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Chlorophyll fluorescence kinetics and response of wheat (*Triticum aestivum* L.) under high temperature stress

Neelu Jain[1]*, GP Singh[1], Rakesh Pandey[2], Ramya P[1], PK Singh[1], Nivedita[1] & KV Prabhu[1]

[1]Division of Genetics; [2]Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110 012, India

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High temperature stress during grain filling period in wheat (*Triticum aestivum* L.) has pronounced effects on yield in major wheat growing agro-ecological zones. The fast chlorophyll fluorescence transients expose the damage to leaf photosynthetic activity under high temperatures. The method relies on the functioning of photosynthetic apparatus of plants under stress; however, these transients have been studied in detached leaves under controlled conditions. Still no reports are available on the potential usefulness of chlorophyll *a* fluorescence kinetics on intact wheat leaves for screening heat stress tolerant genotypes under field conditions. Hence, we conducted a study with the hypothesis that chlorophyll *a* fluorescence parameters and kinetics of plant-attached leaves under field conditions can be useful for screening of heat tolerant genotypes. The experimental population for testing the hypothesis consisted of 20 diverse wheat genotypes grown under timely and late sown environments (terminal heat stress) for two years. The results showed influence of high temperature on the expression of parameters F_o, F_v/F_o, F_v/F_m, and performance index. Analysis of the fast OJIP fluorescence transients indicated that the relative variable fluorescence between steps O and K and between steps O and J were related to high temperature stress tolerance. Five genotypes with consistent performance for better photosynthetic efficiency under thermal stress were observed. The inferences drawn from the present study supported our hypothesis that the analysis of chlorophyll *a* fluorescence transient parameters of plant attached leaves under field conditions can be used as a tool in the selection of wheat cultivars with better thermostability and functioning of photochemical reactions that could sustain photoassimilation and grain dry matter accumulation.

Keywords: Abiotic stress, Chlorophyll fluorescence, Elevated temperature, OJIP kinetics, Photosystem II, Fast chlorophyll fluorescence transients

Wheat cropping systems worldwide have been categorized into 12 mega environments based on the biotic and abiotic characteristics, of which mega environments 4C and 5A symbolize central and eastern parts of India which face severe terminal heat stress[1]. It is well observed that high temperature stress has pronounced effects on wheat growth and yield in major wheat growing agro-ecological zones in India. High temperature due to dry winds in northwestern plains zone and high temperature combined with high humidity in northeastern plains zone significantly reduce yield and quality of wheat[2]. Each 1°C rise of temperature above the ambient temperature during grain filling causes significant loss of grain yield[3]. High temperature leads to decline in physiological processes such as photosynthesis as well as grain starch synthesis[4]. Photosynthetic process is adversely affected due to greater sensitivity of photosystem II (PSII), ATPase and the carbon assimilation pathways[5]. Under heat stress, the photosynthetic electron transport chain cannot use light energy efficiently to produce ATP and NADPH and the structural organization of thylakoid membranes and function of photosystems is also disrupted leading to inhibition of electron transport, generation of reactive oxygen species (ROS) and eventually reduction in photosynthetic efficiency[6].

This altered photochemistry under high temperature stress can be probed with chlorophyll *a* fluorescence which is characterized by a major peak centered around 685 nm attributed mainly to light-harvesting antenna in PSII. The proportion of chlorophyll fluorescence depends on the use of absorbed light in photochemistry and heat dissipation by the leaf[7]. Chlorophyll fluorescence has thus become an useful, rapid and noninvasive tool to study the photosynthetic performance and physiological status of plants under abiotic stresses such as water deficit, reduced sunlight, temperature and salinity[8-10]. The transient in JIP

*Correspondence:
E-mail: neelu_jain25@yahoo.com

kinetics and the associated effects on photosynthetic processes, particularly PSII under variable conditions have been studied in detail in the recent past[11]. Application of chlorophyll *a* fluorescence parameters in QTL studies, interpretation of fluorescence signals, energy partitioning concept, crop phenotyping and other specific applications have been discussed in comprehensive manner[12,13].

