Chlorophyll *a* fluorescence and gas exchange attributes in maize (*Zea mays* L.) differing in drought tolerance: Targets locked

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Abstract

The research was conducted to check the photosynthetic activity of three maize (*Zea mays* L.) cultivars (DTC, EV78 and 6621) by OJIP chlorophyll a fluorescence and gas exchange parameters under drought/water stressed conditions. Morphological attributes like leaf diameter and leaf length were decreased in *Zea mays* L. cultivars. Drought stress also reduced the stomatal conductance thereby reduced the CO2 influx. inducing stomatal closure and reduced CO_2 influx. Among cultivars, DTC had greater water use efficiency (WUE) and photosynthetic rate. Higher PSII quantum yield and chlorophyll contents were also observed in DTC cultivar relative to EV-78 and 6621. The (JIP) analysis from fast chlorophyll a kinetic estimation elucidated acclimatory processes in cultivar DTC. A greater protection of reaction centers which involved the conversion of absorbed energy into electron transport (ET_0/RC) and remainder absorbed energy was safely dissipated (DI/RC) in DTC. Fluorescence differential kinetics revealed that there was greater energetic connectivity among antenna pigments and reaction center in DTC unlike EV78 and 6621 where L-band appeared. Kinetics difference at 3ms showed that DTC had a lower K band appearance which indicated that oxygen evolving complex remained more intact and there was more functional PSII in DTC relative to the other maize cultivars.

Key words: OJIP; Photosystem II; Water use efficiency; Maize; Drought

1. Introduction

Drought is one of the most ubiquitous ecological forces that affect photosynthetic process which is directly linked to crop yield (Blum, 2011). Water limitations reduce cell turgor and stomatal conductance, responsible for reduction in CO_2 assimilation and transpiration rate which disturb the leaf metabolism (Chaves *et al.*, 2009). Smaller rate of assimilation of CO_2 induces imbalance between requirement of electrons for CO_2 assimilation and photochemical activity at photosystem II (PSII) thereby produce (ROS) reactive oxygen species and photoinhibitory harm to reaction centers (Foyer *et al.*, 2012). Plants generates ROS during drought stress which includes (H_2O_2) hydrogen peroxide, superoxide radicals and other freely existing radicals. ROS cause metabolic damages including nucleic acids, membrane lipids and proteins (Blum, 2011). Enzymatic and non-enzymatic

processes are started during combating phase. Beta-carotenes scavenge and binds free radicals comprised of non-enzymatic scavenging system. In enzymatic scavenging systems, different enzymes like peroxidase, catalase facilitate the catalytic transformation of (H_2O_2) Hydrogen Peroxide into (O_2) oxygen and (H_2O) water (Adnane *et al.*, 2015; Farissi *et al.*, 2013). In photosynthetic apparatus, three main sites are affected by deficiency of water. Among these three sites, PSII, photosystem I (PSI) and Benson Calvin cycle (Mehta *et al.*, 2010; Ogbaga *et al.*, 2017). Chlorophyll 'a' fluorescence transients are the most versatile tools to record photosynthetic efficiency. Drought tolerant plants can develop some photosynthetic adaptations such as higher carboxylation efficiency, photoprotective non-photochemical quenching, generation of cyclic electron transport, alternative electron acceptor to cope with drought (Ogbaga *et al.*, 2014). Thus, understanding of photosynthetic responses under water deficit conditions may help in developing drought tolerant crop plants. Of various photosynthetic attributes for checking sensitivity of plant against drought stress is the photosynthetic apparatus's function. Functioning of photosynthetic apparatus is a very beneficial tool for physiological indicator (Baker, 2008; Maxwell and Johnson, 2000; Murchie and Lawson, 2013). Chlorophyll "a" fluorescence indicator can be used for the measurement of photosynthetic efficiency. It also tells us about the association between function and structure of reaction centers and core complexes (Foyer *et al.*, 2012; Strasser *et al.*, 2004). To detect and monitor the environmental stress resistance and tolerance in plants the most widely used technique is a Chlorophyll "a" fluorescence technique.

Among chlorophyll fluorescence techniques, the use of OJIP following JIP test is of most important as it is considered a quick tool which is work under field conditions. OJIP transient cumulatively represents different processes involved in electron transport chain of reduction. The most time taking phase of the rise of fluorescence (I-P), approximately considered at 30-300 ms respectively. IP phase sounds parallel to the re-reduction of P700+ PSI and plastocyanin (PC)+ (Stirbet and Govindjee, 2011). The J-I phase has kinetic properties mostly for the oxidation/reduction of the (PQ) plastoquinone pool. It has been recommended that rise in O-J basically represents the PSII reduction of acceptor side (Stirbet and Govindjee, 2011). JIP test figures out the PSII status under normal and stressed conditions. JIP test extrapolates the dimensions of original fluorescence transients into many biophysical and phenomenological measurements (Strasser *et al.*, 2004). A new parameter, (PI_{ABS}) performance index basically derived from JIP_test calculation is considered to be more sensitive towards drought than the (Qy) quantum yield of photochemistry at primary level (F_v/F_m) (Oukarroum *et al.*, 2007; Strauss *et al.*, 2006). PI_{ABS} is a collective parameter including three independent parameters. First, fully active reaction center's density (RCs), secondly the efficiency of movement of electron by trapped exciton into the electron transport chain and facilitated by the primary acceptor of PSII (QA) and thirdly the probability of photon absorption, trapped in the reaction center (Kumar *et al.*, 2019).

