

COMPARATIVE PHYSIOLOGICAL ASSESSMENT OF SOME EDIBLE OIL-SEED CROPS UNDER DROUGHT STRESS ENVIRONMENT USING FLUORESCENCE AND IR IMAGING TECHNIQUES

FIZA ALI, MUHAMMAD UMAR AND ZAMIN SHAHEED SIDDIQUI*

Stress Physiology and Phenomic Center, Department of Botany, University of Karachi, Karachi-75270, Pakistan

**Corresponding author's email: zaminss@uok.edu.pk*

Abstract

Comparative physiological assessment of some edible oilseed crops under drought stress environments were performed. In this regard, fluorescence and IR thermal imaging techniques were used. Seeds of each sunflower SF0049, SF0054 and soybean NARC-II, William-82 cultivars were germinated. Followed by uniform seedlings of each cultivar were transplanted in rectangular pots of an area 480cm². Three weeks old plants were subjected to drought stress for the next five days. Chlorophyll 'a' fluorescence, IR images, relative water content (RWC) and stomatal conductance (g_s) were recorded. In drought stress, NARC II and William-82 showed 26-31% reduction in RWC as compared to 18-20% reduction in SF0049 and SF0054. Similarly, 67- 87% reduction in stomatal conductance was observed in soybean and 59-72% were recorded in sunflower cultivars. Further soybean cultivars displayed 44.4% to 85.7% decrease in performance index (PI) and dark-adapted quantum yield (Fv/Fm) as compared to 40% to 87% in sunflower cultivars. Maximum yield of photochemistry of PSII and chlorophyll content index were also reduced up to 28-68% in all tested cultivars under drought stress. Non-photochemical quenching, heat dissipation, and leaf temperature were increased over control under drought stress showing maximum in soybean as compared to sunflower. The importance of comparative physiological assessment using fluorescence and IR thermal imaging techniques are discussed.

Key words: Screening, Physiological performance, Stress environment, PSII.

Introduction

Sunflower (*Helianthus annuus* L.) is the fourth largest source of edible oil in the world and its production is still expanding. It was reported that some sunflower cultivars tolerate abiotic stress like salinity and drought (Umar & Siddiqui, 2018). Studies have shown that sunflower yield is reduced up to sixty percent due to abiotic stress (Mazahery-Laghab *et al.*, 2003; Krizmanic *et al.*, 2012). Similarly, soybean is another important oil seed crop contributing significant share in the consumption of edible oil production throughout the world. However, due to ecological extremes like drought and salinity, its yield is greatly reduced (Dai, 2013; Foyer *et al.*, 2016). Studies about the drought stress on oil seed crops are restricted to growth and development.

In drought stress, variation in physiological functions often show a great diversity in plant's life indicating sensitivity and resistibility of the crop plants (Ekmekci *et al.*, 2005; Siddiqui *et al.*, 2014). Moreover, drought is a limiting factor for plants that affect water content, disturb water potential, turgor pressure and stomatal regulations which not only affects photosynthesis but also hamper other metabolism (Jaleel *et al.*, 2008; Siddiqui *et al.*, 2015). For instance, PSII activity and electron flow are hindered due to over excitation and photo-oxidation damage to PSII reaction centers (RC) under drought stress (Akhkha, 2009; Umar *et al.*, 2019).

Chlorophyll fluorescence and IR thermal sensing are non-destructive, advanced, accurate and less time-consuming phenotyping techniques in plant physiology and are being used for the screening of stress tolerant plants under abiotic stress environments. Chlorophyll fluorescence is used to analyze the PSII and PSI activities which demonstrate photosynthetic performance of plants in drought stress (Baker, 2008; Munns *et al.*, 2010;

Siddiqui *et al.*, 2014; Umar *et al.*, 2019). Similarly the leaf temperature is an immediate indicator of internal water status under drought stress. Thus, IR thermography could be a better non-destructive method which may be used to show plant response against the water stress (Sirault *et al.*, 2009). Hence present study on the comparative physiological assessment using fluorescence and IR thermal imaging techniques would be helpful for the rapid screening of oil seed cultivar under drought stress environment rather than detailed experiments.

Materials and Methods

Experimental design and treatment application:

Experiment was carried out during the period 2017-2018 at the Stress Physiology and Phenomic center, Department of Botany, University of Karachi, Pakistan. Two cultivars of each industrially important oil seed crop plants i.e. sunflower (SF0054 and SF0049) and soybean (NARC-II and William-82) were surface sterilized with sodium hypochlorite solution (2%) and then repeatedly washed before sowing. Later seeds were germinated in a seedling tray in the greenhouse at a temperature of 26-28 ± 4°C, 60-70% humidity and a photoperiod of 14/10 hours (day/night). Light intensity varied from 250-400 μmol photon m⁻² s⁻¹ and ten seeds per pot were germinated in rectangular pots. One-week old seedlings were transplanted to the other rectangular pots and seedlings were allowed to grow in natural conditions. Total ten plants for each treatment and control were used. Plants were subjected to drought 15 days after sowing by stop watering and physiological data were examined daily for the next five days. All treatments and control were replicated three times. The whole experiment was repeated twice.

Relative water content: Relative water content (RWC) was measured using Barrs & Weatherley, (1962) method. Fresh weight (FW) of four randomly selected leaves (area; 4 cm²) from each control and drought stress plants was measured at once after harvesting. For turgid weight (TW) sample was kept in 90 mm plastic Petri-plate having distilled water for the next 12 hours. Afterward, leaf samples were dislodged from petri-plate and at once excessive water on the surface was dried using tissue paper to measure turgid weight. For the dry weight (DW) measurement, leaves samples were oven dried at 80°C for 48 hours and weighed. RWC was finally calculated by the formula;

$$\text{RWC} = (\text{FW} - \text{DW} / \text{TW} - \text{DW}) \times 100$$

Chlorophyll fluorescence and stomatal conductance: Chlorophyll fluorescence transients in twenty randomly selected leaves were conducted with chlorophyll fluorescence meter (OS-30 p+ model Opti-Science, USA). Before the measurements, leaves were adopted in 30 minutes dark using clips. Performance index (PI_{abs}), minimal fluorescence (F_o), maximum primary quantum yield of photochemistry of PSII (F_v/ F_o), photochemical quenching (qP), dark adapted quantum yield of PSII (F_v/ F_m) were calculated (Maxwell and Johnson 2000). The JIP test data is used to calculate different parameters of photosystem II (Strasser *et al.*, 2010; Stirbet & Govindjee, 2011).

For the stomatal conductance measurement, twenty randomly selected leaves from each treated and control plants were used. The data was collected from the middle and lower part of the leaf surface using porometer Model SC-1, Decagon.

IR thermal images: Infra-red images of each sunflower (SF0054 and SF0049) and Soybean (NARC-II and William-82) drought stressed plant and control were taken by IR thermal camera; FLIR-E5 (FLIR Systems, USA) before harvesting. The system was optimized in 30 minutes before the measurement and then images were taken later on. Images were directly extracted from the camera into the computer and a report was generated using FLIR Software 2.10.

Chlorophyll content index: The chlorophyll content index of each treated and control leaves samples was recorded using chlorophyll content meter Model CL-01 (Hansatech instruments, UK). Four measurements were taken from each plant.

Statistical analysis

All the data was subjected to statistical analysis and it was calculated using computer-based software packages SPSS Statistics (version 20.0). The Duncan, (1955) multiple range tests ($p \leq 0.05$) were conducted as follow up tests to find the differences among mean values and represented as (ns) non-significant, (*) as significant at $p \leq 0.05$, and (**) $p \leq 0.01$.

