (Zlatev & Yordanov, 2004). Emission of fluorescence from light reaction of photosynthesis is very sensitive indicator of PSII performance (Kalaji *et al.*, 2018). rETR is sensitive to water stress and is slow test and its sensitivity increases with the moderate to severe stresses. The qP represents the proportion of excitations captured by traps and their translation to chemical energy in PSII. The decline in qP was due to the drought induced modulation on the the efficiency of overall photochemical process and functional state of PSII.

qP value indicates the proportion of inactive reaction centres of PSII (Moradi & Ismail, 2007). Decline in qP showed the separation of light harvesting complex from PSII

reaction centre during abiotic stress (Wu *et al.*, 2010). The conversion of light energy into chemical energy is becomes low under drought stress. To improve greater photosynthesis, plant try to absorb more light and unfortunately excessive absorption increased the amount of inactive reaction centre. In this scenario, plant activates its regulatory mechanism, the non-photochemical quenching (NPQ). This tolerance mechanism is believed to be an important mechanism to remove surplus energy quickly. Slight increase in NPQ protect the chloroplast from excessive energy damage during drought stress. It is suggested that S.28111 performing better by using absorbed light to drive better photochemistry and protecting photodamage by increasing NPQ. SF0049 do not converting absorbed light into efficient photochemistry and dissipating more energy. The greater photoprotective process during photosynthesis caused the down regulation of quantum yield of PSII (Φ_{PSII}).

Conclusion

Drought stress negatively affected PSII in sunflower genotypes. The influence of stress was dependent on the duration of water shortage on the genotypes. Primary response of photosynthetic machinery against the drought stress can play a vital role in the plant tolerance. This experiment showed that some parameters of chlorophyll a fluorescence significantly changed in drought environments. The higher deactivation of reaction centres, increased absorption based on CS and ABS, energy trapping and rate of electron transfer decreased under drought stress environments. Chlorophyll 'a' fluorescence parameters showed that photosynthetic machinery of genotype S.28111 was more tolerant as compared to genotype SF0049 under severe drought stress. Despite the more commonly used parameters like Fv/Fm and PI_{ABS} our results indicated that other parameters related to PSII photochemistry are also important to advance our knowledge regarding mechanism of photosynthesis under drought stress in field. Finally, a better understanding of stress related traits could be useful to develop new genotypes of crop plants to gain greater agricultural productivity in arid and semi-arid environments of the world.

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