Drought minimizes the PI_{ABS} (Oukarroum *et al.*, 2007) and lower the conductance of stomata and assimilation of CO_2 in crops. Thus, different JIP test parameters can help in understanding photosynthetic adaptations in stress tolerant crops. For example, while working with transgenic rice overexpressing OsNAC10 (Redillas *et al.*, 2011) revealed that higher drought tolerance in transgenic rice was associated with higher efficiency of energy utilization. Similarly, Oukarroum *et al.* (2007) found greater structural and functional stability in PSII and oxygen evolution complex in drought tolerant barley (*Hordeum vulgare* L.) cultivars. Previously, six maize cultivars were screened in PEG6000 induced drought at the germination and then seedling growth stages. Thus, a drought tolerant cultivar DTC, moderately tolerant cultivar EV-78 and drought sensitive cultivar 6621 were reported (Bashir *et al.*, 2016) when compared on vegetative and reproductive stages of growth, these lines showed same degree of water stress tolerance. We hypothesized that drought tolerant cv DTC has some photosynthetic adaptations under water stress condition, which contributed in greater biomass and growth at tributes like leaf width, leaf length, leaf area, root length shoot length and number of leaves under drought conditions. Thus, the present study was carried out to determine whether DTC indeed has sufficient photosynthetic adaptations to deal with drought stress. In addition, the potential of using some JIP-test parameters derived from fast chlorophyll *a* fluorescence kinetics as reliable criterion for drought tolerance in *Zea mays* L. was examined.

2. Materials and methods

2.1 Plant Material and growth conditions

Seeds of three maize cultivars, DTC, EV78 and 6621 were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Surface sterilization of seeds was done by 5% solution of Sodium Hypochlorite for just 5 minutes before sowing. Each pot (15 cm diameter) was filled with 1kg of garden soil. Five seeds from each maize cultivar were sown in pots. The experiment was arranged as set of completely randomized block design in two treatments (Control and drought) and each cultivar with five replicates. After the onset of seedlings, thinning was done and each pot has only one seedling. Then water stress was imposed on two weeks old plants upto wilting stage and then plants were rehydrated to field capacity. However, during condition, control plants were hydrated continuously upto highest water holding capacity. Then different physiological and biochemical characteristics of maize plants were measured.

2.2 Morphological attributes and Plant Biomass

Shoot length, root length no of leaves, leaf length, leaf diameter and leaf area have been recorded. Root fresh weight, shoot fresh weights, root dry weight and shoot dry weight have been recorded.

2.2 Chlorophyll contents (SPAD values)

Handheld chlorophyll meter was used for recording the total chlorophyll contents (SPAD) of newly developed leaf (**3rd** leaf from upside of the plant) on different days just after the water stress (Minolta SPAD-502 Japan).

2.3 Quantum Yield (QY)

Flourpen (FP 100) was used to measure the quantum yield (QY) of newly developed leaf (3rd leaf from the top of the plant) on different day just after the drought.

2.5 Gas Exchange related parameters

An open system LCA-4 Analytical Development Company, Hoddeson, UK movable infrared gas analyzer was used to study the gas exchange parameters. The following measurements were done from 9:00 to 11.00 am leaf surface area (11.50 cm^2), ambient or surrounding carbon dioxide (CO_2) concentration (C_{ref}) 371µmol/mol, surrounding temperature (45±3°C), leaf chamber temperature (Tch) mottled from 25-28°C, volume gas flow rate of leaf chamber (v) 296 ml /min, molar gas flow rate

of leaf chamber (U) 400 µmol/s, ambient pressure (P) 97.95 kPa, PAR (Q_{leaf}) at leaf surface maximum up to 770 µmol m^{-2} s^{-1}. The data was recorded for sub stomatal carbon dioxide (CO_2) concentration (C_i), net CO_2 assimilation rate (A), transpiration rate (E), water use efficiency (A/E) were made in the fully youngest expanded leaf of each sample.

2.6 Chlorophyll a fluorescence measurement and study of OJIP transients

For measuring chlorophyll a fluorescence FluorPen FP 100 was used on youngest leaf (third leaf). Plants were kept in dark/covered with aluminium foil for 30 minutes, the leaves were wrapped with aluminium foil and it was made sure that photosystem II (PSII) was dark adapted. Collected data was used to measure the OJIP parameters, described by Michael and Strasser, (2001). Kinetics of chlorophyll a fluorescence was measured from 10 µs to 1 s as follows: F_o, the initial fluorescence was recorded at 50µs, L at 150 µs, K at 300 µs, J at 2000 µs and I at 30,000 µs are the intermediates (F_l, F_k, F_j, and F_i, respectively) and P at 500,000µs is the maximum fluorescence (Fm). This was described by the Strasser *et al.* (2004). Chlorophyll 'a' fluorescence transients were double normalized as V_{OK} and change in differences (ΔV_{OK}) of transients with respect to reference and estimated as the L-band. Likewise, differential kinetics as K-band between Fo and Fj was calculated. Chlorophyll fluorescence at O-I phase expressed as V_{OI}. The I-P was determined on two lines: (1) V_{OI} transients normalization between time period of 3×10^4-10^5 µs expressed as V_{OI} and the transient normalization to the time span of 30000 µs 200000 µs equate as V_{IP} (Table 1).

Table 1. Terms used in OJIP and their descriptions.

Sr No.	Terms used in OJIP	Description
1	Fo	Chlorophyll Florescence at o
2	Fi	Chlorophyll Florescence at i
3	Fk	Chlorophyll Flourescence at 300 µs
4	Fj	Chlorophyll Florescence at j
5	Fp	Chlorophyll Florescence at p
6	Area	Area between flouresecence curve (OJIP) and Fm = F
7	Mo	Rate of accumulation of closed reaction centers
8	Vi	Variable Florescence at i
9	Vj	Variable Florescence at j
10	Sm	Multiple turn over
11	Ss	Single turn over

12	N	No. of Quinone redox turn over
13	ABS/RC	Absorption per reaction center
14	TRo/RC	Trapping per reaction center
15	ETo/RC	Electron transport per reaction center
16	DIo/RC	Dissipation per reaction center
17	RC/CSm	Density of reaction center per cross section excited electrons
18	TRo/CSm	Total Trapped energy per cross section excited electrons
19	ABS/CSm	Absorbance per cross section excited electrons
20	ETo/CSm	Electron Transport per cross section excited electrons
21	RC/ABS	Density of reaction center per absorbance
22	PI(csm)	On cross section basis performance index
23	PI$_{ABS}$	On absorption basis performance index

2.7 Statistical analysis

For all characteristics data were presented as mean values with +S.E. Using a COSTAT computer package (Cohort Software, Berkeley, California) a Two Way Completely Randomized Analysis of Variance (2WCR ANOVA) was applied to study the different morpho-physiological characteristics. For the measuring quantum yield of PSII and SPAD three way completely randomized ANOVA was used. (LSD) Least Significant Difference according to Snedecor and Cochran (1980) was got by comparing the mean values of each factor.