Result

Oil seed cultivars were exposed to drought stress and their phenotyping was performed using high tech IR camera and chlorophyll fluorescence. The drought stress showed significant decline in relative water contents (RWC) of treated plants (Fig. 1). Soybean NARC-II showed greater reductions (31%) in RWC as compared to other treated plant cultivars. However, a minimum decrease (18%) was found in SF0054. It was observed that RWC decreased in all the treated plans as the intensity of stress increased (Fig. 1). Greater reduction (87%) in stomatal conductance was observed in NARC-II as compared to other treated plants. Sunflower SF0054 maintained 41% stomatal conductance under drought stress compared to 13% NARC-II, 23% William-82 and 50% in SF0049.

Under drought stress, chlorophyll content index (CCI) showed significant reduction in soybean NARC-II compared to sunflower (Fig. 2). SF0054 showed a greater reduction (61%) in CCI. It was observed that William-82 showed substantially better CCI (28%) against severe drought (5th day). Dissipated energy flux per cross section (DI_o/CS) increased in drought treated plants compared to control (Fig. 2). William-82 showed greater increased DI_o/CS values in stress conditions compared to NARC-II, SF0049 and SF0054.

Drought stress showed substantial decline in maximum quantum efficiency of photosystem II (F_v/F_m) of both oil seeds crop plants (Fig. 3). Maximum decrease in the F_v/F_m was noted on the 5th day of drought. Sunflower cultivars reflected better photochemical efficiency than soybean. NARC-II showed maximum reduction (81.2%) in F_v/F_m compared to William-82, SF0049 and SF0054. Performance index (PI_{abs}) in all tested cultivars was greatly affected by drought stress and showed significant decreases compared to control (Fig. 3). However, sunflower cultivars maintained better PI_{abs} value (83.3 to 85.5%) compared to 44.4 to 85.7% in soybean.

In stress, plants showed a substantial reduction in photochemical quenching (qP) compared to unstressed condition (Fig. 4). Greater reduction was found in soybean varieties NARC-II and William-82 compared to sunflower. SF0054 showed a minimum reduction in qP while NARC-II showed greater reduction under severe drought stress. Non-photochemical quenching (NPQ) showed greater variation in plants when they were exposed to drought stress (Fig. 4). In all tested plants, a substantial increase in NPQ was observed under drought compared to unstressed plants. However, a greater increase in NPQ was recorded in NARC-II compared to William-82, SF0049 and SF0054.

Present results expressed that the OJIP fluorescence curve substantially declined under drought stress (Fig. 5). Sunflower cultivars showed better OJIP fluorescence curve which led to better photosynthetic performance under mild stress compared to soybean. In soybean cultivars OJIP curve had an almost linear under moderate and severe drought stress. The lowest peak of OJIP curve was observed in soybean William-82 under severe drought stress.

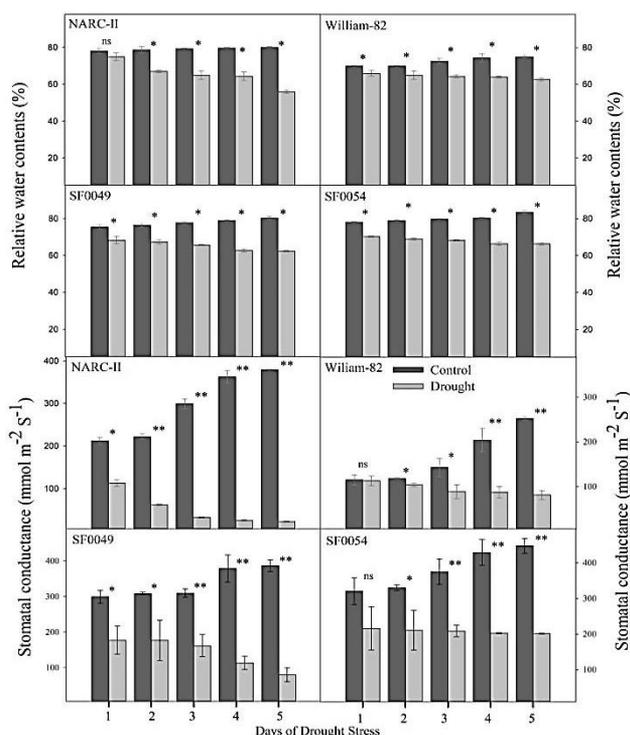


Fig. 1. Changes in RWC and g_s of soybean (NARC-II & William-82) and sunflower (SF0049 & SF0054) genotypes under progressive drought stress environments. Vertical lines on bar represented the standard error whereas asterisk (*) stand for significant difference and (ns) stand for non-significant difference among the control and drought treatment.

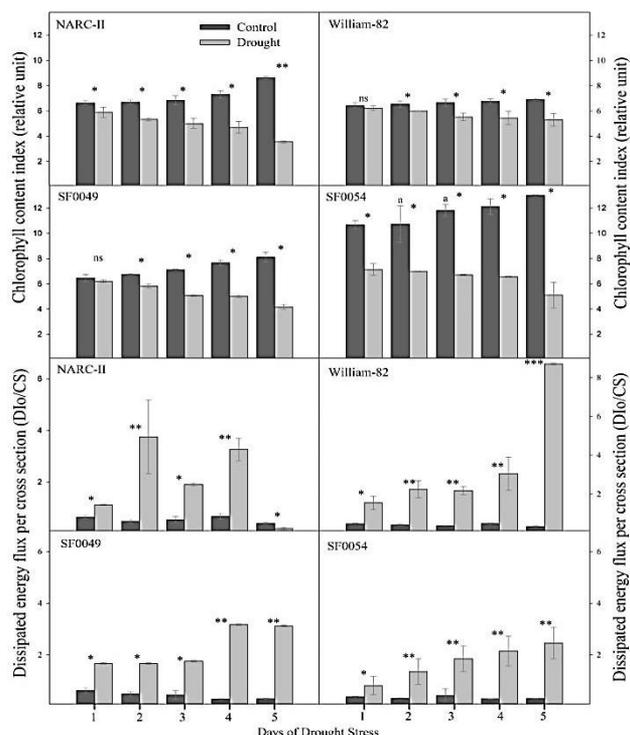


Fig. 2. Changes in chlorophyll and dissipation of soybean (NARC-II & William-82) and sunflower (SF0049 & SF0054) genotypes under progressive drought stress environments. Vertical lines on bar represented the standard error whereas asterisk (*) stand for significant difference and (ns) stand for non-significant difference among the control and drought treatment.

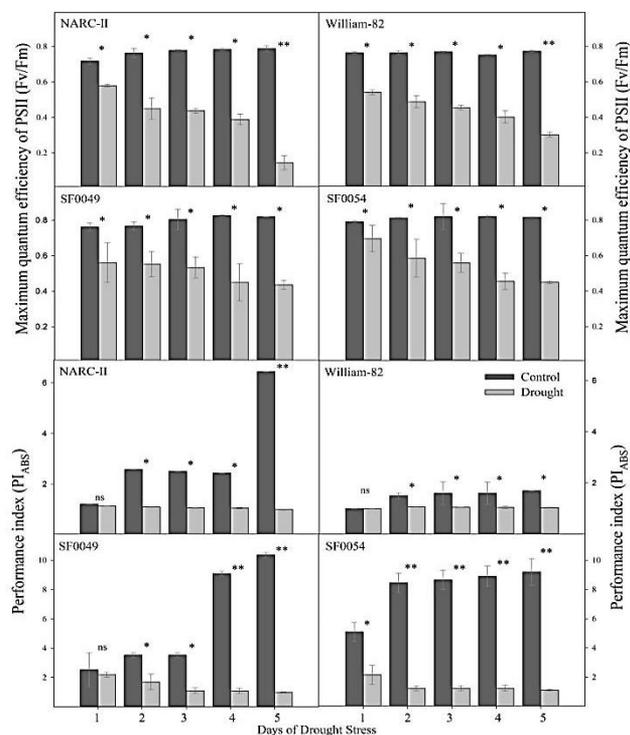


Fig. 3. Changes in F_v/F_m and PI_{ABS} of soybean (NARC-II & William-82) and sunflower (SF0049 & SF0054) genotypes under progressive drought stress environments. Vertical lines on bar represented the standard error and asterisks (* & **) stand for significant differences at 0.05 and 0.01 level respectively whereas (ns) stand for non-significant difference among the control and drought treatment.