Under abiotic stress conditions, an integrated approach for developing varieties with better tolerance, efficient input and wider adaptability is imperative. Reliable phenotyping for high temperature tolerance with parameters that are convenient, nondestructive, rapid and quantitative are desired to improve stress tolerance[8]. Sustenance of photosynthesis under stress conditions is one of the main targets for improving wheat yields. Therefore, chlorophyll fluorescence and pigment contents are explored as suitable tools for assessing plant physiological status[14,15] and to dissect the genetic determinants for yields. The chlorophyll fluorescence transients (OJIP curve) indicate the structural and functional competence of chloroplast membrane bound photosynthetic apparatus in terms of PSII stability, heterogeneity, activity, connectivity and electron transport perturbations[9,11,13]. The polyphasic rise of chlorophyll *a* fluorescence has been reported for evaluating the effects of high temperature stress in soybean[16], beans[17], wheat[6,18] , sorghum[19] and pea[20]. A recent review[12] has also endorsed its potential for screening of high temperature tolerant genotypes. Earlier studies in wheat have shown that high temperature leads to declined variable fluorescence F_v and changed the fast fluorescence kinetics[14,21]. However, all these studies have been based on altered fluorescence kinetics under controlled stress conditions in detached leaves and mostly in a few genotypes as the outdoor field experiments are complex. Brestic *et al*.[22] who evaluated the genotypic variability of PSII thermostability in 30 winter wheat genotypes grown in field conditions, also studied the fluorescence transients in detached leaf pieces of 50 mm length after exposing them to high temperatures in lab conditions in a water bath. However, there is also need for evaluation of the validity of the ealier conclusion[18] that the parameters PI, RC/ABS and (1 – Vj)/Vj showed no genotypic heat stress differences in a large number (41 genotypes) of contrasting wheat genotypes grown and treated for high temperature in greenhouse. Therefore in the present study, we tried to understand the association between chlorophyll fluorescence and physiological response under high temperature stress and explored the usefulness of chlorophyll fluorescence transients as a tool to assess the genotypic variability in field conditions in plant attached intact leaves.

Materials and Methods

Plant materials

Twenty spring wheat cultivars from different production environments (recommended for release) (Table 1) were sown under timely (TS: 16th

Table 1 — Relative change in chlorophyll fluorescence parameters in late sown environments as of timely sown environments at mid-grain filling stage in wheat

Genotype	Recommended Production environments	Relative F_o	Relative F_v/F_m	Relative F_v/F_o	Relative PI_{ABS}
C306	Timely sown rainfed	1.037	0.995	0.977	0.685
HW2004	Timely sown rainfed	0.968	0.949	0.753	0.798
NI5439	Timely sown rainfed	0.992	0.924	0.653	0.576
HI617	Timely sown rainfed	0.936	0.97	0.841	0.762
Halna	Late sown irrigated	0.974	1.007	1.043	0.891
Raj3765	Late sown irrigated	0.908	0.991	0.954	0.957
HI1500	Timely sown rainfed	0.938	1.007	1.046	0.665
HI1544	Timely sown restricted irrigation	0.983	0.996	0.977	0.978
HI1531	Timely sown restricted irrigation	1.045	1.045	1.244	1.354
PBW175	Timely sown rainfed	0.923	0.959	0.807	0.813
HD2987	Timely sown rainfed/ restricted irrigation	1.032	0.964	0.816	0.641
NW2036	Late sown irrigated	1.066	0.998	0.984	0.903
WH730	Timely sown irrigated	0.991	0.906	0.610	0.404
WL711	Timely sown irrigated	0.954	0.950	0.766	0.514
WH157	Timely sown irrigated	1.002	0.987	0.932	0.731
HUW510	Late sown irrigated	0.920	1.009	1.044	1.298
DBW43	Timely sown irrigated	1.137	0.983	0.912	0.730
HD2888	Timely sown rainfed	1.242	1.007	1.035	1.124
Babax	Timely sown irrigated	1.036	0.972	0.865	0.790
Excalibur	Timely sown irrigated	1.052	0.913	0.642	0.700

November, 2012-13 and 18th November, 2013-14) and late sown (LS: 5th January, 2013 and 5th January 2014) conditions taken as control and temperature stress environments, respectively for two years. Of these twenty cultivars, Babax and Excalibur were parents of international mapping populations, obtained from CIMMYT nurseries while the rest were released varieties from India except germplasms DBW43 and WH730.

