A quick method to screen high and low yielding wheat cultivars exposed to high temperature

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Abstract In tropical countries, high temperature stress is the major abiotic stress, which controls the productivity and yield of crop plants. Two high yielding and low yielding genotypes of durum wheat were selected for detailed analysis of their photochemical efficiencies. In low yielding genotypes (Malvi local and Sawyer local), the whole primary photochemical reactions are affected before and after heat stress. The results show that low yielding genotypes show less efficiency in the usage of the available excitation energy. This is a case study to establish use of chlorophyll a fluorescence measurement as an effective tool to screen plants for their stress tolerance. The study is important for stress physiology and may be useful for assessment of stress tolerant plants.

Keyword Wheat genotypes · High temperature · Chlorophyll fluorescence

Abbreviations

F₀ Initial fluorescence
F_m Maximum fluorescence
F_v Variable fluorescence

Introduction

Wheat is the most important cereal grown in the world, especially in the tropical countries. Heat stress is an important environmental factor that limits the productivity of many

crops and affects the quality and quantity of crop yield in tropical countries (Georgieva et al. 2000). Heat stress affects photosynthetic processes significantly by causing several changes in the structure and function of the photosynthetic apparatus. Elevated temperature results in reduced photosynthetic metabolism (Biswal et al. 2011), photosynthetic capacity and photochemical efficiency (Percival 2005). First effect of stress in a leaf is often seen in the form of damage to photosystem II (PSII) (Li et al. 2009, see review by Mathur et al. 2014).

Chlorophyll fluorescence has been used in several studies to detect the stress tolerance in different genotypes in response to heat stress in many plant species (Slabbert and Kruger 2011). Chlorophyll fluorescence is generally used to provide a rapid, non-invasive diagnostic method for detecting and quantifying damage to the leaf photosynthetic apparatus, particularly in relation to PS II response to environmental stress before any of the visual symptoms appear (Mehta et al. 2010; Mathur et al. 2011; Murchie and Lawson 2013). The JIP-test is a tool to analyze polyphasic rise of Chl *a* fluorescence transient (OJIP labeled phases). It helps to investigate the efficiency of electron transport (Christen et al. 2007) and also to analyze the structural integrity of chloroplasts. The shape of OJIP is universal for all higher plants and at a given moment, is determined by the physiological state of the sample. The JIP-test is based on the analysis of the rise in polyphasic fast chlorophyll *a* and is a method for studying the relationship between light-dependent reactions and ChlF. The JIP-test (OJIP) name comes from the specific points on the induction curve that is formed by the recorded ChlF signal and is suitable to the gradual reduction of Q_A—the primary electron acceptor of PSII. The J-I part of the curve corresponds to the reduction of the secondary electron acceptor Q_B, plastoquinone (PQ), cytochrome (Cyt b₆f), and plasto cyanine (PC). The increase in ChlF in the I-P part of the induction curve is typically attributed to the reduction of electron transporters of the PSI acceptor-side: ferredoxin (Fd), intermediary acceptors,

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and the NADP. OJIP analysis is a tool for determining the correlations between the structure and functions of the photosynthetic apparatus, which is useful in evaluating plant vitality (Kalaji et al. 2014).

One of the major goals for plant breeders is to develop genotypes with a high yield potential and the ability to maintain yield across environments. Since rate of photosynthesis is directly proportional to total yield of a crop, parameters related to photosynthesis, particularly PSII, can be exploited to gain more information about the components that provide stability under stress conditions and hence high yield. For this reason a detailed study of various photochemical events associated with PSII and their stability under heat stress conditions has been carried out. High and low yielding wheat cultivars, already differentiated on the basis of traditional parameters were subjected to Chl fluorescence analysis. This study will establish details of Chl *a* fluorescence measurements which can help in screening of crop plants for their stress tolerance very quickly and in a single step.

Materials and methods

Plant material and growing conditions

A field study was carried out during *rabi* season 2010–11 at Indian Agricultural Research Institute, Regional Station, Indore (M.P.) India. The experimental field is situated between 22°37' N latitude to 75°50' E longitude at 557 m above mean sea level having semi-arid and humid climate with a temperature range of 23 to 44 °C and 7 to 29 °C in summer and winter seasons, respectively. Sowing was done in beds having length of 2.5 m in two rows plot with a spacing of 18 cm in RBD with three replications for each sowing. Recommended agronomical practices were followed to raise the good crop and all care was taken to minimize any variations due to environmental and cultural conditions.

Measurement of fast kinetics of Chl *a* fluorescence

The polyphasic chlorophyll *a* (Chl *a*) fluorescence was measured using a Plant Efficiency Analyzer (PEA, Hansatech, King's Lynn, Norfolk, UK) during vegetative stage (controlled condition) and post anthesis (grain filling conditions) i.e. after facing high temperature conditions. Leaves were dark adapted for 15 min before fluorescence measurements. Details can be seen in Mathur et al. (2011). Energy pipeline models were prepared using the software Biolyzer HP 3 (the chlorophyll fluorescence analyzing program by Bioenergetics Laboratory University of Geneva, Switzerland).