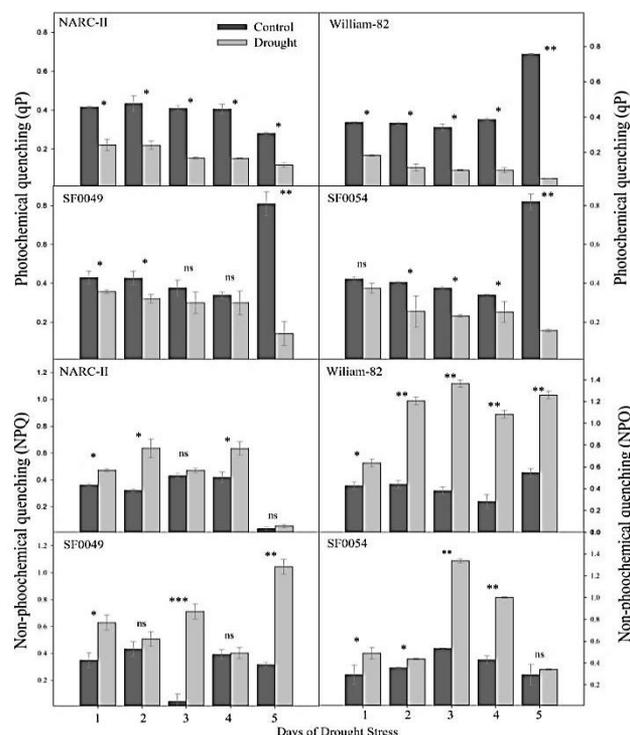


Fig. 4. Changes in q_P and NPQ of soybean (NARC-II & William-82) and sunflower (SF0049 & SF0054) genotypes under progressive drought stress environments. Vertical lines on bar represented the standard error and asterisks (* & **) stand for significant differences at 0.05 and 0.01 level respectively whereas (ns) stand for non-significant difference among the control and drought treatment.

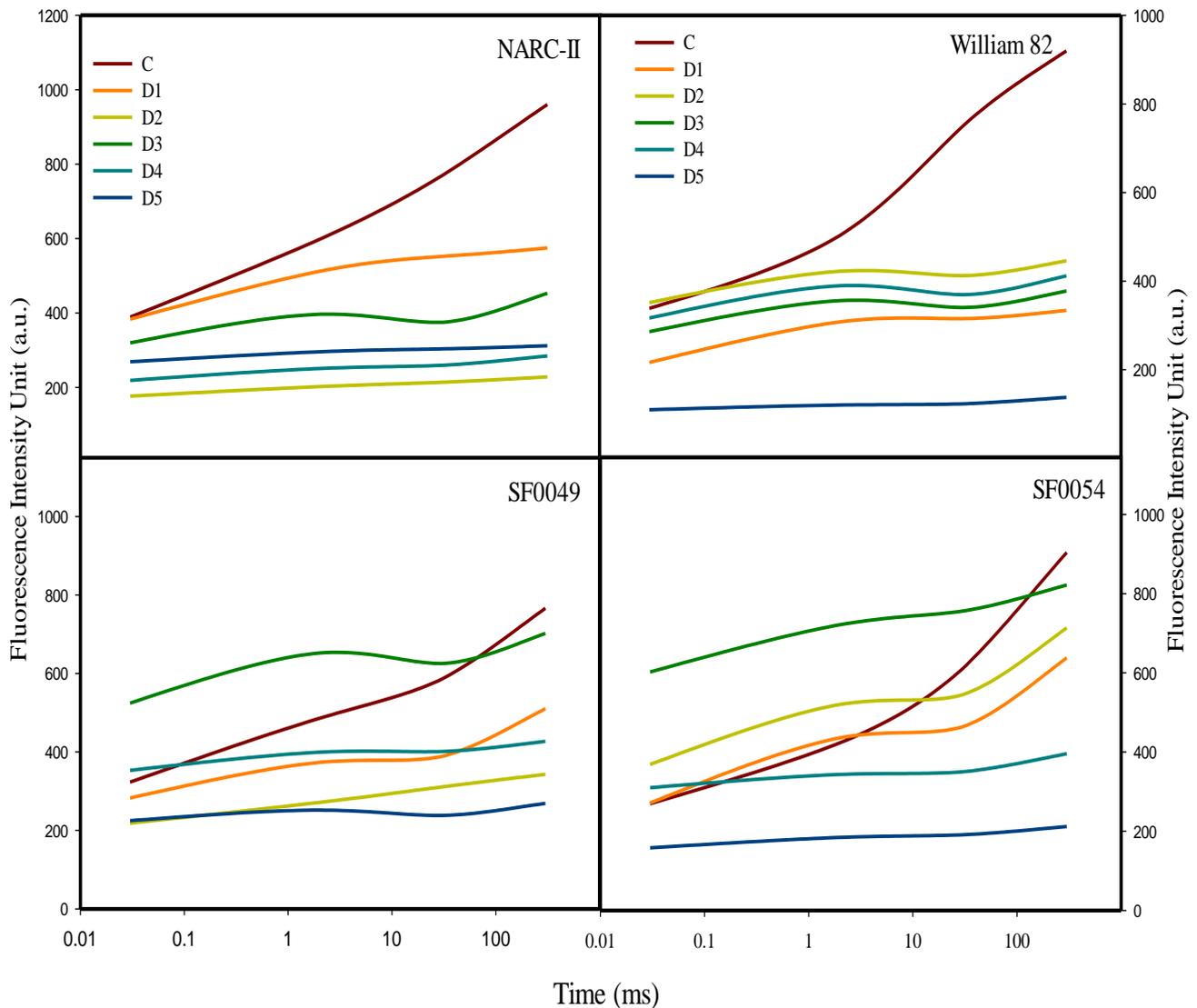


Fig. 5 Chlorophyll a fluorescence induction curve (OJIP rise) of soybean (NARC-II & William-82) and sunflower (SF0049 & SF0054) genotypes under progressive drought stress environments. D1 to D5 represents the days of drought.

Result showed that minimal fluorescence (F_0), Ratio of variable fluorescence at K-step to the amplitude $F_J - F_0$ (W_k), efficiency with which a PSII trapped electron was transferred until PSI acceptors ($1 - V_i$), quantum yield baseline (F_0/F_m) increased in stress condition compared to control in soybean and sunflower cultivars (Figs. 6 and 7). The greater increment in the F_0 was observed in sunflower cultivars under mild drought stress compared to soybean. Results showed that William-82 had the highest W_k values in stress condition among the tested plants. Comparatively, W_k was greatly increased in soybean compared to sunflower cultivars. NARC-II showed lowest increments in F_0/F_m values in stress condition as compared to William-82, SF0049 and SF0054.