3. Results and Discussion

Fresh and dry biomass of *Zea mays* cultivars (DTC, EV78 and 6621) significantly ($P \le 0.001$) minimized due to water stress (Table 2). These cultivars significantly differed when plotted their values on graph from one another (Fig. 1) DTC cultivar showed the least reduction in biomass after drought, whereas minimum values of all the growth parameters were observed in 6621 cultivar. In addition, EV-78 cultivar exhibited in between values of these growth attributes (Fig. 1). Similarly, shoot and root length was highest in DTC cultivar after drought in comparison with the other cultivars (Fig. 1).

Table 2. Mean squares from the analysis of variance (ANOVA) of the data for shoot f. wt., shoot d. wt., root f. wt., root d. wt. (g/plant), shoot length, root length, leaf area of cv. DTC, cv. EV78 and cv. 6621 of *Zea mays* L., grown in control and drought.

Source of variance	df	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d. wt.	Shoot length	Root length	Leaf area	No. of leaves
Cultivars	2	824.6***	91.6***	89.2***	6.1***	747.1ns	25. 6*	234.07***	8.7***
Drought	1	1583.1***	175.8***	141.1***	40.9***	9810.2***	283.7***	215.7**	12.04***
Cvs x Drought	2	62.6***	6.9***	7.3***	0.01***	1039.03*	9.7ns	37. 5ns	0.2ns
Error	24	2.087	0.231	26.3	0.125	227.9	8.35	18. 6	0.4
Total	29								

*, **, *** significant at 0.01, 0.05, 0.001 levels respectively while ns = non-significant.

No. of leaves per plant minimized significantly ($P \le 0.001$) in these cultivars by imposing of drought (Table 2). However, DTC cultivar had leaves per plant greater in number as compared to others after drought (Fig. 1). Primary mechanism to adaptive response of different plants to cope with drought stress is a plant physical growth (Sapeta, 2013). Our results showed significant loss in fresh and dry biomass, shoot length, root length and number of leaves of three cultivars (DTC, EV-78 and 6621) in response to drought. The cultivars responded differently. DTC maize cultivar produced better growth and biomass accumulation under water stress while EV-78 maize cultivar was dynamic with respect to physical growth and considered moderately drought tolerant cultivar. As drought progressed, leaf rolling, wilting and maximum biomass reduction were observed in 6621 maize cultivar which is considered the drought sensitive cultivar. To reduce usage and increase in absorption of water, plants under drought stress often decline their rate of growth and production of biomass (Martin and Stephens, 2006). Reduction in leaf number and leaf area

was a drought tolerance strategy for the plants with a consequent reduction in transpiration (Blum, 2005).

Total chlorophyll contents were decreased in drought effected plants of maize cultivars relative to their respective control plants. Chlorophyll contents was higher in DTC cultivar while chlorophyll contents were lower in 6621 cultivar recorded on days 2, 5, 6, 7, 9, 11 after the onset of drought. Quantum yield of photosystem II also reduced under drought stress on 2, 5, 6, 7, 9, 11 days after drought stress (Table 3, Fig. 1). Chlorophyll contents under drought stress decrease in sensitive species. Such procedure has been seen under drought stress in sensitive cultivars (Ogbaga et al., 2014). Chlorophyll contents have been reduced due to drought as measured by SPAD (Papasavvas et al., 2008) (Fig. 1).

Table 3. Mean square (MS values) from the data of analysis of variance (ANOVA) for quantum yields and SPAD of cv. DTC, cv. EV78 and cv. 6621 of *Zea mays* L. on 2, 5, 6, 7, 9, 11 days after drought, grown in control and drought.

Source of variance	df	Quantum yield	SPAD
Day	1	7.601***	6392.53***
Cvs	2	0.116***	301.38***
Drought	5	0.703***	154.21***
Drought x Day	2	0.282***	313.30***
Cvs x Drought	5	0.008ns	15.70ns
Day x Cvs	10	0.011***	38.17*
Drought x Day x Cvs	10	0.005***	24.48
Error	864	0.009	16.31
Total	899		

*, **, *** significant at 0.01, 0.05, 0.001 levels respectively while ns = non-significant.

Net assimilation rate of carbon dioxide (A), concentration of sub-stomatal carbon dioxide (C_i) and rate of transpiration (E) decreased significantly ($p \leq 0.001$) due to drought stress (Table 4). However, greater loss in these gas exchange parameters were observed in cv. 6621 after drought, while the reverse was true for drought tolerant DTC cultivar (Fig. 1). Drought tolerant DTC cultivar had lower transpiration but higher C_i values compared with the other cultivars (Fig. 1). Furthermore, drought tolerant DTC possess greater water use efficiency (WUE=A/E) under severe drought conditions as compared with the moderately drought tolerant and drought sensitive cultivars of maize in which WUE severely reduced (Fig. 1). Overall, DTC cultivar had higher photosynthetic rate that is linked with maximum C_i, low rate of transpiration and higher rate of WUE than cv. EV-78 and cv. 6621. Induction of drought reinforced the negative impact on photosynthesis as a consequence of leaf tissue dehydration directly linked with soil drought. The first response of leaf tissues to water stress is commonly manifested in closing of stomata restricted to CO_2 diffusion linked with a decrease in mesophyll conductance of CO_2 limited by drought (Chaves et al., 2009; Flexas et al., 2002; Medrano et al., 2002)

Table 4. Mean squares (MS values) from the data of analysis of variance (ANOVA) for E (mmol H_2O m^{-2} s^{-1}, A (µmol CO_2 m^{-2} s^{-1}), WUE (A/E) µmol CO_2/mmol H_2O and Ci (µmol CO_2 m^{-2} s^{-1}) of cv. DTC, cv. EV78 and cv. 6621 of *Zea mays* L., grown in control and drought.