Statistical analysis

Mean values of grain yield and other various yield parameters were calculated using standard statistical procedures by Gomez and Gomez (1984).

Results and discussion

In an earlier study, 102 genotypes of durum wheat were subjected to high temperatures after anthesis, facing high temperatures in the day with mean daily temperatures higher than 32 °C. On the basis of several parameters like number of grains (NG), biomass (BM), harvest index (HI) in %, hectoliter weight (HW), grain weight (GW), grain yield (GY), two high yielding and two low yielding genotypes were selected (Table 1). The high yielding genotypes were regarded as heat tolerant (HI-8691, HI-8638) genotypes which showed better yield even after facing high temperature stress. The low yielding genotypes were heat sensitive genotypes (Malvi local, Sawyer local) whose yield decreased dramatically after facing high temperature stress. Detailed analysis and comparison of photochemical efficiency of PS II was carried out in two high yielding and two low yielding genotypes in order to understand the physiological basis for stress tolerance as well as to check if the results from Chl fluorescence are consistent with results obtained by traditional parameters.

The plants exhibit a polyphasic rise called O-J-I-P fluorescence transient; the O to J phase (ends at ~2 ms), the J to I phase (ends at ~30 ms) and I to P phase (ends at ~500 ms). The shape of the O-J-I-P fluorescence rise has been related to a major change in the photosynthetic electron transport (Joly and Carpentier 2009). Chlorophyll *a* fluorescence transient was measured before (Fig. 1a) and after stress (Fig. 1b) in high yielding and low yielding genotypes of wheat to evaluate the effect of high temperature on the photochemical efficiency of PS II. It is evident from the Fig. 1a that the shape of the curve is different in high and low yielding genotypes indicating their different efficiencies to carry out photochemical events. However after heat stress, the photochemical efficiency of high yielding genotype is not affected while a significant decrease is observed in low yielding genotype (Fig. 1b). This clearly indicates that low yielding genotypes is much more susceptible to heat stress. Various parameters like Fv/Fm, Fv/Fo, tfm, area and PI were derived from induction curves of High and low yielding genotypes (Fig. 2).

The parameter Fv/Fm represents the maximum quantum yield of primary photochemistry of PS II (maximum photochemical efficiency of PSII in the dark-adapted state) and is used as a sensitive indicator of plant photosynthetic performance (Kalaji et al. 2011). The decrease in Fv/Fm ratio under heat stress is considered to reflect the damage to PSII

Table 1 Various parameters on the basis of which high yielding and low yielding genotypes of wheat were selected

S.No	Genotypes	Traits						
		NG	BM	HI	HW	GW	SDS	GY
1	HI 8691	62.4	63.8	36.6	80.3	48.6	36.3	23.3
2	HI 8638	58.7	54.3	36.6	81.0	45.1	37.7	19.7
3	Malvi Local	34.9	53.6	19.8	71.2	35.4	31.0	10.6
4	Sawer Local	37.2	50.0	22.9	74.5	38.3	27.7	11.3
5	S.E	2.00	6.25	1.79	0.53	0.84	0.71	2.09

NG Number of grains, BM Biomass, HI Harvest index in %, HW Hectoliter weight, GW Grain weight, SDS Sedimentation value, GY Grain yield

(Srivastava et al. 1997). The results showed that Fv/Fm was much less reduced (very slight change) in high yielding genotypes than in low yielding genotypes during grain filling stage or stressed condition (Fig. 2). It indicates high yielding genotypes have a higher stability of PS II under heat stress.

Fv/Fo ratio, which reflects the structural alterations in PS II (Havaux and Lannoye 1985) decreased significantly in low yielding genotypes during heat stressed conditions (or grain filling stage) compared to high yielding genotypes (Fig. 2). The ratio Fv/Fo reflects the efficiency of electron donation to PS II reaction center and the rate of photosynthetic quantum

conversion at PS II reaction center (Babani and Lichtenthaler 1996). Area over the fluorescence induction curve between Fo and Fm is proportional to the pool size of reduced PQ on the reducing side of PS II. If the electron transfer from reaction center to quinone pool is blocked, this area will be dramatically reduced. As compared to tolerant genotypes the area over the fluorescence curve was decreased by 59 %, 50 % in sensitive genotypes (Fig. 2). In tolerant genotypes a significant increase in this area was observed indicating increase in pool size of the reduced PQ pool.