Experimental site and weather details

The study was conducted at experimental farm of Indian Agricultural Research Institute, New Delhi (latitude 280 38'23"N, longitude 770 09'27"E and altitude 228.61 m above mean sea level) during 2012-13 and 2013-14 crop seasons. The experiment was laid out in field in 3 rows of 3 m each with 20 cm row to row spacing in two replications. To raise a good crop, the cultivation practices prescribed for wheat in northwestern Indogangetic plains under irrigation were followed.

Temperature variables for the wheat cycle of both the crop seasons are shown in Fig. 1. Anthesis of genotypes in timely sown environments was observed during 7th to 9th standard meteorological weeks while for late sown, it was during 11th to 13th weeks in 2013. During 2014, anthesis of genotypes in timely sown environments was recorded between 6th to 9th weeks and in late sown between 11th to 13th weeks. In 2013, the weekly mean maximum temperature during the anthesis in timely sown crop (7th to 9th week) was between 21.7 to 25.2°C and in the late sown environment (11th to 13th week) was in the range 29.7 to 30.1°C. The weekly mean maximum temperature during the anthesis in timely sown crop (6th to 9th week) was between 19.0 to 22.4°C and in the late sown environment (11th to 13th week) was in the range 26.4 to 30.9°C during the crop year 2014.

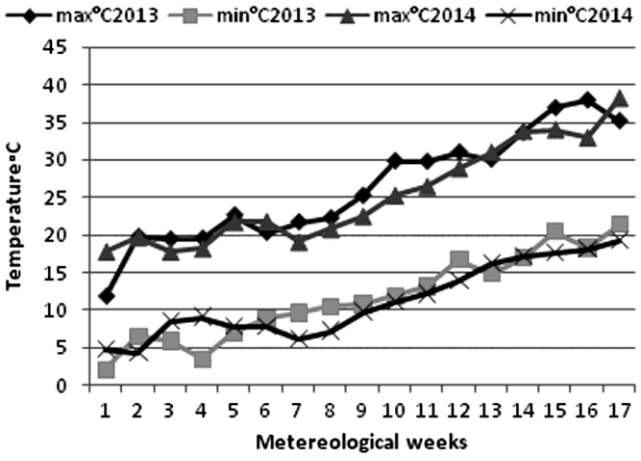

Fig. 1 — Maximum and minimum temperatures for the wheat growth cycle

Chlorophyll fluorescence measurements

Chlorophyll fluorescence were measured on flag leaves with a portable fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd. UK) at the midgrain filling stage (milking or Z75 to 77[23]) in both control and temperature stress trials. The measurements were taken on three randomly selected plants for each variety in both the environments. The measurements were taken on multiple days during Z75 to Z77 stage. The flag leaves were adapted to darkness for 25 min using clips and a strong 1s light pulse was applied by light emitting diodes. The leaves exhibited a polyphasic chlorophyll *a* fluorescence rise on light illumination (3000 µmol m^{-2} s^{-1}) and fluorescence transient was recorded as the steps labeled O, J, I and P. Fluorescence transients started with origin O at 0.05 ms (milliseconds) and reached peak at P =30 ms with intermediate steps named as J and I at 0.3 ms and 2 ms, respectively. The chlorophyll fluorescence parameters studied included F_v/F_m which indicates the maximum quantum efficiency of PSII, F_v/F_o which represents the maximum yield of primary photochemistry of PSII, and performance Index (PI) which indicates the internal force of the sample to resist constraints from outside. PI was expressed as PI_{ABS} (absorbance) and PI_{Total}. Other fluorescence parameters were analyzed using the JIP test kinetics[24].

