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Research Article

EVALUATION OF DROUGHT TOLERANCE IN *TRITICUM AESTIVUM* L. THROUGH THE ANALYSIS OF POLYPHASIC CHLOROPHYLL FLUORESCENCE KINETICS

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ABSTRACT
Water shortage is a critical problem touching plant growth and yield in arid and semi-arid region. Photosynthesis is one of the key processes to be affected by water deficits, via decreased CO_2 diffusion to the chloroplast and metabolic constraints. Understanding the physiological basis of drought tolerance will be helpful in can play a major role in stabilizing crop performance under drought and in the protection of natural vegetation. The functional state of photosynthetic apparatus is a useful physiological indicator to study the sensibility of plants to environmental stresses. Therefore, present investigation was carried out to evaluate the drought-induced changes in photosynthetic apparatus of *Triticum aestivum* L. variety GW-366. Drought stress induced a significant decrease in the maximum quantum yield of primary photochemistry (F_v/F_m) and electron transport system in thylakoid membrane. On the other hand, light harvesting complexes of plants were found highly tolerant against drought condition. The results also reveal that the polyphasic chlorophyll fluorescence OJIP technique can be beneficial for screening various varieties of crop plants against different biotic and abiotic stresses.

Keywords: *Triticum aestivum L., Drought Stress, Chlorophyll a Fluorescence, JIP Test*

INTRODUCTION
Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields. Among the environmental stresses, drought stress is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Water deficit affects many morphological as well as physiological processes associated with plant growth and development. These changes include reduction of water content, diminished leaf water potential (Ψw), closure of stomata and a decrease of cell enlargement and plant growth. Drought stress reduces plant growth by affecting photosynthesis, respiration, the membrane stability index (MSI) and nutrient metabolism (Jaleel *et al.*, 2008). Drought not only decreases the leaf photosynthetic rate but also regulates the interaction of plant carbon uptake and environmental resources, which is termed the resource use efficiency.
The inhibition of photosynthesis or biochemical processes linked to photosynthesis represent the physiological state of the plant and therefore measurement of photosynthesis can be used as an indicator of environmental stress effects (Lichtenthaler and Rinderle, 1998). It has been concluded that measurement of an induced change of the photosynthesis process could be useful to monitor the presence of environmental stresses and the measurements of variable chlorophyll *a* fluorescence from intact plants offer several parameter values that are very useful for understanding the effects of environmental stresses on the photosynthesis (Govindjee, 1995).
Under drought conditions, *in vivo* chlorophyll *a* fluorescence analyses have provided extensive information about structure and function of the photosynthetic machinery. The F_V/F_M ratio is a parameter which allows detection of any damage to PS II and possible photoinhibition (Ahmed *et al.*, 2002). Changes in the proportion of photochemical and energy-dependent quenching lead to alteration of fluorescence kinetics under drought stress (Zlatev and Yordanov, 2004). Chlorophyll *a* fluorescence emitted from the chloroplast thylakoid membrane is often used as a very sensitive intrinsic indicator of the photosynthetic reaction in photosystem II (Ahmed *et al.*, 2002). The analysis of fluorescence has

become a powerful and non- destructive tool in photosynthesis research and plant stress detection (Strasser and Butler, 1977; Genty *et al.*, 1989; Havaux *et al.*, 1991; Strasser and Govindjee, 1992; Srivastava and Strasser, 1996; Clark *et al.*, 2000; Strasser and Stribet, 2001; Strasser and Tsimilli-Michael, 2001; Parihar and Soni, 2016; Soni *et al.*, 2016).

The reactions of plants to drought stress depend on the intensity and duration of stress as well as the plant species and its stage of growth, therefore, in the present studies, efforts were carried out to understand the effects of water deficit condition on photosynthetic performance of *T. aestivum* L. var. GW-366. Through Chlorophyll *a* fluorescence O-J-I-P analysis, we assessed various photosynthetic parameters *i.e.* F_0, F_M, F_V/F_M, Specific (ABS/RC, TR/RC, ET/RC and DI/RC) and phenomenological fluxes (ABS/CS, TR/ CS, ET/ CS and DI/ CS), performance index (PI) during exposure of various intensity of drought stress on photosynthetic performance of *T. aestivum* L. var. GW-366.