Sm is the normalized total complementary area above the O-J-I-P transient. Sm was increased slightly in high yielding species under heat stress or in grain filling stage while in low~yielding species (heat sensitive genotypes) it decreases significantly (by 45 and 31 % in Malvi local, Sawer local) (Fig. 2). Increase in Sm in high yielding species under stressed condition suggested that more energy is needed to close all RCs in high yielding species than in low yielding species (Strasser et al. 2000). Tfm which reflects the time to reach maximum fluorescence (ms) decreased significantly (~72 %) in low yielding genotypes during heat stress conditions (or grain filling stage) while in high yielding genotypes only a slight decrease was observed (Fig. 2). Time-dependent turnover number of Q_A (N) indicates how many times Q_A has been reduced in the time span from 0 to Tfm. In the present study, a significant decrease in the value of N was observed in low yielding species under heat stress or in grain filling stage while it increases in high yielding species indicating the change in plastoquinone (PQ) pool size (Fig. 2).

Some of the parameters which are calculated using the JIP-test are related to energy fluxes for light absorption (ABS), trapping (TR) of excitation energy and electron transport (ETR) per sample area called cross-section (CS) and can be visualized by means of dynamic energy pipeline leaf model of the photosynthetic apparatus (Kruger et al. 1997). The high and low yielding genotypes were compared on the basis of energy fluxes as shown in Figs. 3 and 4. The parameter ABS/CSm indicates absorption of photon flux by antenna chlorophyll molecules of active and inactive reaction center of PS II per excited cross-section of leaf area and is represented by the

Fig. 1 Fluorescence induction curves in high yielding (HI-8691, HI-8638) and low yielding (Malvi local, Sawer local) genotypes of wheat. **a** Before stress (vegetative stage), **b** after high temperature stress (during grain filling)

Fig. 2 Changes in various parameters obtained from fluorescence induction curves (as shown in Fig. 1) in high yielding and low yielding genotypes of wheat before and after heat stress

dark-adapted Fm. In high yielding genotypes there was no significant change in this ratio while a decrease in ABS/CSm in low yielding genotypes at high temperature was observed. It indicates a decrease in the efficiency of absorption energy of

PSII per excited cross-section (reflects increased density of inactive reaction centers). TRo/CSm indicates maximum trapping flux per excited active cross section leaf area resulting in the reduction of Q_A to Q_A^- . This damage was very less in high yielding HI-8691 while in high yielding HI-8638 the trapping of excitation energy per CS was slightly increased. Maximum trapping flux reduces in low yielding genotypes compared to high yielding genotypes which indicate that the active reaction

Fig. 3 Energy pipeline leaf model of phenomenological fluxes (per excited cross-section) in high yielding genotypes before and after stress. **a** HI-3691 before stress, **b** HI-3691 after stress, **c** HI-8638 before stress **d** HI-8638 after stress. Parameters ABS/CSm, TRo/CSm, ET/CSm and Dlo/CSm indicate absorption, maximum trapping flux beyond Q_A , electron transport and dissipation per excited cross-section leaf area, respectively. Each relative value is drawn by the width of the corresponding arrow, standing for a parameter. Empty and full black circles indicate the percentage of active and non active reaction centers of PS II respectively

Fig. 4 Energy pipeline leaf model of phenomenological fluxes (per excited cross-section) low yielding genotypes. **a** Malvi local before stress, **b** Malvi local after stress, **c** Sawyer local before stress, **d** Sawyer local after stress. Other details same as in Fig. 3

centers are converted into inactive RC that ultimately reduces the efficiency of trapping. ETo/CSm increases in high yielding genotypes while it reduces significantly in low yielding genotypes. The decreased ratio of ETo/CSm in low yielding genotypes reflects a decline in PS II activity and indicates inactivation of reaction centre (RC) complexes and the OEC. In low yielding genotypes, an increase in DIO/CSm (dissipation energy expressed per cross-section) at high temperature indicates that loss of energy is more and energy available for photochemistry is reduced under stress conditions.

By this study, we want to emphasize that by monitoring a single parameter of chlorophyll fluorescence, a quick assessment of a particular genotype as stress tolerant/stress sensitive can be done. It takes months to get data to differentiate between high and low yielding cultivars if conventional parameters like number of grains, Biomass, harvest index etc. are measured. Here we worked on pre-tested high yielding and low yielding genotypes to prove that this technique is equally authentic as compared to traditional parameters, but quicker. In addition, we have also come to know that which process of photosynthesis is affected in low yielding genotypes. This will help to plan further strategies to improve yield in low yielding cultivars.

Conclusion

In low yielding durum wheat species the whole primary photochemical reactions are affected before and after heat stress as evident from decreased values of several fluorescence parameters like Fv/Fm, Fv/Fo, Tfm etc. On the other hand high yielding genotypes HI 8691 and HI 8638 showed much stability in these parameters before and after heat stress conditions. They cope up with heat stress to maintain photosynthetic activity for survival under unfavorable conditions and show an increase in overall performance. High yield may be associated with better energy usage efficiency. This knowledge can allow quick selection of plants with a higher potential photosynthetic efficiency (vitality) during heat stress conditions, to be used successfully during breeding programmes in wheat.

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