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ORIGINAL RESEARCH

Biophysical characterization of drought tolerance in Wheat (*Triticum aestivum* L.) through polyphasic chlorophyll fluorescence OJIP analysis

Sunita PARIHAR[1] and Vineet SONI[1]*
[1]Plant Bioenergetics and Biotechnology Laboratory, Department of Botany, Mohanlal Sukhadia University,
Udaipur-313001, Rajasthan, India
*Corresponding Author email: vineetsonijnu@gmail.com

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ABSTRACT

Drought tolerance is the essential trait that needs to be incorporated in cereal crops, particularly those grown under the rainfed cultivation. Understanding the biophysical basis of drought tolerance will be helpful in developing selection strategies for improving various crop varieties. Therefore, present investigation was aimed to evaluate the drought-induced changes in photosynthetic machinery of drought-tolerant (Harshita: HI-1531) and drought-susceptible (Raj-4037) varieties of wheat (*T. aestivum* L.). Maximum quantum efficiency of PS II photochemistry (F_v/F_m) parameter was found sensitive to drought stress in Raj-4037 as compare to HI-1531. Study of other parameters *i.e.* specific energy fluxes (per QA-reducing PSII reaction center- RC), phenomenological fluxes (per cross section-CS), quantum efficiencies and density of active reaction centers indicates the higher potential of drought tolerance in HI-1531 variety of *T. aestivum.*

KEY WORDS: *Drought, Triticum aestivum L., Chlorophyll fluorescence, JIP-test*

Introduction

Drought stress is one of the main abiotic stress factors that negatively influence the growth, development and productivity of plants. It has numerous effects on plants by a rapid closure of stomata which leads to a reduced CO_2 concentration in leaves that ultimately limits photosynthetic activity by direct inhibition of ATP synthase (Tezara *et al,* 1999) and Rubisco enzyme (Carmo-Silva *et al.,* 2012). In particular, PSII has been shown to be very sensitive to drought stress (Havaux *et al.,* 1987; Toivonen and Vidaver, 1988; He *et al.,* 1995). Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and a powerful tool to study the plant's response to environmental stress (Yoshida *et al.,* 2015, Filek *et al.,* 2015). Chlorophyll fluorescence kinetics reflects the photosynthetic efficiency of plants and provides wealth of information on the relationship between structure and function of photosystem II (PS II) reaction center (RC) and core complexes (Govindjee 1995). Recently, a fast and reliable method for screening procedures of plant tolerance against environmental stresses has been introduced and developed further, the so-called OJIP test (Strasser *et al.,* 2004). The OJIP test developed by translates the fluorescence measurements of the transients (O–J–I–P) into several phenomenological and biophysical expressions that quantify PS II function. It has already been used to identify drought-tolerance in maize (Shao *et al.,* 2010), wheat (Huseynova, 2012), Grape (Wang *et al.,* 2012), sorghum (Jedmowski *et al.,* 2013), barley (Oukarroum *et al.,* 2007; Jedmowski *et al.,* 2015), etc.

Understanding the biophysical basis will be helpful in developing selection strategies for improving drought tolerance in various crop varieties. Therefore, in the present study, a comparative chlorophyll *a* fluorescence OJIP analysis was carried out in drought-tolerant (Harshita: HI-1531) and susceptible (Raj-4037) varieties of wheat (*T. aestivum* L.). Efforts were also made to understand the physiological basis of drought tolerance in drought-tolerant variety.

Materials and Methods

Plant Materials and Growing Conditions

T. aestivum L. varieties Raj-4037 and HI-1531 were evaluated concerning their ability to endure drought stress. Seeds were obtained from Maharana Pratap Agriculture University and Technology (MPUAT), Udaipur (Rajasthan, India) and were sown *in vivo* in germination trays containing 50% clay, 25% sand, and 25% humus under controlled conditions at 15 ^{o}C under a 12 h photoperiod. Prior to sowing, surface sterilization of seeds was done with 0.1% $HgCl_2$ followed successive washings with distilled water. Seedlings were watered twice a day.

Drought Treatment

The germinated plants of both wheat varieties were equally well watered for 3 weeks prior to exposure to drought stress treatment. After 3 weeks, at the stage of 2 fully developed leaves (Fig. 1 a, b), the plants were divided into two sets (each of 100 plants), out of which one set was subjected to drought stress by withholding of water supply, while the second set was watered regularly and served as a control.

Fluorescence measurements

Chlorophyll *a* fluorescence O-J-I-P transients were recorded after 5 days of drought stress treatment in the growth chamber at 20°C under dim green light with a Plant Efficiency Analyzer, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (peak at 650 nm) of 3000 $\mu molm^{-2}s^{-1}$ (sufficient excitation intensity to ensure closure of all PSII RCs to obtain a true fluorescence intensity of F_m) provided by a high intensity LED array of three light emitting diodes. A total measuring time of one second was used thought out the experiments.