MATERIALS AND METHODS

Plant Materials and Growing Conditions: *T. aestivum* L. var. GW-366 was evaluated concerning their ability to endure drought stress. Seeds were obtained from Maharana Pratap Agriculture University and Technology, Udaipur (Rajasthan, India) and were sown *in vivo* in germination trays containing 50% clay, 25% sand, and 25% humus under controlled conditions at 15°C under a 12 h photoperiod (Figure 1A). 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 plant of wheat variety was equally well watered for 3 weeks prior to exposure to drought stress treatment. After 3 weeks, at the stage of 2 fully developed leaves (Figure 1), 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.

Measurement of Polyphasic Chlorophyll Fluorescence Kinetics: Chlorophyll *a* fluorescence O-J-I-P transients were recorded alternate days until the visible appearance of severe drought symptoms 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.

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). The extracted and technical parameters, specific energy fluxes (per reaction center), phenomenological energy 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 analysis has recognized to be a gentle method for the discovery and quantification of changes induced in the photosynthetic apparatus. Various abiotic stresses such as drought can, directly or indirectly, influence the photosynthetic activity of leaves and as a consequence alter the Chlorophyll a fluorescence kinetics (Epitalawage *et al.*, 2003; De Ronde *et al.*, 2004). The study of modifications in chl fluorescence kinetics gives thorough information on the organization and function of the photosynthetic machinery, especially PSII (Strasser and Strasser, 1995). In the present study, effects of drought stress on photosynthetic apparatus of *T. aestivum* var. GW-366 was studied through the analysis of modulated chlorophyll fluorescence, rapid fluorescence induction kinetics and the polyphasic fluorescence transients.

Mild drought stress (water deficit condition upto 2 days) could not influence the F_0 and F_M (Figure 1B). The fluorescence intensity decreased in response to moderate and severe drought conditions. An increase in stress intensity causes a significant declined the F_M. A reduction in the fluorescence yield of leaves can be accredited to a blockage of electron flow at oxidizing site of PS II (Congming and Vonshak, 2002).

The decline in F_M and fluorescence intensity at J, I and P steps may be due to these reasons, (i) Byhang-up of PS II donor side which results in the aggregation of P^{+}_{680} (Govindjee, 1995) and (ii) Or due to a decline in the pool size of Q_A^-. As compared to control leaves the area over the fluorescence curve was dramatically decreased in stressed leaves.

F_V/F_M also is more thoughtful factor for analyzing physiological damage to the photosynthetic machinery exposed to drought stress. This parameter is widely considered to be an important indication of plant photosynthetic performance under drought stress condition. Severe drought conditions dramatically decreased the F_V/F_M ratio from 0.801 (control) to 0.262 (after 8 days of drought stress treatment) in GW-366. Centritto *et al.*, (2005) found that F_V/F_M declined only under situations of severe water deficit. The distinguished decrease in F_V/F_M ratio may recommend a disruption in the electrons transport rate in PSII, exciting the accumulation of a population of reduced Q_A^-.

Various parameters of JIP test are associated to energy fluxes for light absorption (ABS), trapping (TR) of excitation energy and electron transport rate (ET) per sample area called cross-section (CS) and can be imagined by means of dynamic energy pipeline leaf model of the photosynthetic apparatus (Kruger *et al.*, 1997).

In the present study, drought caused a remarkable reduction in density of active reaction centers (RC/CS) in *T. aestivum* var. GW-366 (Figure 1C, D). A decrease in RC/CS ratio reflects that the density of active RCs is converted into inactive RCs. ABS/CS (absorbance per cross section) is the number of photons absorbed by an excited PS II cross-section.

Water deficit condition also increased absorption per reaction center (ABS/RC) and dissipated energy per reaction center (DI/RC) in plants growing under drought condition (Figure 1 E, F). Increase in ABS/RC shows that active reaction centers enhanced their light harvesting efficiency by raising antenna size with increasing drought duration.