The Maximal fluorescence (F_m) quantum yield of electron transport flux from Q_A to Q_B ($1 - F_J/F_m$), quantum yield of electron transport flux until the PSI electron acceptor ($1 - F_V/F_m$), Probability that a trapped electron transfers an electron into the electron transport ($1 - V_J$), Electron transport rate through PS II (F_m/F_0) and

Electron transport flux from Q_A to Q_B per PSII (ET_0/CS) decreased in all tested cultivars under drought stress environment (Figs. 6 and 7). In comparison, sunflower cultivars had better F_m , F_m/F_0 and $1 - F_J/F_m$ values compared to soybean. William-82 showed greater reduction in $1 - V_J$ values in stress condition. NARC-II had shown lowest ET_0/CS values in stress condition compared to William-82, SF0049 and SF0054.

IR thermography of drought exposed to soybean and sunflower cultivars were performed (Fig. 8). Red color in images displayed higher temperature (low RWC) while blue color expressed lower temperature (higher RWC) in leaf. Plants under drought stress exhibited the greater temperature compared to unstressed plants. Data obtained from thermal images have shown that soybean plants exhibited higher temperature under drought conditions compared to sunflower (Fig. 8). William-82 exhibited highest average leaf temperature under severe drought stress compared to other tested cultivars. The mean temperatures of the IR images were presented in Table 2.

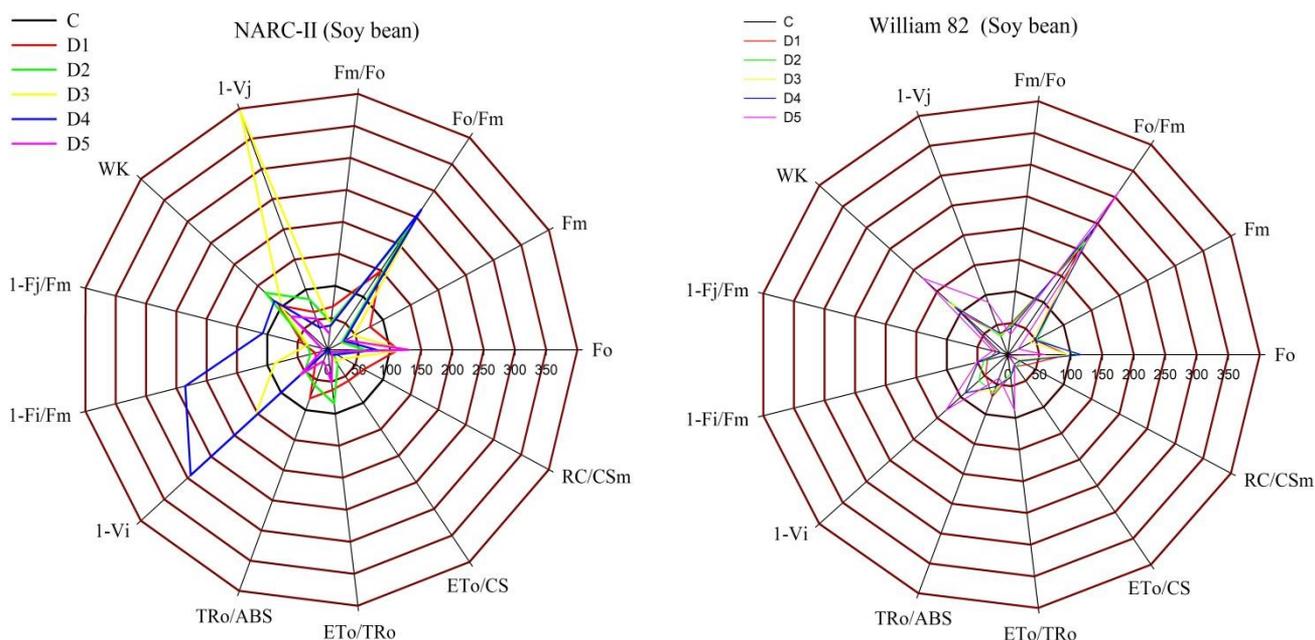


Fig. 6. A radar plot showed the changes in selected parameters of chlorophyll a fluorescence of Soybean plants under various drought stress environments. Percent values are presented over control plants (values of control plants were considered as 100 percent).

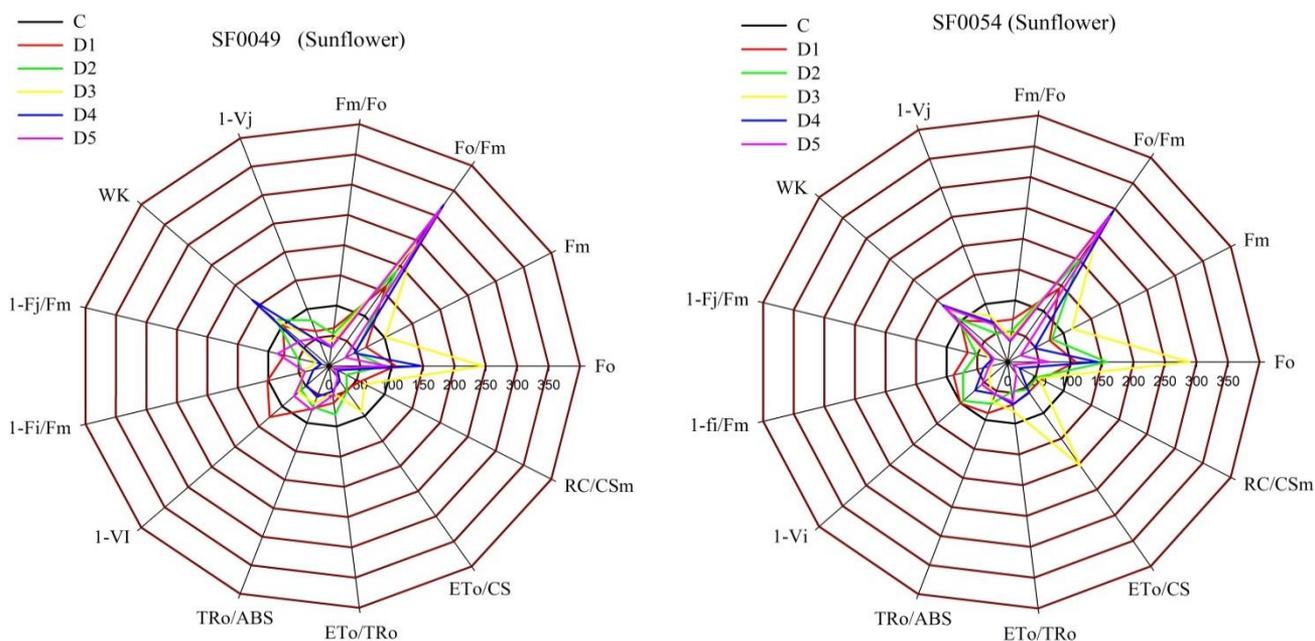


Fig. 7. A radar plot showed the changes in selected parameters of chlorophyll a fluorescence of sunflower plants under various drought stress environments. Percent values are presented over control plants (values of control plants were considered as 100 percent).

Discussion

To make the screening technique quicker and easier against drought stress in oilseed plants, chlorophyll fluorescence and IR thermal imaging technique has been used. In this study we focused on the damage in photosynthetic machinery and heat dissipation under drought stress environments. Chlorophyll *a* fluorescence technology allows to get evidence on the photosynthetic electron transport chain including PSII donor side, electron transfer between PSI and PSII acceptor side (Strasser *et al.*, 2010; Kan *et al.*, 2017).