Source of variance	df	A	Ci	E	WUE (A/E)
Cultivars	2	43.26***	1704.5**	0.28 ns	13.01***
Drought	1	182.54***	4201.38***	1.02**	2.66ns
Cvs x Drought	2	34.82***	297.05ns	0.02ns	3.807*
Error	12	2.32	209.16	0.07	0.63
Total	17				

*, **, *** significant at 0.01, 0.05, 0.001 levels respectively while ns = non-significant.
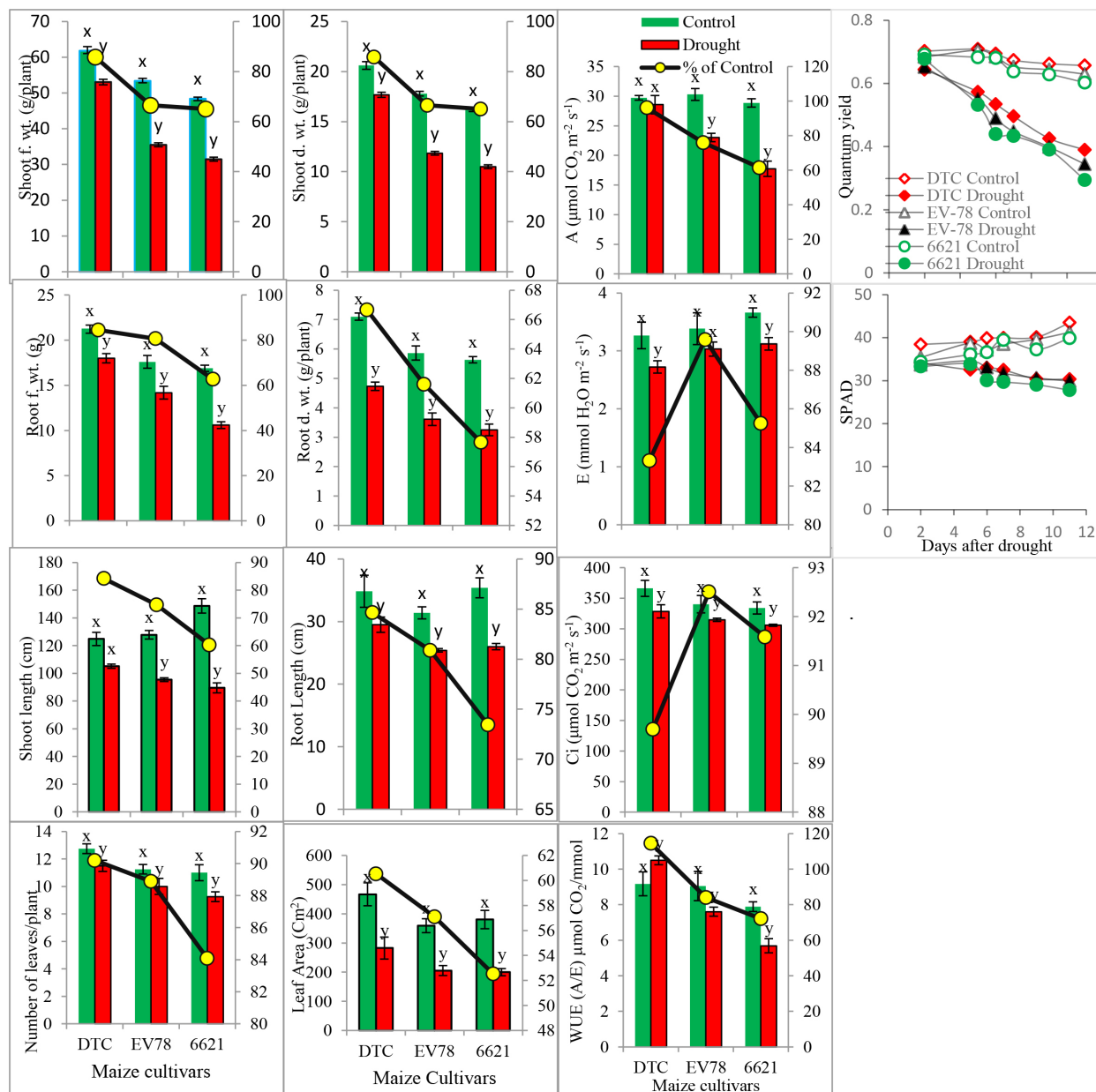
Fig 1. Shoot fresh weight (g/plant), shoot dry weight (g/plant), root fresh weight (g/plant), root dry weight (g/plant), shoot length (cm), root length (cm), leaf area (cm^{-2}), no. of leaves, A (μmol CO_2 m^{-2} s^{-1}), Ci (μmol CO_2 m^{-2} s^{-1}), E (mmol H_2O m^{-2} s^{-1}) and WUE (A/E) μmol CO_2/mmol H_2O, SPAD and QY on periodic basis of cv. DTC, cv. EV78 and cv. 6621 of *Zea mays* L., grown in control and drought stressed conditions.