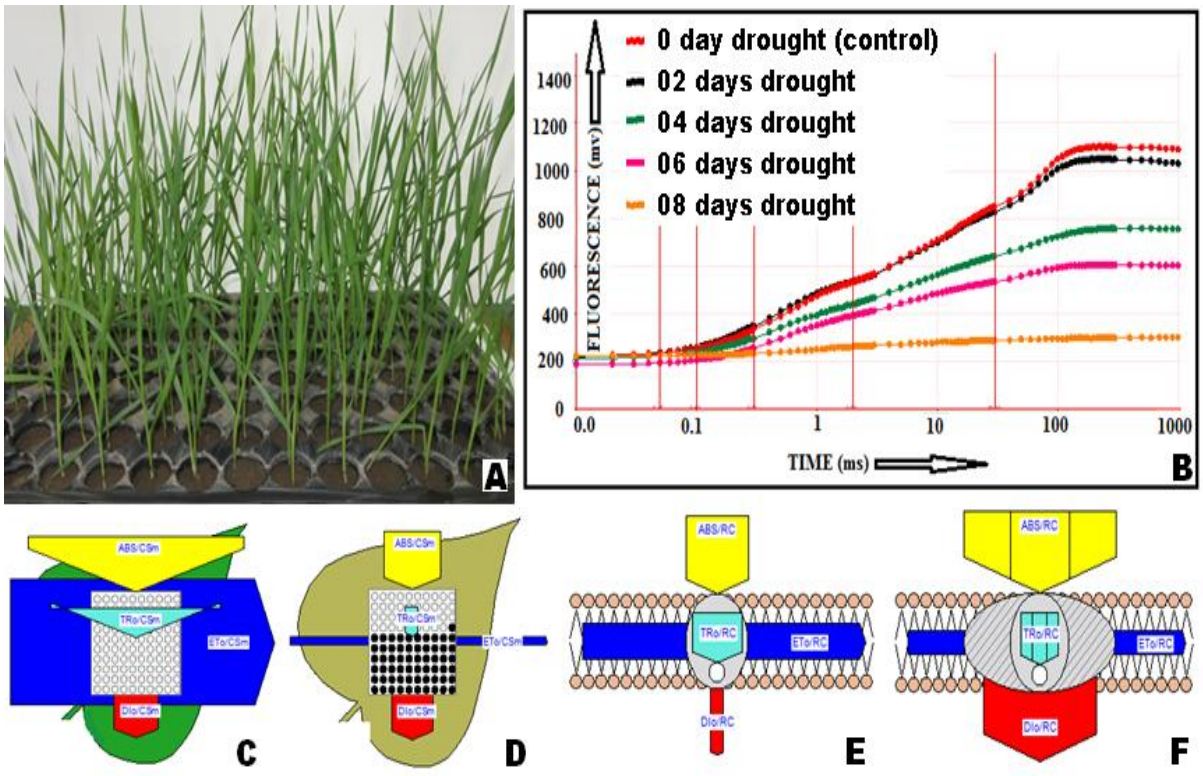

Figure 1: Three Weeks Old Plants of *T. Aestivum* var. GW-366 (A); Chlorophyll Fluorescence OJIP Curve (B); Leaf Model of Controlled (C) and Drought Stressed Plants; Pipeline Model of Active Reaction Centers of Controlled (E) and Drought Stressed (F) Plants

Absorption efficiency per cross section (ABS/CS) could not alter in drought stressed plants (Figure 2), which shows the resistant capability of light harvesting complexes in *T. aestivum* var. GW-366. It may also due to the enhancement the light harvesting activities of remaining active reaction centers. A drastic increase in DI/CS was also observed in the present investigations. Enhancement in DI/CS reflects the inhibition of electron transfer system in thylakoid membrane (ET/CS).

An increase in DI/CS (dissipation energy per cross-section) in drought stress condition directs that loss of energy is more and energy accessible for photochemistry is also reduced under stress conditions.