Data analysis

Fluorescence transients were measured with increasing time intervals beginning from 0.05 ms (O) to 0.3 ms (J) to 2 ms (I) to 30 ms (P) and finally saturation at S. The data points were initially normalized between points O-J, and O-K, subsequently ΔW_{OJ} and ΔW_{OK} were calculated and analyzed as graphs. The L bands between 100-200 µS due to relative variable fluorescence at steps O and K, $[W_{OK} = (F_t-F_o) / (F_k-F_o)]$ and K bands between 200-400 µS due to relative variable fluorescence at steps O and J, $[W_{OJ} = (F_t-F_o) / (F_J-F_o)]$, were visualized[9]. The L and K bands are indicative of stability of photosynthetic system. The differences in relative variable fluorescence ΔW ($\Delta W_{OK} = W_{OK\ late\ sown} - W_{OK\ timely\ sown}$ and $\Delta W_{OJ} = W_{OJ\ late\ sown} - W_{OJ\ timely\ sown}$) were calculated between control and stressed genotypes to determine the efficiency of PSII units.

The data was recorded on the thousand grain weight (TGW) and grain yield for all the 20 genotypes. Relative change for fluorescence parameters was computed as values of late sown environments as of timely sown environments.

Results

Chlorophyll fluorescence analysis during first year

The genotypes showed differential fluorescence transient values in both control and stress environments. The fluorescence parameters were strongly influenced by temperature stress (Table 1). The expressions F_v/F_o, F_o/F_m, maximum quantum efficiency of PSII (F_v/F_m) and performance index exhibited significant differences. Genotypes HW2004, NI5439, Halna, Raj3765, HI1500, HI1544, WH157 and HUW510 exhibited lower F_o levels under stress. F_v/F_m values decreased under stress in most of the genotypes except Halna, HI1500, HI1531, HUW510 and HD2888. Mean fluorescence values for most of the parameters showed significant LSDs in the two environments (Table 2).

Performance index (PI) revealed differences with higher sensitivity as compared to other fluorescence parameters in stress environments. PI_{ABS} decreased in all the varieties except Raj3765, HI1544, HD2987, HUW510 and HD2888 under elevated temperature stress as compared to control reflecting their ability to perform better (Fig. 2). These varieties reflected higher relative efficiency of the three PI components—reaction centres per antenna (RC/ABS), relative efficiency of primary photochemistry (φPo/1-φPo), and electron transport activity (Ψo/1 – Ψo) (Table 2). The overall PSII functioning measured by PI was decreased by 17.4% under stress.

The differences of O and K fluorescence kinetics in both control and stress environments led to the appearance of the hidden L band at around 0.10 µs in the wheat genotypes (Fig. 3A). The normalized fluorescence transient values between F_o (50 µs) and F_k (300 µs) revealed the positive amplitude of L band in 14 genotypes. The remaining six genotypes, Raj3765, Halna, HI1531, HUW510, DBW43 and HD2888 exhibited lower ΔW_{ok} values implying an increase in the excitation energy transfer or connectivity between different PSII units under temperature stress. The relative fluorescence intensities at steps O and J visualised the K-band among the genotypes. The appearance of the K-band at around 0.2 µS indicates the relative changes in PSII donor side on being exposed to high temperatures. The genotypes Raj3765, HI1544, HI1531, HUW510, DBW43 and HD2888 reflected negative transient values of K band (Fig. 3B).