From the double normalized fluorescence transients and differential kinetic analysis between O and P, fluorescence at OJ step decreased in both tolerant drought cultivar (DTC) and in moderately tolerant (EV-78) cultivars whereas there was an increase in fluorescence at OJ step in water stress sensitive (6621) cultivar (Fig. 2). However, at JI step, greater increase and decrease in fluorescence of 6621 and DTC cultivars was observed, respectively (Fig. 2). Similarly, from double normalized differential kinetics at different steps, it is obvious that more positive L-band appeared in water stress sensitive cultivar (6621) as compared with moderately tolerant maize cultivar (EV-78) and a negative band appeared in tolerant drought cultivar (DTC) (Fig. 3).
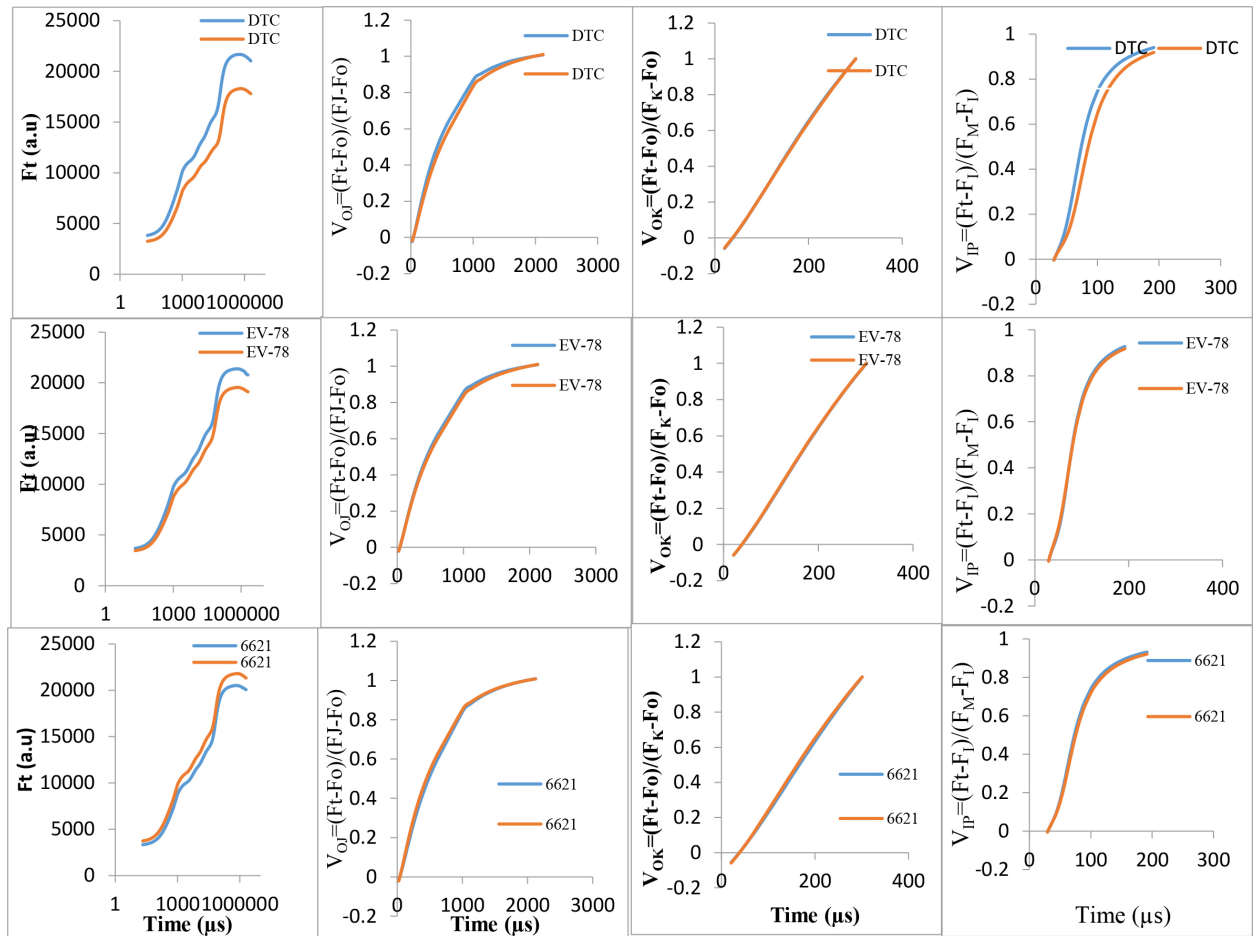
Fig 2: Chlorophyll "a" fluorescence transients were recorded in dark adapted (15 mins) *Zea mays* L. cultivars (DTC, EV78 and 6621) under control and drought conditions. Raw chlorophyll 'a' fluorescence transients showing its intensities recorded between time interval (1-10^6 µs). Fluorescence transients between "O" and "J" points were double normalized between Fo and Fj upto 2000 µs by using formula Voj = (Ft-Fo)/(Fm-Fo). Fluorescence transients between Fo and Fk with respect to time interval (30-300 µs) Vok = (Ft-Fo)/(Fk-Fo). Fluorescence transients double normalized between the initial (I) and peak (P) ends by using formula VIP = (Ft-Fi)/(Fm-Fi).
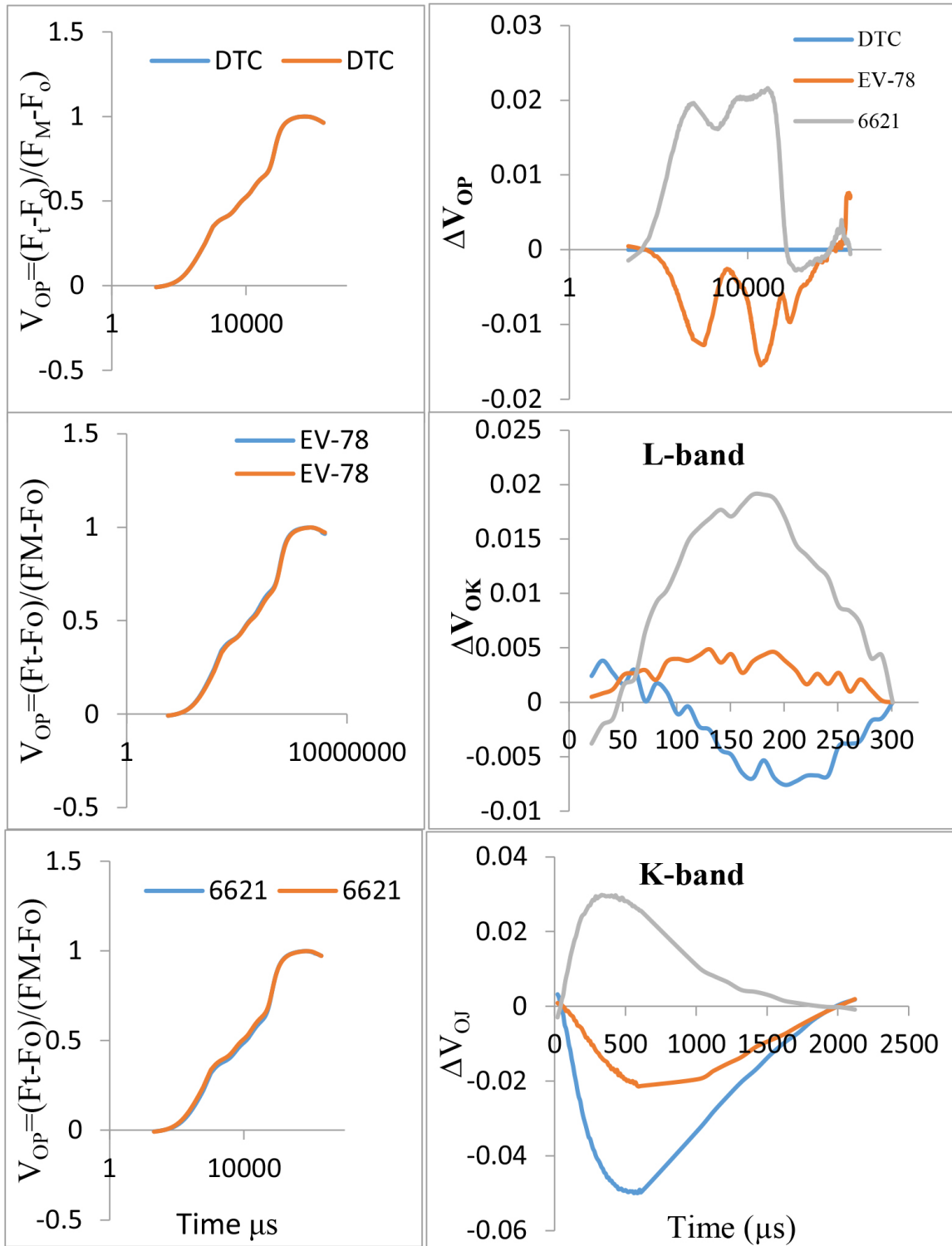
Fig. 3. The chlorophyll "a" fluorescence transients were recorded in *Zea mays* L. cultivars (DTC, EV78 and 6621) under control and drought conditions. Fluorescence transients double normalized between the origin (O) and peak (P) ends by using formula VOP=(Ft-Fo)/(Fm-Fo). Flourescence kinetic differences of V_{OP}, V_{OK}, and V_{OJ}.