The results of this study suggest that the photosynthetic machinery of variety GW-366 has tolerant light harvest complexes against drought stress.

On the other hand, the drought sensitive electron transfer system develops low potential to tolerate severe drought stress condition in *T. aestivum* var. GW-366.

The results also indicate that the polyphasic chlorophyll technique can be beneficial for drought tolerance monitoring and screening in plant breeding programmes and this will support to design further strategies to improve yield in drought stressed areas.

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

Data Extracted from the Recorded Fluorescence Transient O-J-I-P	
$F_0 \cong F_{50\mu s}$	minimal fluorescence, when all PS II reaction centers are open (at t=0)
$F_m = F_P$	maximal fluorescence, when all PS II reaction centers are closed
$F_{100\mu s}$	fluorescence at 100µs
$F_{300\mu s}$	fluorescence at 300µs
$F_J \equiv F_{2ms}$	fluorescence at the J-step (2 ms) of O-J-I-P
$F_I \equiv F_{30ms}$	fluorescence at the I-step (30 ms) of O-J-I-P
$F_V \equiv F_M - F_0$	maximal variable fluorescence

Specific Energy Fluxes (Per Q_A-Reducing PSII Reaction Center - RC)	
$ABS/RC = M_0 \cdot (1/V_J) \cdot (1/\phi_{Po})$	absorption flux per reaction center
$TR/RC = M \cdot (1/V_J)$	trapped energy flux per reaction center
$ET/RC = M \cdot (1/V_J) \cdot \psi_0$-	electron transport flux per reaction center
DI/RC = (ABS/RC) - (TR/RC)	dissipated energy flux per reaction center

Phenomenological Energy Fluxes (Per Excited Cross Section – CS)	
ABS/CS	absorption flux per cross section
$TR/CS = \phi_{Po} \cdot (ABS/CS)$	trapped energy flux per cross section
$ET/CS = \phi_{Po} \cdot \psi_0 \cdot (ABS/CS)$	electron transport flux per cross section
DI/CS = (ABS/CS) – (TR/CS)	dissipated energy flux per cross section

Density of Reaction Centers	
$RC/CS = \phi_{Po} \cdot (V_J/M_0) \cdot ABS/CS$	density of reaction centers (Q_A-reducing PSII reaction centers)

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)

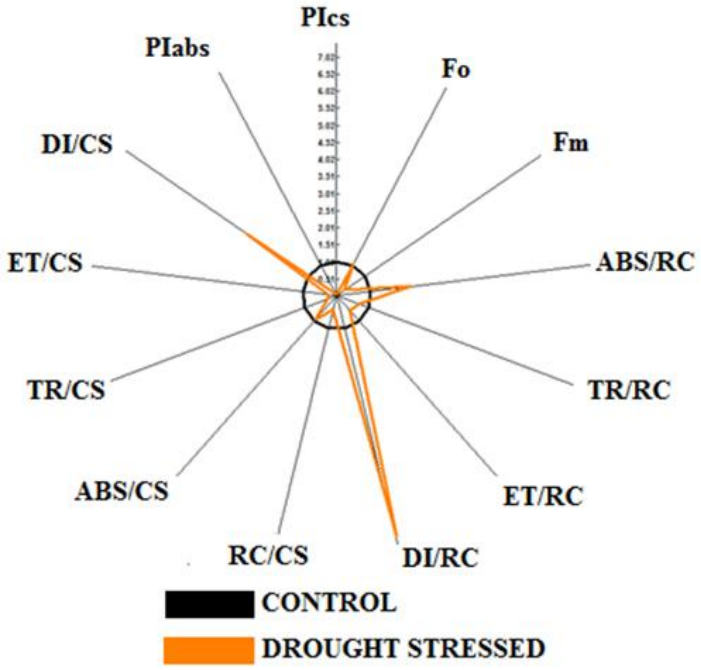

Figure 2: Radar Plot Showing Comparative Analysis of Various Photosynthetic Parameters in Controlled and Drought Stressed Plants

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