RWC and g_s used to examine the stress tolerance in different cultivars and is a better indicator of leaf water status. Study revealed that RWC and g_s were reduced under salt and drought stresses (Umar & Siddiqui, 2018). Crop plants which retain RWC under drought stress are supposed to be tolerant. Sunflower variety SF0054 retained greater RWC and had better g_s compared to other cultivars in drought stress. Studies revealed that decline in RWC was related to cell membrane properties and its adaptability to environmental changes such as drought (Liu *et al.*, 2002; Blokhina *et al.*, 2003). RWC is a good indicator of drought tolerance or adaptation in various plant species (Siddiqui *et al.*, 2014; Naz *et al.*, 2020).

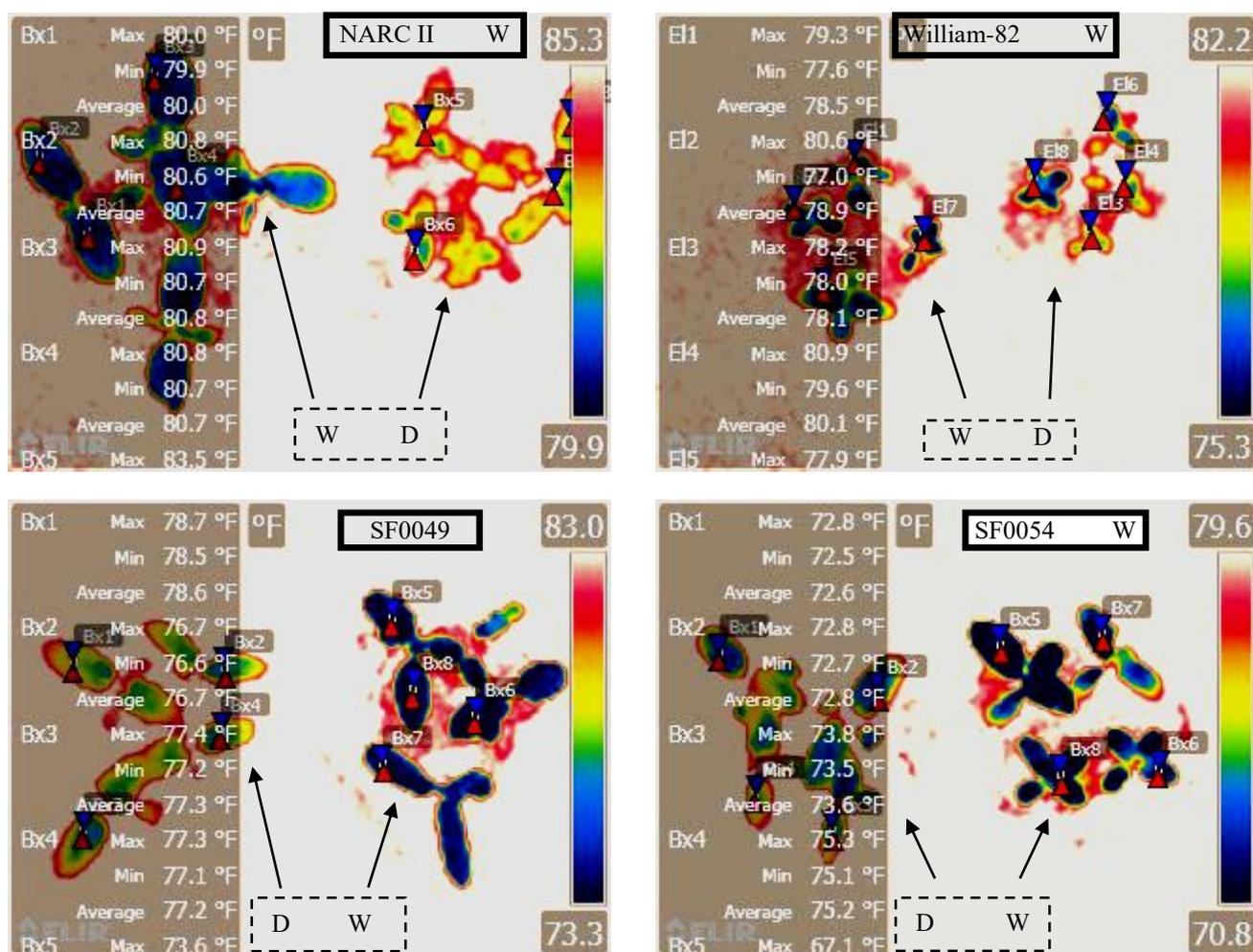


Fig. 8. High tech IR images of sunflower (SF0049, SF0054) and soybean genotype (NARC-II, William-82) under stressed (5 days drought = D) and unstressed environments (W). Images were taken by FLIR camera E5 series. (Report generated by FLIR Software 2.10).

Table 1. Nomenclature and definitions commonly used parameters by the JIP-test for the analysis of chlorophyll *a* fluorescence transient OJIP with their physiological significance.

Fluorescence parameter	Physiological significance
F _o	Minimal recorded fluorescence intensity when all PSII RCs are open
F _m	Maximal recorded fluorescence intensity when all PSII RCs are closed
F _o /F _m	Quantum yield baseline
F _m /F _o	Electron transport rate through PSII
1-V _J	Efficiency with which a PSII trapped electron is transferred from Q _A to PQ
1-V _I	Efficiency with which a PSII trapped electron is transferred until PSI acceptors
1-F _j /F _m	Quantum yield of the electron transport flux from Q _A ⁻ to PQ
1-F _i /F _m	Quantum yield of the electron transport from Q _A ⁻ to final PSI acceptors
W _k	Ratio of variable fluorescence at K-step to the amplitude F _J -F _o
TR _o /ABS	Maximum quantum yield for primary photochemistry (at t=0)
ET _o /TR _o	Probability that a trapped excitation moves an electron further than Q _A ⁻
ET _o /CS	Electron transport flux per cross section
RC/CS _m	Density of reaction centre (Q _A reducing PSII reaction Centre)

CCI showed significant reduction in all tested cultivars. The soybean cultivars showed greater reduction in CCI compared to sunflower. Furthermore, Photosynthetic electron transport and PSII reaction centers were damaged under drought stress. To identify the stress induced damage on the acceptor side of PSII, the JIP parameter D_{io}/RC was observed. Results

indicated that some RCs were inactivated and the dissipation per RC was enhanced under drought stress. D_{io}/CS was increased in soybean compared to sunflower. Results from CCI and D_{io}/CS suggested that soybean had a greater inactive reaction center and found to be sensitive against drought stress compared to sunflower cultivars.

Table 2. Alterations in temperature of sunflower and soybean genotypes in response to drought stress environments.

Genotypes		Max. temperature	Min. temperature	Avg. temperature
NARC II	Control	81.9	82.1	82.3
	Drought	82.8	81.8	82.6
William-82	Control	63.2	62.4	62.8
	Drought	66.7	67.0	67.4
SF0049	Control	77.0	74.3	75.7
	Drought	81.7	80.8	81.2
SF0054	Control	71.1	68.1	69.6
	Drought	76.7	74.0	73.35

Therma CAM researcher Pro version 2.10 software was used

Various parameters of JIP-test are sensitive to drought stress and explain the physiological status of the plants. Fv/Fm is an important indicator of photo-inhibition under stressed environments. Drought stress showed substantial declines in Fv/Fm in both oil seeds plants. In comparison, sunflower cultivars showed better photochemical efficiency than soybean cultivars. It was reported that PSII efficiency was greatly reduced under environmental stresses (Umar *et al.*, 2019; Umar & Siddiqui, 2020). Lower Fv/Fm indicated photochemical inactivation and damaged reaction centers in soybean plants under drought stress. PI_{abs} are very sensitive stress indicators that detect the stress in plants even before the visible symptoms appear on the leaves (Clavel *et al.*, 2006; Christen *et al.*, 2007). PI_{abs} could be a better parameter to monitor and evaluate the effects of drought stress on the overall photosynthetic performance (Van Heerden *et al.*, 2007). Sunflower cultivars showed better PI_{abs} than soybean plants. Among the tested cultivars, SF0054 showed much better result under drought stress.