Chlorophyll fluorescence analysis during second year

Fluorescence parameters also revealed significant differences during the consecutive crop season 2013-14 for the 20 genotypes. Significant LSDs were found

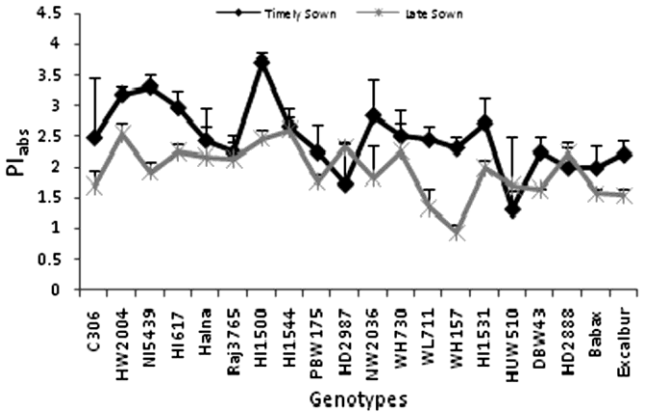

Fig. 2 — Photosynthetic Performance index values for wheat genotypes in timely and late sown environments

Table2 — Genotype means for fluorescence parameters in timely sown versus late sown environments

Fluorescence parameters	Timely sown environments	Late sown environments	LSD	SD	Timely sown environments	Late sown environments	LSD	SD
	2012-13				2013-14			
F_v/F_o	4.59±0.11	4.05±0.10	0.30	***	3.215±0.13	3.083±0.09	0.037	*
F_v/F_m	0.819±0.003	0.800±0.004	0.011	**	0.748±0.01	0.738±0.01	0.04	ns
F_o/F_m	0.199±0.003	0.180±0.004	0.011	**	0.195±0.001	0.187±0.003	0.051	ns
PI_{ABS}	2.47±0.12	1.94±0.09	0.321	**	2.32±0.05	2.13±0.10	0.17	*
RC/ABS	0.40±0.006	0.38±0.005	0.017	**	0.39±0.003	0.372±0.001	0.015	*
φPo/1- φPo	4.59±0.11	4.05±0.1	0.307	***	4.78±0.09	4.43±0.11	0.23	**
Ψo/1 – Ψo	1.311±0.034	1.23±0.03	0.097	*	1.22±0.02	1.19±0.03	0.03	*

[*significant at 0.05; **at 0.01; and, *** at 0.001 probability level, respectively. ns:non-significant; LSD: Least significant difference; and SD: significance of difference]

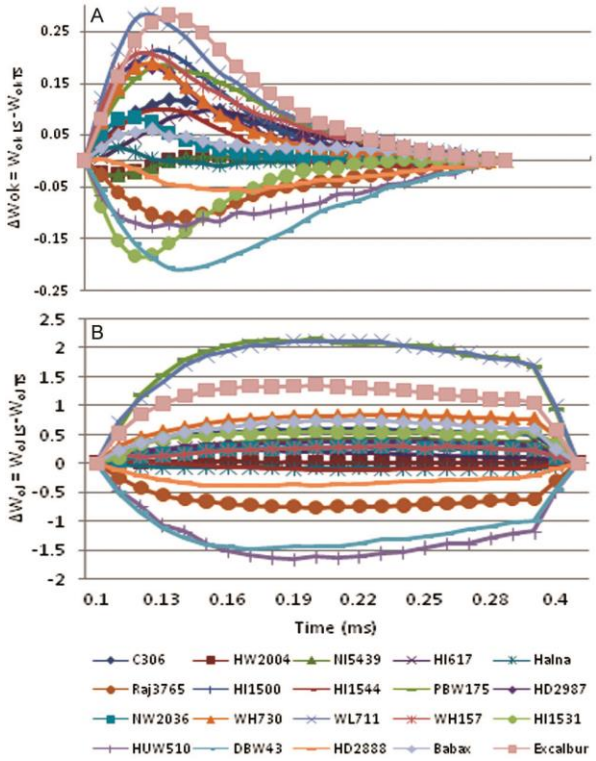

Fig. 3 — Chlorophyll fluorescence transient curves represented as difference kinetics of late sown and timely sown environments during 2013. (A) Relative variable fluorescence between steps O and K expressed as $\Delta Wok = W_{ok\ LS} - W_{ok\ TS}$;and (B) Relative variable fluorescence between steps O and J expressed as $\Delta W_{oJ} = W_{oJ\ LS} - W_{oJ\ TS}$