Although a positive K-band appeared in drought sensitive cv. 6621 due to drought stress, a more negative K-band appeared in tolerant drought cultivar (DTC) than in moderately drought tolerant EV-78 (Fig. 3). Same cultivars response was also analyzed at kinetic difference (ΔV_{op}) of OP phase (Fig. 3). Alterations due to drought stress in biophysical and phenomenological energy fluxes using JIP test are introduced as radar plot (Fig. 4). The increase in rate of accumulation of reaction centers that are closed (Mo) due to cyclic drought in drought sensitive cultivar 6621 compared with tolerant (DTC) and moderately tolerant (EV-78) (Fig. 4). There were significant

differences (at $p \leq 0.05$) in the maximum quantum yield of photochemistry at primary level (Φ_{Po} or F_v/F_m) among the cultivars at various drought levels but DTC maintained its Fv/Fm than cultivar 6621(Fig. 4). The pool size of electron carriers or multiple turnover Q_A reduction events (S_m) was higher in water stressed plants of 6621 followed by the EV-78. Whereas it was lower in DTC plants under drought stress. In addition, number of Q_A redox turnover until F_m (N) was maximum in drought sensitive cultivar (6621) followed by the drought tolerant cultivar (DTC) but number of redox turnover of Q_A was observed in moderately tolerant cultivar (EV-78) (Fig. 4).
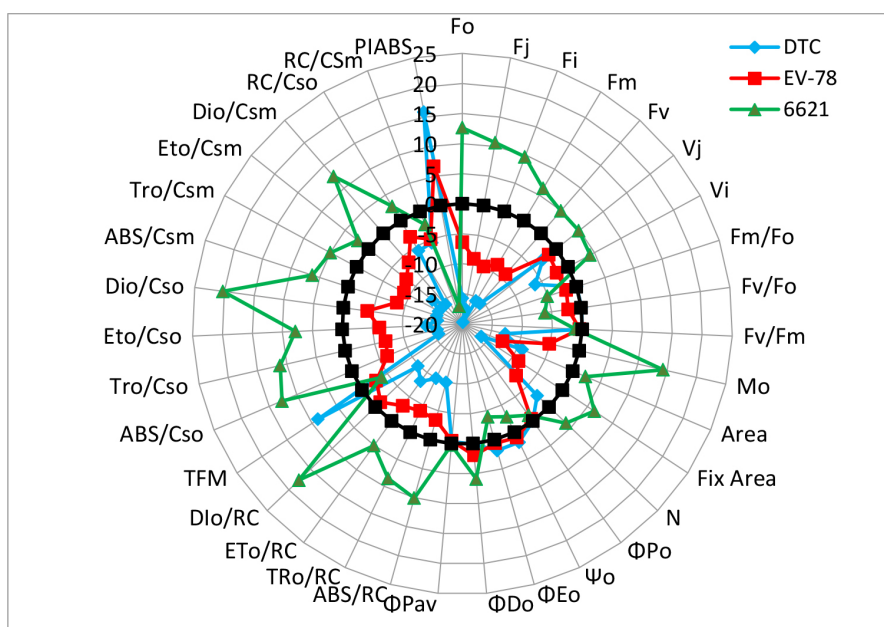

Fig. 4. A Radar plot illustrating different OJIP chlorophyll 'a' fluorescence transients in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV78 and 6621) under control and drought conditions.

Table 5. Percent (%) of controls of Fm/Fo, Fv/Fo, Fv/Fm, Fo, Fj, Fi, Fm, Fv, Mo, Vj, Vi, Sm, Ss, φPo, ABS/RC, TRo/RC, ETo/RC, DIo/RC, RC/CSm, ABS/Csm, PIABS, RC/ABS, φpo/(1-φpo), Ψo/(1-Ψo), PICSo, PICSm of cv. DTC, cv. EV78 and cv. 6621 of *Zea mays* L. when plants were subjected to drought stress.