The soybean cultivars had shown lower qP values as compared to sunflower under drought stress. Lower qP values indicated the proportion of inactive PSII reaction centers and separation of light harvesting complex-II from PSII reaction (Moradi & Ismail, 2007; Wu *et al.*, 2010). The reaction performance in which light energy converted into chemical reactions was insufficient under stressed conditions. Consequently, plants seek to absorb additional light to compensate for the greater yield. However, damaging impact of extra light could be rapidly activated by a regulatory mechanism called non-photochemical quenching (Horton *et al.*, 2005). The drought stress increased the NPQ in sunflower and soybean cultivars compared to control. The greater NPQ in soybean cultivars indicated damages RC that were not accepting light due to photo-oxidative damage under drought stress. Under environmental stress, the NPQ increased which showed an adaptive mechanism of energy dissipation protecting the photosynthetic apparatus against light induced damages (Netondo *et al.*, 2004). It was noticed the NPQ provided better protection to the chloroplast from excessive energy damage under drought stress.

To investigate the photosynthetic efficiency, chlorophyll 'a' fluorescence can be a useful tool that reflect the efficiency of electron transport between PSII and PSI under abiotic stresses (Strasser *et al.*, 2010; Oukarroum *et al.*, 2015; Kan *et al.*, 2017). The

photochemistry of PSII activity can be measured through JIP test. The OJIP transient and JIP-test are important indicators to study the stress induced responses in crop plants. The 'O' level is minimal fluorescence when all Q_A is in an oxidized state. Results had shown that drought stress decreased the minimal fluorescence in all soybean cultivars whereas sunflower cultivars showed higher 'O' level only in moderate drought stress (3 days drought). OJIP reflects the fluorescence rises from its lowest fluorescence level 'O' to a 'J' and J to I step can be varied due to reduction of Q_A by PSII. This rise represents the photochemical reduction of Q_B. When electron transport from Q_A to Q_B was repressed by drought stress afterward Q_A abridged quickly, leading to a substantial increase in J step due to photochemical quenching. Soybean and sunflower cultivars showed decline in J-I step under drought stress. It was suggested that drought stress confine the electron transport efficiency from Q_A in oil seed crop plants. This could be a reason by which soybean cultivars displayed poor photosynthetic efficiency as compared to sunflower cultivars. In the last I-P step, soybean plants did not show the rise in fluorescence while sunflower cultivars exhibited rise in this step. I to P rise related to the potential of trans-thylakoid in photosystem I (Papageorgiou & Govindjee, 2004). Lower I to P level, suggested that stress-induced damages caused reduction in PSI efficiency to accept electrons and reduced PQ pool size in soybean cultivars under drought stress. Results obtained from the OJIP curve confirmed that soybean plants had greater stress induced damage under drought stress as compared to sunflower plants. Moreover, sunflower showed better photosynthetic activities as compared to soybean plants under moderated and severe drought stress. Mehler's reaction has a protective role in the efficient cycling of H₂O during water shortage (Lovelock & Winter, 1996). It is suggested that the greater susceptibility of soybean cultivars under drought stress might be due to the lower ability of Mehler's reaction.

Spider plot: In the OJIP test, the K and J steps indicated the inhibition of the water splitting complex and electron transport chain after Q_A in PSII. Increase in K-step and W_K (relative variable fluorescence at K-step to the amplitude FJ-Fo) are the specific indicator of the injury to the water splitting complex or they express the photo-inhibition of PSII donor under drought stress (Brestic & Zivcak, 2013). In this study, the W_K was increased under drought stress environments (Figs. 6 and 7). However, genotype NARC-II showed reduction under severe drought stress whereas it increased under mild drought. Our results expressed that drought stress destroyed the water splitting complex and restricted the efficiency of electron transport on the PSII donor side. As the water splitting complex is responsible for the photo-oxidation of water in light dependent reaction, it was suggested that the oxidation of water molecules was impaired in NARC-II and under drought stress. Fo/Fm increased under drought stress in both soybean and sunflower cultivars. Efficiency with which a PSII trapped electron moved from Q_A to Q_B (1-VJ) was significantly decreased in stressed plants compared to control.

Genotype Soybean had greater reduction in 1-VJ in stressed environments compared to sunflower cultivars. It was suggested that drought stress restricts the efficiency of electron transport further from Q_A in soybean. This might be the reason through which soybean showed poor photosynthetic efficiency compared to sunflower. The F_m/F_o which represents the electron transport rate through PSII was decreased in stress condition as compared to control. There was a significant difference among the cultivars in terms of

F_m/F_o . Among the tested cultivars, NARC-II and William-82 had lower F_m/F_o under drought stress conditions as compared to sunflower cultivars. Results showed that F_o was increased in sunflower cultivars under moderate drought stress (D3 and D4). F_o indicates the integrity of photosynthetic machinery and loss of energy transfer to RC from antenna complex (Baker, 2008). It was suggested that sunflower plants maintained the integrity of photosynthetic machinery under drought stress as compared to soybean plants.