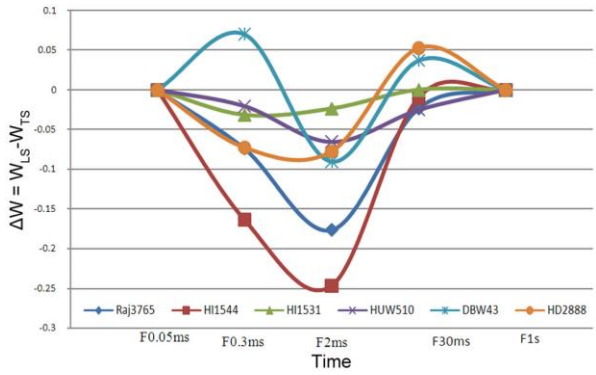

Fig. 4 — Fluorescence kinetics in six wheat genotypes as difference kinetics of stress and non-stress environments during 2014.

among initial (F_o) and maximum (F_m) fluorescence between the two environments (Table 2). The fluorescence transient values at times 2.0 ms (I) and 30 ms (P) revealed most significant changes under stress conditions. After 0.3 ms (J), the fluorescence signals declined in genotypes undergoing temperature stress with an exception of Raj3765, HUW510, HI1544, HI1531 and HD2888 (Fig. 4). The fluorescence signals in the five stressed genotypes were in accordance with that obtained in the previous year indicating least effect on their PSII in the stressed environment. The five genotypes again revealed negative amplitudes of L and K bands except DBW43.

Table 3 — Correlation coefficients with TGW and yield in late sown environments

Fluorescence Parameters	2012-13		2013-14	
	TGW	Yield	TGW	Yield
F_o	–0.46*	0.23	0.2	0.42
F_m	–0.18	0.46*	0.43	0.41
F_v/F_m	0.47*	0.36	0.49*	0.32
F_v/F_o	0.47*	0.38	0.46*	0.18

* significant at P=0.05

TGW showed significant negative correlation with F_o (–0.46*) in stress environments during 2013 (Table 3), while F_v/Fo and F_v/F_m showed significant correlation with TGW in both the years.

Discussion

Chlorophyll fluorescence transient is an useful tool in determination of effects of various adverse factors such as high temperature, moisture stress, salinity or nutrition stress on plants in the recent past[9,11,25]. Heat stress impairs PSII due to changes in thylakoid membranes, and release of Mn from manganese-stabilizing proteins in the PSII reaction centre[12]. Damaging of PSII leads to lower electron transfer rate and longer time to reach maximum fluorescence intensity. Dark adaption of a photosynthetic sample allows induction of a curve known as O-J-I-P transient that provides information about the structure and function of photosynthetic apparatus. The JIP test evaluates a number of parameters to characterize the photosynthetic status which depends on the excitation energy dissipation, energy trapping and further transport of electron between the two photosynthetic systems. The O-J part of fluorescence indicates closure of PSII due to reduction of Q_A, J-I relates to reduction of electron acceptor Q_B, plastoquinone and cytochrome, I-P relates to reduction of electron transporters such as ferredoxin and NADP. Under stress, the oxygen evolving complex inhibits and the electron transport gets blocked. Limitations in these processes can be identified using the chlorophyll fluorescence and electron transport inhibitors[25,26]. The present study aimed at evaluating chlorophyll fluorescence as criteria for identification of stress

tolerant lines was therefore found promising under field conditions.