The JIP test

The Chlorophyll a fluorescence transient O-J-I-P was analyzed according to the JIP- test (Strasser and Strasser, 1995; Strasser and Tsimilli-Michael, 2001; Soni and Strasser, 2008). The extracted and technical parameters, specific fluxes (per reaction center), phenomological fluxes (per cross section), quantum efficiencies or flux ratios, density of reaction centers and performance indexes were calculated by using the equations of JIP- test (Table: 1).

Results and Discussion

Chlorophyll *a* fluorescence has been proven to be a very useful, non-invasive tool for the study of the photosynthetic apparatus and more specifically the performance of PSII (Krause and Weis 1991, Strasser *et al.,* 2000). The JIP test is suggested as a powerful tool to probe the behavior of the photosynthetic apparatus under various biotic and abiotic stresses, as the shape of the chlorophyll fluorescence OJIP transient is highly sensitive to all kind of environmental stresses (Tsimilli-Michael *et al.,* 1999, Strauss *et al.,* 2006). In present study, drought-tolerant (Harshita: HI-1531) and susceptible (Raj-4037) varieties of wheat were studied through chlorophyll *a* fluorescence OJIP analysis to understand the physiological basis of drought tolerance in drought-tolerant variety of wheat. When exposed to saturating actinic light, both control and drought stressed plants showed a typical polyphasic rise in Chlorophyll *a* fluorescence started from the initial Fo intensity and increased to the highest intensity (Fm). Drought stress remarkably changed the OJIP transient of both wheat varieties (Fig. 2 a).

An increase of antenna size (ABS/RC: total absorption per active RC), may either indicate that (i) a fraction of active RCs is inactivated e.g., by being transformed to non-Q_A-reducing centers, or (ii) the functional antenna has increased in size. In the first case, the TR/RC could not be affected (since it refers only to the active RCs) and, thus, TR/ABS would decrease inverse of ABS/RC. In the second case, TR/ABS would proportionally follow the ABS/RC and, thus, TR/ABS is not affected. In the present study, drought stress drastically increased ABS/RC and TR/RC in both wheat varieties. A remarkable decline in TR/ABS was also

Table 1: Formulae and glossary of terms used by the JIP-test for the analysis of Chlorophyll *a* fluorescence transient OJIP emitted by dark-adapted photosynthetic samples

Extracted and technical fluorescence parameters	
$F_o = F_{50\,\mu s}$	fluorescence intensity at 50 μs
$F_{100\,\mu s}$	fluorescence intensity at 100 μs
$F_{300\,\mu sp}$	fluorescence intensity at 300 μs
F_J	fluorescence intensity at the J step (at 2 ms)
F_I	fluorescence intensity at the I step (at 30 ms)
F_M	maximal fluorescence intensity
Specific fluxes or specific activities	
$ABS/RC = M_0 \cdot (1/V_J) \cdot (1/\phi_{Po})$	Absorption flux per reaction center
$TR_0/RC = M_0 \cdot (1/V_J)$	Trapped energy flux per reaction center
$ET_0/RC = M_0 \cdot (1/V_J) \cdot \psi_0$	Electron transport flux per reaction center
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux per reaction center
Phenomenological fluxes or phenomenological activities	
ABS/CS	Absorption flux per cross section
$TR_0/CS = \phi_{Po} \cdot (ABS/CS)$	Trapped energy flux per cross section
$ET_0/CS = \phi_{Po} \cdot \psi_0 \cdot (ABS/CS)$	Electron transport flux/ cross section
$DI_0/CS = (ABS/CS) - (TR_0/CS)$	Dissipated energy flux per cross section
Quantum efficiencies or flux ratios	
$\phi_{Po} = TR_0/ABS = [1 - (F_o/F_m)] = F_v/F_m$	Maximum quantum yield for primary photochemistry (at t= 0)
Density of RCs	
$RC/CS = \phi_{Po} \cdot (V_J/M_0) \cdot ABS/CS$	Density of reaction centers (Q_A-reducing PSII reaction centers)

observed in both wheat varieties. These findings suggest that changes took place both in the fraction of RCs transformed to non-Q_A reducing centers and in the functional antenna size. However, photosynthetic parameters i.e. ABS/RC, TR/RC and TR/ABS were found more sensitive to drought stress in Raj-4037 as compared to HI-1531.

Phenomenological fluxes (ABS/CS, TR/CS, ET/CS, DI/CS) severely reduced in Raj-4037 as compared to HI-1531 (Fig 2 B). Performance index (PI) declined to 4% and 10 % in Raj-4037 and HI-1531 respectively when subjected to drought stress. Drought also drastically declined density of active reaction centers (RC/CS) in Raj-4037 (Fig 2 c, d) when compared to HI-1531 (Fig 2 e, f).