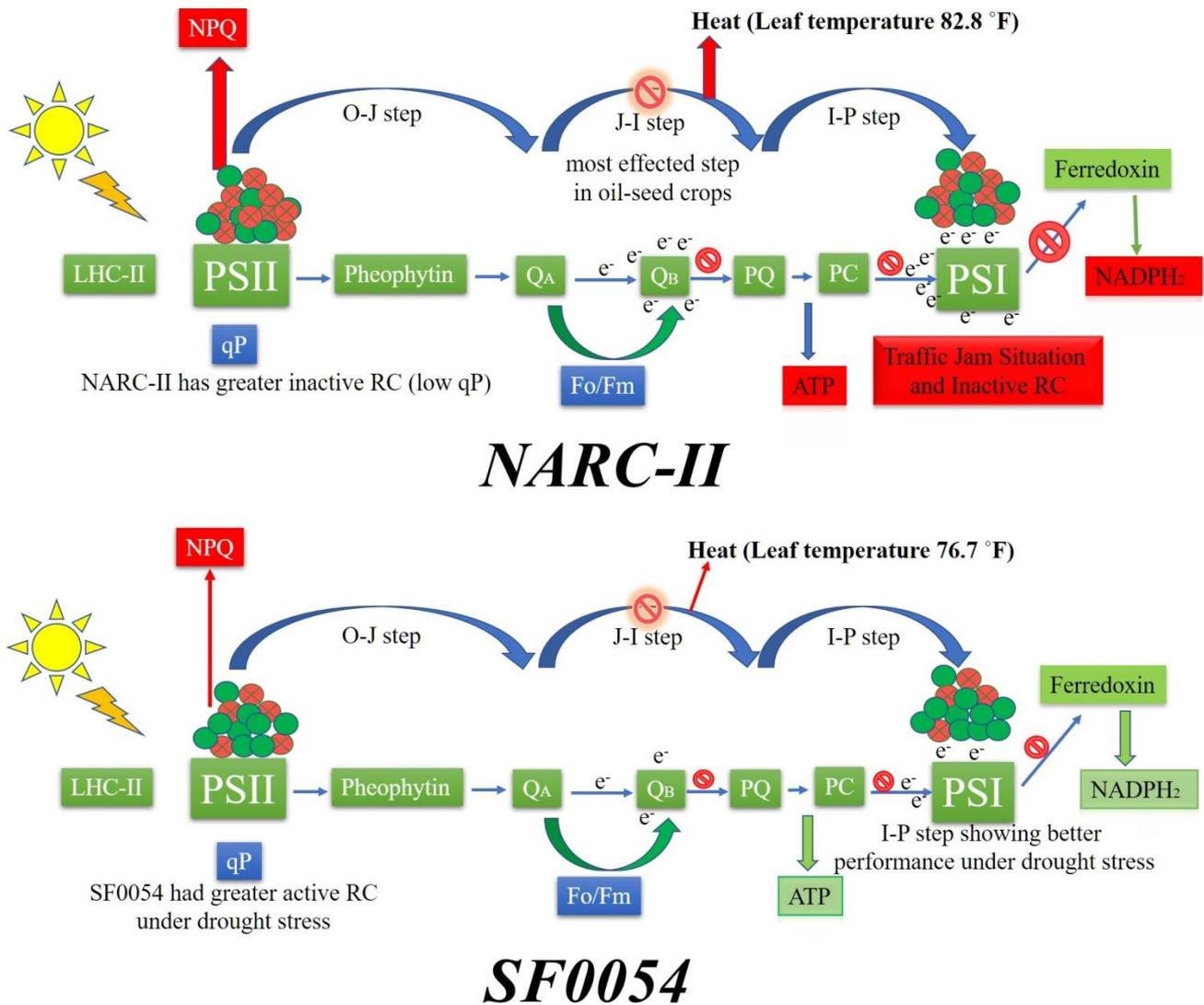


Fig. 9. A schematic presentation of chlorophyll a fluorescence under various drought stress environments and their relationship with IR thermography. Symbols in electron transport: Blue arrows expressed the direction of electron flow during photosynthesis, red arrows shown the dissipation or heat loss (thick red arrows represented greater dissipation), green boxes indicated the steps of electron acceptor/donor, parameters were highlighted with blue boxes. green circles for active RCs and red circles for inactivated RCs. The biological significance of abbreviations was explained in Table 1.

IR images of oil seed crops reflected the heat dissipation under drought stress (Fig. 8). IR images of leaf temperature respond directly to progressive drought. In this study, sunflower plants showed lower leaf temperature (blue color) compared to soybean plants under drought stress environments. Leaf temperature in sensitive cultivars increases under drought stress. The lower leaf temperature in sunflower plants might be due to the greater water use efficiency and higher RWC. Sensitivity of IR thermal

camera not only recognized the stress tolerant cultivars but was also non-invasive, less time consuming. It was presumed that drought stress caused increased thermal dissipation (Df_o/CS and NPQ) resulting in high leaf temperature. Drought stress affects the g_s , F_v/F_m , PI_{ABS} and RWC are few attributes that are correlated with leaf temperature (Siddiqui *et al.*, 2014; Kwon *et al.*, 2015). Furthermore, stomata play a key role in supporting the leaf moisture which results in lower down leaf temperatures

under abiotic stress (Jones *et al.*, 2009). Removal of water molecules through stomata needs energy to change from liquid state to vapour. Transpiration has cooling effects on plant leaf because the hottest molecule escapes from the plant. At this stage, transpiration becomes the most important factor for overall plant temperature under drought stress. It is seen that the sudden plant response under stress is the transpiration reduction in order to minimize the water loss eventually increase in leaf temperature (Brennan *et al.*, 2007; James *et al.*, 2008; Woo *et al.*, 2008). Therefore, it can be suggested that cutback in RWC and g_s may induce more heat dissipation that causes a rise in leaf temperature. It is recommended that use of IR thermography in screening stress tolerance related studies provides quick evaluation and chlorophyll fluorescence give the photosynthetic explanation of stress tolerance mechanism in plants.

Key findings of this research were presented in a model (Fig. 9). It was evident from the study drought stress destroyed the water splitting complex and disturbed the ratio of photochemical to non-photochemical quantum efficiency and thereby affected electron transport efficiency (F_m/F_o , F_v/F_o and W_k). Further, the drought stress impaired the functional state of plants and inactivated the reaction center that leads to greater dissipation (ABS/RC, D_iO/CS and NPQ). The transport of electrons from Q_A to Q_B (F_v , 1-VJ and Mo/VJ) and primary electron acceptor of PSI were the most affected sites of photosynthetic electron transport. The most affected site of electron transport in oil seed crop plants were Q_B and PSI, both sites had a traffic jam situation due to their acceptor side failing to accept electrons under drought stress environments (Fig. 9). The leaf temperature represented in the model might be altered due to the NPQ or heat dissipation under drought stress. Model was prepared based on two cultivars that had consistent results.

Conclusions

In conclusion, various fluorescence parameters indicated that drought stress reduced the photosynthetic capacity of soybean and sunflower cultivars but there were no specific indicators among the chlorophyll fluorescence that detected the early stress in both oil seed crops plants. However, IR thermal sensing camera can identify the sensitive plants during stress initiation. The responses of the oilseed crop photosynthetic apparatus against drought stress showed different patterns. Moderate drought stress did not affect the IP phase in all the oilseed cultivars except NARC-II where greater decline was observed, which was related to the reduction of PSI efficiency to accept electrons. It is concluded that physiological assessment of crop plants using IR-thermography and chlorophyll fluorescence may provide the quick and accurate explanation of drought stress tolerance in different plant cultivars.

Acknowledgment

The authors acknowledge financial support from HEC Research project #6586/Sindh/NRPU/R&D/HEC2015. We are also grateful to the Department of Botany, University of Karachi, seed manager of FMC Corporation (Pakistan), and Seed Certification Department of Pakistan for providing seeds and support for this study.