Parameters	Maize Cultivars		
	DTC	EV-78	6621
Fm/Fo	102.5474	98.2027	94.91126
Fv/Fo	103.1183	97.81207	93.88629
Fv/Fm	100.5308	99.56949	98.80599
Fo	84.24783	93.59627	112.6518
Fj	82.55973	91.00184	110.6543
Fi	80.27699	90.21179	109.6974
Fm	84.41996	91.45109	106.2535
Fv	84.45867	90.98482	104.9669

Mo	87.34364	94.87491	114.3312
Vj	97.17219	98.45308	104.8355
Vi	93.79181	97.90466	104.1825
Sm	108.0207	96.98211	97.06923
Ss	110.6721	104.424	92.85838
φPo	99.9755	99.56949	98.80599
ABS/RC	90.08966	96.58467	110.0736
TRo/RC	90.06015	96.09563	108.5274
ETo/RC	91.82678	96.83959	105.0597
DIo/RC	90.2004	98.85972	117.6471
PIABS	115.8238	106.6396	82.88959
RC/ABS	110.0559	103.5361	90.84833
φpo/(1-φpo)	99.58279	97.64492	93.2921
Ψo/(1-Ψo)	104.1217	102.5668	92.66851
PICso	97.50442	96.85438	88.23527
PICsm	100.1915	98.1394	82.14598

(TR_0/RC) trapping flux/ reaction center and absorption flux per reaction center (ABS/RC) declined in DTC followed by EV-78 in response to drought whilst greater increase in these characteristics was examined in cv. 6621 (Fig. 4). Electron transport ET_0/RC reduced with drought in the cultivars with respect to control with lesser decrease in ET_0/RC observed in drought resistant maize cultivar (DTC). While cv. 6621 exhibited the maximum increase under drought stress EV-78 cultivar was in between the tolerant drought cultivar (DTC) and sensitive drought cultivar (6621) regarding ET_0/RC. Drought significantly ($P \leq 0.05$) increased the dissipated energy flux per reaction center (DIo/RC) and the highest value was recorded in drought sensitive maize cultivar (6621). While the tolerant (DTC) was the least in DI_o/RC, moderately drought tolerant (EV-78) presented moderate change in increasing divergence of waste energy (Fig. 4). Drought ($P \leq 0.001$) reduced the RC/Cs_m but this effect was more pronounced in 6621 than in the others maize cultivars. The ABS/Cs_m was also ($P \leq 0.05$) reduced with drought in DTC (16%) and EV-78 (9%) while an increase of approximately 6% was observed in 6621 with respect to control (Table 5). The ABS/Cs_m generally decreased under drought in tolerant DTC and moderately tolerant EV-78 maize cultivars but increased in the drought sensitive maize cultivar 6621. TR_o/Cs_m and ET_o/Cs_m showed a significant ($p \leq 0.05$) trend upon drought imposition. In the tolerant DTC and moderately tolerant EV-78 maize cultivars, there were 16 and 9% reduction in TR_o/CS_m and 14 and 8% decrease in

ET_o/Cs_m, respectively. These parameters were maximum in the drought sensitive 6621. Highest value of DI_o/Cs_m (13%) was observed in 6621 whereas it declined 16 and 7% in DTC and EV-78 respectively. The density of reaction centers (RC/ABS) declined (9.2%) in 6621 but increased 10% in DTC in response to drought. EV-78 maintained the density of reaction center RC/ABS under drought. Significant changes ($P \leq 0.01$) was determined in indexes of performances including $PI_{(ABS)}$ and $PI_{(csm)}$. Comparison among these three cultivars DTC, EV-78 and 6621 maize cultivars showed that DTC (drought tolerant) increased $PI_{(ABS)}$ to 12.86 % while it decreased to 21% in 6621 (drought sensitive) under drought stress (Table 5). In addition, EV-78 (moderately tolerant) kept its $PI_{(ABS)}$ to control. Maximum reduction in $PI_{(csm)}$ was observed in drought sensitive 6621 but in the drought tolerant DTC, this parameter increased and remained almost constant in the moderately tolerant EV-78 (Chowdhury *et al.*, 2013).

Chlorophyll 'a' fluorescence is a useful parameter for the estimation of PSII bioenergetics in photosynthesis. In this study, fluorescence induction was sensitive to water stress. High F_0 values observed in 6621 cultivar might be due to many reasons such as increase in number of inactive RCs capable of slowing or depressing the reduction of quinone A (Q_A) at primary level and thus favor rise in fluorescence level at F_0 as cited by (Kalaji *et al.*, 2011). Increased V_J recorded in the same cultivar under drought can occur due to gathering of reduced Q_A (Q_A^-) pool capable of restricting

electron transport beyond Q_A^- as explained by (Redillas *et al.*, 2011). Chlorophyll a fluorescence double normalized data between Fo and F_m (V_{op}) build on single and multiple turn over stages in electron transport chain. First, O-J phase shows the photochemical reduction of Q_A in PSII reaction centers, the gradual changes in transients under four drought cycles were compared for V_{OK} (between 50 and 300 µs). It is clear from the results that positive K-band appeared in drought sensitive cv. 6621 but a more negative K-band in drought tolerant DTC than in moderately drought tolerant EV-78 (Fig. 3). The difference in V_{OJ} (ΔV_{OJ}) under drought in drought tolerant (DTC), in not excessive tolerant EV-78) and sensitive cultivar maize cultivars (6621) exhibiting negative and positive K-bands (Fig. 3) suggest the ability of the cultivar to resist drought-induced imbalance between the electrons at the donor and acceptor sides of PSII. But the distinct K-band under drought might be due to less efficiency of oxygen-evolving complex (OEC) resulting an imbalance between the electron flow from the OEC to the RC and towards the PSII acceptor side in the direction of PSI. Results indicate that that more positive L-band appeared in water stress sensitive 6621 compared with moderately tolerant EV-78 while a negative band appeared in the drought tolerant cultivar DTC (Fig. 3). The recorded variations in the amplitude of L-band (150 µs) showed that plants affected by drought were able to inhibit dissociation of light harvesting complex II (LHC II) from photosystem II (PSII) complex via the maintenance of energetic connectivity (Oukarroum *et al.*, 2007; Oukarroum *et al.*, 2009). However, an attenuated L-band in DTC suggests an adaptive mechanism against the loss in energetic connectivity which can occur on the verge of drought acclimation (Fig. 3) (Heerden *et al.*, 2007). During water stress, rise in the positive amplitude of L-band suggests that PSII units are least assembled together thus less energy is being exchanged among the independent PSII units (Oukarroum *et al.*, 2009; Redillas *et al.*, 2011). The O-I part shows the kinetic properties for reduction or oxidation of the plastoquinone pool (PQ), whereas the I-P phase indicate the changes in electron flux from reduced plastoquinol (PQH_2) to the final electron acceptor of PSI. The negative response of ΔV_{OI} recorded at four drought cycles indicated that 6621 was unable to maintain the reduction rate of PQ (capturing of exciton to the reduction of PQ) whereas reverse was true DTC. A positive increase

