EVALUATION OF DROUGHT-INDUCED MODULATION IN PHOTOSYNTHETIC PERFORMANCE OF TRITICUM AESTIVUM VARIETY LOK-1

Sunita Parihar and Vineet Soni

Plant Bioenergetics and Biotechnology Laboratory, Department of Botany, Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India

*Author for Correspondence

ABSTRACT

Drought stress is a global issue that influences growth and productivity of plants. The main objective of this study was to identify indicators related to drought tolerance through fast polyphasic chlorophyll fluorescence kinetics in wheat var. LOK-1. Various parameters such as initial fluorescence (F₀), the maximum primary yield of photosystem II (Fv/Fm), maximum quantum yield of photosystem II (Fv/FM), performance index, energy pipeline models were measured in response to drought stress. The results indicate that the plants have capability to maintain photosynthetic photosynthetic performance under mild drought stress.

Keywords: Triticum aestivum L. variety LOK-1, Photosystem II, Chlorophyll fluorescence kinetics, Drought tolerance.

INTRODUCTION

Drought is one of the major abiotic stresses decreasing crop development and production (Jiang and Zhang, 2004). Usually, drought stress happens when the available water in the soil is decreased and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Khajeh et al., 2003). Drought also plays a serious role in affecting cereal grain production and quality. In the present time, with a growing population and global climate change; the situation is becoming more dangerous (Hongbo et al., 2005).

Various adaptations and fast actions are essential to cope with drought stress. Plants under dry conditions modulate their metabolism process, resulted in many physiological and biochemical modifications (Ort, 2001). Usually, plants respond to early drought stress by a rapid closure of stomata which decreases the CO₂ transmission rate. This leads to a decreased CO₂ concentration that finally restricts photosynthetic machinery by direct inhibition of the photosynthetic enzyme RuBisCO (Haup-Herting and Fock, 2000) or ATP synthase (Tezara et al., 1999). Among drought responses, photosynthesis is the crucial processes to be affected by drought stress (Chaves, 1991). Evaluating changes in photosynthetic performance can give information on the drought tolerance of a plant. For this purpose, chlorophyll fluorescence has become a broad, non-destructive technique to study changes in the photosynthetic machinery (Nunes et al., 2008; Silvestre et al., 2014). Chlorophyll fluorescence analysis has been commonly applied as a non-destructive technique to investigate the photosynthetic efficiency of plants, under stress conditions. Definitely, the information of fluorescence signals gives complete information on the status and function of Photosystem II (PSII) reaction centers, both the donor and acceptor sides and light harvesting antenna complexes. Over the last few years, numerous techniques have been suggested to improve plant performance in drought conditions. In recent times, the chlorophyll a fluorescence OJIP transient has been used to measure the damage of the photosynthetic machine under drought stress. The OJIP transient is definite by O, J, I, and P steps, parallel to the redox states of PS II and PS I (Strasser et al., 2000, 2004).

The “JIP test” developed by Strasser et al., (1995) based on the theory of “energy flow” through thylakoid membranes (Strasser et al., 2000), converts the fluorescence measurements of the transients (O–J–I–P) into many phenomenological and biophysical parameters that measures PS II functions. Each letter explains a specific variation in the curve and this permits the analysis of the structural and functional parameters that finally shows the activity of the whole photosynthetic machinery (Strasser et al., 2004).
The JIP-test analyze the polyphasic rise of the chl fluorescence OJIP transient and has been established to examine in vivo the “vitality” of plants and the adaptive behavior of the photosynthetic apparatus to drought stress (Tsimilli-Michael et al., 1996; Strasser et al., 2004). The chlorophyll fluorescence method is potentially useful for developing varieties for drought tolerance. To enhance crop production, it is crucial to find out the machinery of plant responses to drought conditions with the vital aim of developing drought tolerant crops in the water limited regions. On the basis of various parameters of fluorescence induction curves, \( F_V/F_M \) ratio, performance index (PI), and pipeline models, efforts were carried out to understand the effects of drought stress on photosynthesis in wheat var. LOK-1.

**MATERIALS AND METHODS**

**Plant Materials and Growing Conditions:** *T. aestivum* L. var. LOK-1 was 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°C under a 12 h photoperiod (Fig. 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.

**In Vivo 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, 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.

**Analysis of polyphasic chlorophyll fluorescence kinetics:** Chlorophyll a fluorescence O-J-I-P transients were recorded till 6 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.). Chlorophyll a fluorescence transient was induced by a red light (peak at 650 nm) of 3200 mol m\(^{-2}\) s\(^{-1}\) given by the PEA over an array of six light-emitting diodes. Fluorescence data were analyzed as per the equations of JIP test proposed by Strasser and Strasser, (1995) (Table: 1).