References

- Akhkha, A. 2009. The effect of water stress on photosynthesis, respiration and relative chlorophyll index of the desert plant *Calotropis procera*. *Biosci. Biotechnol. Res. Asia.*, 6: 653-658.
- Baker, N.R. 2008. Chlorophyll fluorescence: a probe of photosynthesis *In vivo*. *Ann. Rev. Plant Biol.*, 59: 89-113.
- Barrs, H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.*, 15: 413-428.
- Blokhina, O., E. Virolainen and K.V. Fagerstedt. 2003. Antioxidative damage and oxygen deprivation stress. *Ann. Bot.*, 91: 179-194.
- Brennan, J.P., A.G. Condon, M. Van-Ginkel and M.P. Reynolds. 2007. An economic assessment of the use of physiological selection for stomatal aperture-related traits in the CIMMYT wheat breeding program. *J. Agric. Sci.*, 145: 187-194.
- Brestic, M. and M. Zivcak. 2013. PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. In: (Eds.): Rout, G.R. & A.B. Das. *Molecular Stress Physiology of Plants*. Springer, Dordrecht, Netherlands. pp. 87-131.
- Christen, D., S. Schönmann, M. Jermini, R.J. Strasser and G. Défago. 2007. Characterization and early detection of grapevine (*Vitis vinifera*) stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. *Environ. Exp. Bot.*, 60: 504-514.
- Clavel, D., O. Diouf, J.L. Khalfauoui and S. Braconnier. 2006. Genotypes variation in fluorescence parameters among closely related groundnut (*Arachis hypogaea* L.) lines and their potential for drought screening programs. *Field Crop Res.*, 96: 296-306.
- Dai, A. 2013. Increasing drought under global warming in observations and models. *Nat. Clim. Chang.*, 3: 52-58. doi: 10.1038/nclimate1811.
- Duncan, D.B. 1955. Multiple range and Multiple F-test. *Biometrics.*, 11: 1-42.
- Ekmekci, Y., A. Bohms, J.A. Thomson and S.G. Mundree. 2005. Photochemical and antioxidant responses in the leaves of *Xerophyta viscosa* Baker and *Digitaria sanguinalis* L. under water deficit. *Zeitschrift für Naturforschung C.*, 60: 435-443.
- Foyer, C.H., H.M. Lam, H.T. Nguyen, K.H. Siddique, R.K. Varshney, T.D. Colmer, W. Cowling, H. Bramley, T.A. Mori, J.M. Hodgson, J.W. Cooper, A.J. Miller, K. Kunert, J. Vorster, C. Cullis, J.A. Ozga, M.L. Wahlqvist, Y. Liang, H. Shou, K. Shi, J. Yu, N. Fodor, B.N. Kaiser, F.L. Wong, B. Valliyodan and M.J. Conside. 2016. Neglecting legumes has compromised global food and nutritional security. *Nature Plants.*, 2: 16112. doi: 10.1038/nplants.2016.112.
- Horton, P., M. Wentworth and A. Ruban. 2005. Control of the light harvesting function of chloroplast membranes: The LHCI aggregation model for nonphotochemical quenching. *FEBS Lett.*, 579: 4201-4206.
- Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *C. R. Biol.*, 331: 42-47.
- James, Ra, V.O.N., S. Caemmerer, A.G. Condon, A.B. Zwart and R. Munns. 2008. Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat. *Funct. Plant Biol.*, 35: 111-123.
- Jones, H.G., R. Serraj, B.R. Loveys, L. Xiong, A. Wheaton and A.H. Price. 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. *Fun. Plant Biol.*, 36: 978-989.

- Kan, X., J. Ren, T. Chen, M. Cui, C. Li, R. Zhou, Y. Zhang, H. Liu, D. Deng and Z. Yin. 2017. Effects of salinity on photosynthesis in maize probed by prompt fluorescence, delayed fluorescence and P700 signals. *Environ. Exp. Bot.*, 140: 56-64.
- Krizmanic, M., I. Liovic, A. Mijic, G. Krizmanic, B. Simic, T. Duvnjak, M. Bilandzic, R. Marinkovic, D. Gadzo and A. Markulj. 2012. Effect of environment on quantitative traits of new OS sunflower hybrids. *Sjemenarstvo.*, 29: 121-135.
- Kwon, T.R., K.H. Kim, H.J. Yoon, S.K. Lee, B.K. Kim and Z.S. Siddiqui. 2015. Phenotyping of plants for drought and salt tolerance using Infra-red thermography. *Plant Breed. Biotech.*, 3: 299-307.
- Liu, Y., G. Fiskum and D. Schubert. 2002. Generation of reactive oxygen species by mitochondrial electron transport chain. *J. Neurochem.*, 80: 780-787.
- Lovelock, C.E. and M.K. Winter. 1996. Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical tree species. *Planta.*, 198: 580-587. doi: 10.1007/bf00262645.
- Maxwell, K. and G. Johnson. 2000: Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.*, 51: 659-668.
- Mazahery-Laghab, H., F. Nouri and H.Z. Abianeh. 2003. Effects of the reduction of drought stress using supplementary irrigation for sunflower (*Helianthus annuus*) in dry farming conditions. Pajouheshva-Sazandegi. *Agron. Hort.*, 59: 81-86.
- Moradi, F. and A.M. Ismail. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Ann. Bot.*, 99: 1161-1173.
- Munns, R., R.A. James, X.R.R. Sirault, R.T. Furbank and H.G. Jones. 2010. New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. *J. Exp. Bot.*, 61: 3499-3507.
- Naz, H., N.A. Akram, and H. Kong. 2020. Assessment of secondary metabolism involvement in water stress tolerance of quinoa (*Chenopodium quinoa* Willd.) Subjected to varying water regimes *Pak. J. Bot.*, 52: 1553-1559. DOI: [http://dx.doi.org/10.30848/PJB2020-5\(8\)](http://dx.doi.org/10.30848/PJB2020-5(8))
- Netondo, G.W., J.C. Onyango and E. Beck. 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci.*, 44: 806-811.
- Oukarroum, A., F. Bussotti, V. Goltsev and H.M. Kalaji. 2015. Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* plants under salt stress. *Environ. Exp. Bot.*, 109: 80-88.
- Papageorgiou, G.C. and Govindjee. 2004. Chlorophyll *a* Fluorescence. A Signature of Photosynthesis. Pp. 818. Springer, Dordrecht.
- Siddiqui, Z.S., J.I. Cho, D.B. Park, G.S. Lee, T.H. Ryu, H. Shahid, M. Umar and S.C. Park. 2015. Field assessment of CaMsrb2 transgenic lines in a drought stress environment. *Turk. J. Bot.*, 39: 973-981.
- Siddiqui, Z.S., J.I. Cho, S.H. Park, T.R. Kwon, B.O. Ahn, K.S. Lee, M.J. Jeong, K.W. Kim, S.K. Lee and S.C. Park. 2014. Physiological mechanism of drought tolerance in transgenic rice plants expressing *Capsicum annuum* Methionine sulfoxide reductase B2 (*CaMsrb2*) gene. *Acta Physiol. Plant.*, 36: 1143-1153.
- Sirault, X.R.R., R.A. James and R.T. Furbank. 2009. A new screening method for osmotic component of salinity tolerance in cereals using infra-red thermography. *Fun. Plant Biol.*, 36: 970-977.
- Stirbet, A., and Govindjee. 2011. On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. *J. Photochem. Photobiol. B: Biol.*, 104: 236-257.
- Strasser, R.J., M. Tsimilli-Michael, S. Qiang and V. Goltsev. 2010. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochim. Biophys. Acta. Bioenergetics.*, 1797: 1313-1326.
- Umar, M. and Z.S. Siddiqui. 2018. Physiological performance of sunflower genotypes under combined salt and drought stress environment. *Acta Bot. Croat.*, 77: 36-44.
- Umar, M. and Z.S. Siddiqui. 2019. Responses of photosynthetic apparatus in sunflower cultivars to combined drought and salt stress. *Photosynthetica.*, 57(2): 627-639. DOI: 10.32615/ps.2019.043.
- Umar, M. and Z.S. Siddiqui. 2020. Florescence assessment of sunflower genotypes against drought stress environment. *Pak. J. Bot.*, 52: 1181-1188. DOI: [http://dx.doi.org/10.30848/PJB2020-4\(29\)](http://dx.doi.org/10.30848/PJB2020-4(29)).
- Van Heerden, P.D.R., J.W. Swanepoel and G.H.J. Krüger. 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO₂ assimilation. *Environ. Exp. Bot.*, 61: 124-136.
- Woo, N., M.R. Badger and B. Pogson. 2008. A rapid non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. *Plant Methods.*, 4: 27.
- Wu, X.X., H.D. Ding, J.L. Chen, H.J. Zhang and W.M. Zhu. 2010. Attenuation of salt-induced changes in photosynthesis by exogenous nitric oxide in tomato (*Lycopersicon esculentum* Mill. L.) seedlings. *Afr. J. Biotechnol.*, 9: 7837-7846.

(Received for publication 2 July 2019)