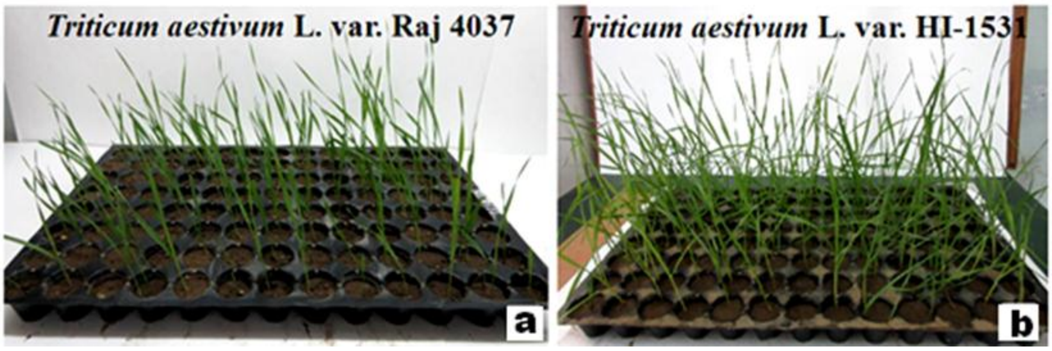

Fig. 1: Three weeks old plants of *T. aestivum* var. Raj-4037 (a); and HI-1531 (b)

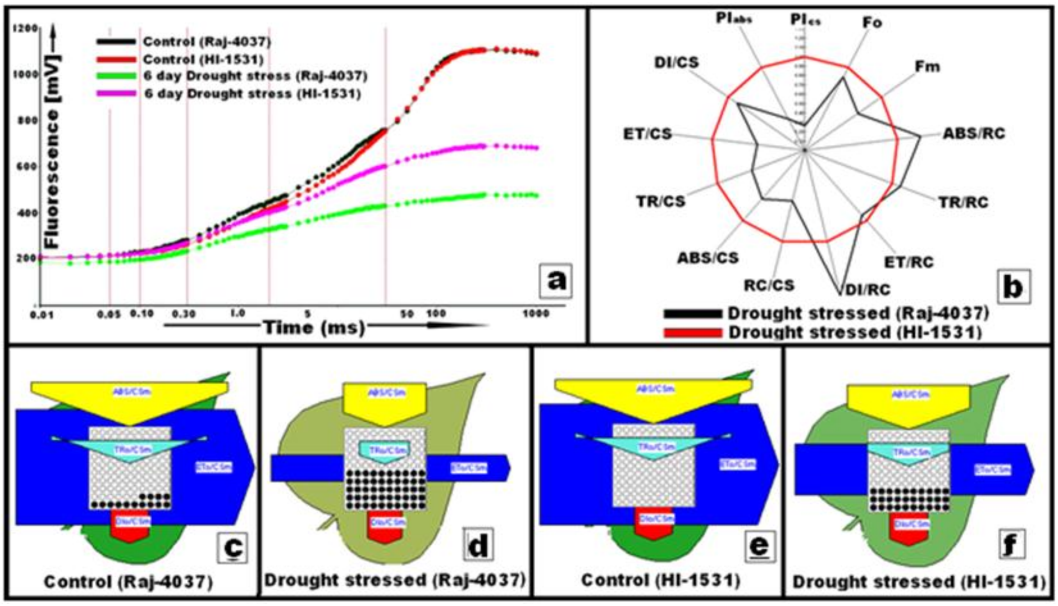

Fig. 2: (a) showing chlorophyll fluorescence OJIP curve of controlled and drought stressed plants of T. *aestivum* var Raj-4037 and HI-1531; (b) Radar plot showing comparative analysis of various photosynthetic parameters in drought stressed plants of Raj-4037 and HI-1531; (c, d) Leaf model showing various phenomenological fluxes (ABS/CS, TR/CS, ET/CS, DI/CS) in Raj-4037 and HI-1531 (e, f)

The maximum quantum yield for primary photochemistry (Fv/Fm) was declined from 0.817 (control) to 0.623 (after 5 days of drought stress treatment) in Raj-4037. On the other hand, HI-1531 exhibited low reduction in Fv/Fm (0.816 to 0.704). Low variation in Fv/Fm means that there is no loss in the yield of PSII photochemistry. The results of this study suggest that the photosynthetic machinery of variety HI-1531 has high potential to tolerate drought stress as compared to Raj-4037 variety of *T. aestivum* L. Our results also suggest that the measurement of maximum quantum yield for primary photochemistry (Fv/Fm) and performance index (PI) are appropriate criteria for the diagnosis drought stress in wheat.

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