**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**

<table>
<thead>
<tr>
<th>Extracted and technical fluorescence parameter</th>
<th>( F_0 = F_{50 \mu s} )</th>
<th>fluorescence intensity at 50 ( \mu )s</th>
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<tbody>
<tr>
<td>( F_J )</td>
<td>fluorescence intensity at 2 ms</td>
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<tr>
<td>( F_I )</td>
<td>fluorescence intensity at 30 ms</td>
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<tr>
<td>( F_M )</td>
<td>maximal fluorescence intensity</td>
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<thead>
<tr>
<th>Specific fluxes or specific activities</th>
<th>( \text{ABS/RC} )</th>
<th>Absorption flux per reaction center (RC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{TR}_0/\text{RC} = M_0 \cdot (1/V_J) )</td>
<td>Trapped energy flux per RC</td>
<td></td>
</tr>
<tr>
<td>( \text{ET}_0/\text{RC} = M_0 \cdot (1/V_J) \cdot \psi_0 )</td>
<td>Electron transport flux per RC</td>
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<tr>
<th>Phenomenological fluxes or phenomenological activities</th>
<th>( \text{ABS/CS} )</th>
<th>Absorption flux per cross section</th>
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<tbody>
<tr>
<td>( \text{TR}_0/\text{CS} )</td>
<td>Trapped energy flux per cross section</td>
<td></td>
</tr>
<tr>
<td>( \text{ET}_0/\text{CS} )</td>
<td>Electron transport flux/ cross section</td>
<td></td>
</tr>
<tr>
<td>( \text{DL}_0/\text{CS} = (\text{ABS/CS}) - (\text{TR}_0/\text{CS}) )</td>
<td>Dissipated energy flux per cross section</td>
<td></td>
</tr>
</tbody>
</table>

| Quantum efficiencies or flux ratios | \( \Phi_{P0} = [1 - (F_0/F_m)] = F_V/F_m \) | Maximum quantum yield for primary photochemistry |

| Density of RCs | \( \text{RC/CS} \) | Density of reaction centers |
RESULTS AND DISCUSSION
The analysis of the kinetics of chlorophyll fluorescence has been a non-invasive technique used broadly for the investigation of plant responses under various stress conditions such as salinity, chilling, high temperatures, nutrient deficiency and drought (Greaves and Wilson, 1987; Brennan and Jefferies, 1990; Hakam et al., 2000). In the present study, chlorophyll fluorescence analysis was undertaken to understand the physiological basis of drought tolerance in wheat var. LOK-1. The chlorophyll fluorescence intensity started from the initial (F₀) intensity and increased to the highest intensity (Fₘ) in both control and drought-stressed plants (Fig. 1b). The shape of the O-J-I-P transient is very sensitive to drought stress (Strasser and Tsimilli-Michael, 2001; Sayed, 2003; Van Heerden et al., 2003, Govindachary et al., 2004). In the present study, a wide variation between chlorophyll fluorescence OJIP kinetics of control and drought stressed plants was observed. The fluorescence intensity decreased in response to only in moderate or severe drought conditions. Under severe drought stress condition, the shape of the O-J-I-P transient changed in wheat leaves to decrease in Fₘ, resulting in lowering of Fᵥ.

Figure 1. (a) Three years old plants of T. aestivum var. Lok-1, (b) chlorophyll a fluorescence induction curves of control and drought-stressed plants, Thylakoid membrane and leaf models of control (c,d) and drought stressed plants (c,d) of T. aestivum var. Lok-1.

The O-J phase of the chlorophyll fluorescence transient is related to a photochemical reduction of the primary Quinone acceptor Qₐ in PS II reaction center and is sensitive to severe stress. Schansker et al., (2005) studied that the I-P phase depends on both, electron flow through PS I and a transient block of electron flow on the acceptor side of PS I which depends on PS I content and suggests a change in the
ratio between PSI and PSII. $F_v/F_m$ is considered to be a sensitive indicator of plant photosynthetic performance under drought stress condition. The maximum quantum yield for primary photochemistry ($F_v/F_m$) was declined from 0.737 (control) to 0.025 (after 6 days of drought stress treatment) in LOK-1. In present study, it was noted that all component, except light harvest complexes (LHC), of photosynthetic apparatus of Lok-1 variety are highly resistant to drought stress. An increase in ABS/RC, TR/RC and ET/RC was observed with drought treatment (Fig. 1c,e). On the other hand, a slight reduction in ABS/CS, TR/CS and ET/CS was observed in plants subjected to water deficit condition (Fig. 1d,f). 6 days drought treatment caused a drastic reduction in the density of active reaction centers (RC/CS) in LOK-1 variety of wheat. Despite decline in RC/CS, the remaining active reaction centers enhanced their efficiency to maintain photosynthesis process in LOK-1 variety.

Leaf models suggest that the light harvesting complexes of plants are highly sensitive to drought stress. Degradation of LHCs might be the root cause of decline of photosynthesis in LOK-1 variety of wheat under water deficit condition. In future, through breeding programmes, the drought tolerance efficiency of Lok-1 variety of wheat can be enhanced by increasing drought resistance of LHCs in $T. aestivum$ var. Lok-1.

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