Thermal stress can lead to a significant change such as increase in F_o or decrease in F_m[27]. In our study, F_o showed a significant decrease in tolerant genotypes with the increase in grain yield, reflecting its ability to be an effective parameter in screening for thermal stress. F_o was observed to be correlated with drought sensitivity and grain yield[28,29]. Some workers also concluded that the recording of F_o-temperature curves could provide a rapid means of determining heat tolerance in durum wheat[30]. The increase in F_o in some of the genotypes can be interpreted as irreversible damage to PSII caused by uncontrolled dissipation of heat energy that produces an excess of excitation energy[31], or an inhibition of electron flow from reduced Q_A to Q_B[32]. The results showed significant differences for F_v/F_m, Fv/F_o and F_o/F_m between control and stress environments. In previous studies, F_v/F_m and F_v/F_o have been reported as informative parameters in determining the physiological status of PSII during screenings for high temperature stress in controlled environments[6,18]. The significant response of fluorescence parameters to the TGW and grain yield also reflected a greater photosynthetic efficiency. The study indicates that temperature stress induced changes in chlorophyll fluorescence parameters can differentiate individual genotypes under natural stressed environments.

Many studies have reported photosynthetic performance index as a sensitive measure of stress[33,34]. PI measured for wheat genotypes under drought correlated well with the drought tolerance estimated by grain yield. Our study corroborates with the findings[9] that the expressions of PI and its log values (Driving Force) are more sensitive to stress compared to F_V/F_m ratios. We observed relatively higher PI values among tolerant genotypes to that reported in barley[33] and in wheat[6]. The lower values of PI obtained in sensitive genotypes may be due to absorption of energy by the inactive reaction centers, lower photochemical yield and reduction in the electron transfer from Q_A^- to the intersystem electron acceptors as suggested earlier[35].

The typical chlorophyll fluorescence induction kinetics reflects the overall performance of photosynthetic apparatus[11,24]. High temperature induced destruction of Mn cluster of PSII reaction step leads to appearance of K step. In the OJ phase, the changes in the L band (100-200 µs) and K band (200-400 µs) could be helpful in predicting the tolerance of varieties to high temperature. Change in the JIP fluorescence curves during the first 300-400 µS provided information on the energetic connectivity of PSII units and evaluates the reduction capacity of PSII units in the form of L and K bands. The six genotypes exhibiting lower ΔW_{ok} values during 2013 were tolerant under high temperature conditions and the better energetic connectivity of their PSII units corresponds to higher photosystem efficiency. Genotype Halna is known for heat avoidance mechanism (early maturing at 126-134 days) and therefore exhibited little amplitude of L band without specific peaks. Raj3765 and HUW510 are heat tolerant varieties and are grown as national check in the areas influenced by terminal heat stress. HD2888 is used as national check for varietal trials in rainfed environments (Sourced from compilation of All India coordinated varietal improvement program). We could also observe the positive deviations of K bands in heat susceptible genotypes indicating greater damage to their oxygen evolving complex (OEC) under elevated temperatures. The positive amplitude of K band exhibited the inhibition of OEC probably due to higher intensity of electron flow from acceptor to donor leading to the damage of reaction centers under high temperatures[9] and has been observed in many plants such as wheat[6,21], *Prunus*[34], and pea[20]. The appearance of L and K bands in this study revealed the integrity status of photosystems on being exposed to high temperatures. The negative deviations observed in five genotypes in stress environments during both years indicated the potential to cope with stress due to their greater stability of OEC and electron transport from PSII to PSI for driving energy synthesis. The relatively higher performance index in some of the genotypes depicts the superior functionality of both photosystems under stress environments.

Rapid, reliable and precise screening for suitable physiological parameters is a pre-requisite for breeding heat tolerant varieties. Chlorophyll fluorescence has emerged as one of the quick and precise techniques for evaluating the performance of genotypes undergoing stress. Previous studies on chlorophyll fluorescence are limited to controlled environments. We have sucessfully assessed the usefulness of chlorophyll fluorescence kinetics in differentiating genotypes under field conditions. Five genotypes with better and stable photosynthetic

efficiency under thermal stress were observed. Thus, chlorophyll fluorescence parameters appears to be a useful selection criterion to evaluate thermostress tolerance in field. However, furthur investigations are needed to study the heritability of trait to be used as sole selection criteria. Further, improved understanding of the underlying mechanisms governing the fluorescence transients can facilitate its application in large scale field screening studies.

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