in I-P phase gives information of higher pool of final electron acceptors of PSI. A decreased IP-phase in DTC and EV-78 may be a part of adapting process attempting to cope up with drought stress to maintain PQ pool size. However, an apparent decrease in IP-phase at drought because of severe decline in leaf water status might mean the attainment of threshold level to drought acclimation. Connection of PSI dependent control of PSII activity under drought stressed conditions has been showed in previous work (Gomes *et al.*, 2012; Stirbet and Govindjee, 2011). Appearance of attenuated peak under drought stress therefore indicates that I-P phase of OJIP transients is apparently caused by PS-I associated limitations.

As regards maximum quantum yield of primary photochemistry (F_v/F_m), there was decrease in the three cultivars (Fig. 1). But cultivars also differed significantly in this character; however, DTC had higher quantum yield relative to the other cultivars. Plant scientists describe such occurrence to be a part of photo protection mechanism for reduction of light absorbance by decreasing chlorophyll contents (Galmes *et al.*, 2007).The F_V/F_M insensitivity to drought is a well-known occurrence. F_v/F_m insensitivity considers the inversal changes in original fluorescence (F_o) and maximum fluorescence (F_m). However, the ratio F_v/F_m would not change markedly (Fig. 4) (Elsheery and Cao, 2008). Transfer of electrons from photosystem II (PSII) to Q_A then PQ to photosystem I (PS I) electron acceptor can be characterized according to their sensitivity to drought stress and ranked as φRO > φEo> φPO.

The increased in the ratio of absorbance per reaction center (ABS/RCs) under drought refers to only active photosystem II (PSII) reaction centers which may be due to the inactivation of some photosystem II (PSII) reaction centers (Lu *et al.*, 2001) and then again regrouping from inactive to active antennas of photosystem II (PSII) reaction centers (Heerden *et al.*, 2003).In spite of greater absorbance per reaction center (ABS/RC), very high rate of efficient separation excitations that are untrapped (DI_o/RC) values under drought with higher level transport of electron per PSII reaction centers in 6621 was observed. However, DI_o/RC decreased in DTC. Thus, this reduced dissipation in cv. DTC might be an adaptation against drought stress. ET_o/RC was higher in 6621 compared with the others under drought. Down regulation of this parameter might have stopped over reduction of electron

transport chain and make easy loss of excessive energy in order to minimize the photo oxidative damage in the thylakoid membrane (Heerden *et al.*, 2007). Acclimation response to drought stress in 6621 showed significant increment in DI_o/Cs_m unlike EV-78 and DTC where there was less release in DI_o/CS_m. Again, this suggests a defending response of 6621 involving the release of excess energy as DI_o/Cs_m. This action contributes to the photoprotection of PSII by decreasing the active reaction

center density. The behavior of other phenomenological fluxes particularly ET_o/Cs_m was reduced due to water stress and might have significantly affected the performance indexes $PI_{(csm)}$ and $PI_{(ABS)}$. The response of reduction in $PI_{(csm)}$ of drought sensitive cv. 6621 was affected maximally than other maize cultivars (DTC and EV-78). DTC was shown to be good in $PI_{(ABS)}$ while 6621 was least in $PI_{(ABS)}$ (Fig. 4). EV-78 on the hand, was in between the values of $PI_{(ABS)}$.

Fig 5. Experimental picture showing the phenotypic differences among tolerant (DTC) Moderately tolerant (EV78) and drought sensitive (6621) cultivar. Morphological, photosynthetic and gas exchange parameters strongly and significantly support the differences among cultivars shown in this picture.

4. Conclusion

Photosynthesis in the maize cultivars under drought stress was repressed by stomatal closure which limited the sub-stomatal diffusion of CO_2. Leaf gas exchange relations (Ci, A, E and WUE) and chlorophyll a fluorescence technique revealed stomatal limitation under drought conditions. Photosystem II performance was down-regulated via the addition of inactive reaction centers (RC_s), decline in the accumulation of reduced Q_A (Q_A^-) pool, decrease in electron transport beyond Q_A^-, disruption in the regular electron transport activity S_m (number of electron carriers/electron transport chain), N (turn-over number), $ET_o/$ RC, ET_o/Csm and increase in heat wastage, However, structural probity and stability of PSII were largely maintained by the drought tolerant and moderately tolerant maize cultivars DTC and EV-78- as reflected by $\Delta V_{OP}, \Delta V_{OK}$ (L- band) and ΔV_{OJ} (K- band) under drought stress compared with the drought sensitive cultivar-6621. Overall, the present research focused on the photosynthetic performance specifically the chlorophyll a fluorescence

parameters of maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) as a cereal food crop under drought stress conditions (Fig. 5). Our results could provide insights into future characterization of photosynthetic traits aimed at increasing yield for drought prone areas.

Compliance with ethical standards

NA

Conflict of Interest

The authors declare no conflict of interest.

Author's contribution

Conceptualization of research (HRA, ZUZ, SM); Designing of the experiments (HRA, ZUZ, SM); Contribution of experimental materials (HRA, ZUZ, SM, MA, NB); Execution of field/lab experiments and

data collection (MA, NB, AA, TI); Analysis of data and interpretation (MA, SA, ASK, MK, IR, ZA, MJ, MI); Preparation of the manuscript (MA, NB).

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