

## Effects of salt stress on photosystem II of canola plant (*Barassica napus*, L.) probing by chlorophyll a fluorescence measurements

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### Abstract

The changes of Chl a fluorescence induction curve in response to different salinity levels in Canola (*Barassica napus*, L.) plants were investigated. The canola seedlings were exposed to salt stress (100 mM and 200 mM NaCl) for 7 days and Chl a fluorescence induction kinetics was analysed. By using the OJIP-test, various parameters like  $V_j$ , area,  $N$ ,  $\phi_{P_0}$ ,  $\phi_{E_0}$ ,  $\psi_0$ , RC/ABS and  $PI_{ABS}$  were calculated and compared at different salinity levels and compared with the control plants. The results showed that the parameters related to the donor site of electron in PSII [ $F_v/F_0$ ,  $\phi_{P_0}$  and  $\phi_{P_0}/(1-\phi_{P_0})$ ] were constant and unaffected by salt stress. In contrast, the parameters dependent on the acceptor site of the electron such as  $V_j$ , area,  $N$ ,  $\phi_{E_0}$ ,  $\psi_0$  and  $\psi_0/(1-\psi_0)$  significantly decreased with an increase in salt concentration. Also, the multi parametric index  $PI_{ABS}$  (performance index) showed enough sensitivity to salt stress as a useful parameter to screen the activity of photosynthetic apparatus in canola plant. Based on our results, we suggest that in response to salt stress the electron acceptor site of PSII, especially at fractions of the transfer of electron from  $Q_A$  to  $Q_B$  and  $Q_A$  redox turnovers is more affected. In conclusion, this fraction of electron transport chain of PSII is possibly the first and major target of salt stress at photosynthetic apparatus in canola plant.

**Keywords:** Canola (*Barassica napus* L.); chlorophyll a fluorescence; OJIP-test, photosystem II; salt stress

### 1. Introduction

Canola (*Brassica napus* L.) is an important annual oil seed crop for both industrial and nutritional purposes and recently has served as a source to produce bio-diesel fuel [1-3]. Canola, similar to many other crop species is affected by adverse environmental conditions. Salinity in soil and water has long been identified as one of the various kinds of stresses limiting the crop production [1, 4, 5]. According to FAO (2008), about 800 million hectares of land are damaged by salinity and 0.25 to 0.5 million hectares are lost from production every year as a result of salt accumulation [6]. The negative effects of salinity on plant growth and development are associated with low osmotic potential of soil solution, changes in nutrient uptake, specific sodium and chloride ions effects or a combination of these factors [1, 4, 7]. These effects lead to a wide variety of morphological, biochemical and physiological changes at cellular, tissue, organ and whole plant levels [7, 8]. Previous studies indicated that the photosynthetic apparatus is one of the major target sites of abiotic stresses [9-14]. High salt conditions result in decreasing the efficiency of photosynthesis that seems related to

photosystem II (PSII) complex [9, 10, 13, 14]. Plants response to salinity depends on PSII ability to respond to this stress [10]. It has been reported that salinity reduces the quantum yield of PSII electron transport, the amount of light energy that reaches the reaction centers, and PSII mediates oxygen evolution activity [9-14]. Chlorophyll a (Chl a) fluorescence is a very small fraction of the dissipated energy from the photosynthetic apparatus. It has been proven that Chl a fluorescence in the spectral region 680-740 nm is almost exclusively emitted by PSII [15, 16]. Hence, the kinetics of Chl a fluorescence can provide very useful information about the structure, conformation and function of PSII complex. Chl a fluorescence analysis is a rapid and useful non-invasive tool to monitor the PSII behavior in intact plants under different conditions [15-19]. Chl a fluorescence intensity in dark-adapted photosynthetic organisms shows a characteristic variation with time after illumination known as fluorescence transient [15, 16]. The fluorescence transient shape is a polyphasic curve during illumination labeled as O-J-I-P. This curve rises from an initial low value  $F_0$  (the minimal fluorescence) to  $F_j$  (the fluorescence value at 2 ms) and  $F_I$  (the fluorescence value at about 20-30 ms) and a peak of fluorescence  $F_p$  (the maximal

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fluorescence or Fm) [15, 16, 18, 19]. The shape of the O-J-I-P transient has been found very sensitive to stress caused by changes in different conditions [9, 14, 20]. Among various methods to analyse Chl a fluorescence transient, the OJIP-test is highly studied for *in vivo* investigation of intact photosynthetic apparatus [9, 10, 12, 14, 18, 19, 21]. This test translates the shape changes of the O-J-I-P transient to quantitative changes of the several parameters which are based on the theory of energy fluxes in thylakoid membrane. These parameters are light absorption (ABS), trapping of excitation energy (TR) and conversion of excitation energy to photosynthetic electron transport (ET) per reaction center (RC) or per sample area called cross-section (CS) [15, 16, 19]. The OJIP test can be used to investigate, point by point, the PSII fractions change, because PSII behavior changes when the plants are exposed to environmental stresses. This test can translate the physiological states of PSII to biophysical parameters under different conditions [16].

The aims of this study were investigation on the target site of salt stress on PSII in Canola plant, the effects of salinity on various fractions of donor and acceptor sites of electron transport in PSII and demonstration of the changes of Chl a fluorescence induction curve and related parameters during exposure to salinity levels.

## 2. Materials and methods

### a) Plant materials and growth conditions

Canola plant (*Brassica napus*, L. cv. Likord) was used for experiments. The seeds were allowed to germinate for 48h on wet filter paper placed in Petri dishes. The germinated seeds were transferred to hydroponic cultures. The tubes supporting the plants were placed on floating rafts, on the surface of 10-litre tanks filled with 0.1 strength modified Johnson's solution [22]. The pH was adjusted and maintained at  $6 \pm 0.3$  by adding  $\text{CaCO}_3$  powder.

All experiments were carried out in a growth chamber (Conviron E16, Canada) with 16h light/8h dark regime at  $24^\circ\text{C}/20^\circ\text{C}$ , 70% humidity and a light intensity of  $200\mu\text{mol. m}^{-2}. \text{s}^{-1}$ .

There were ten plantlets in each hydroponic tank with 3 replications for each salt treatment. The plantlets were grown up to three leaf stages and then NaCl treatment was given to them.

### b) Salt treatments

Three salinity levels i.e. Control, 100 mM and 200 mM NaCl were applied to the nutrient solution. After 7 days, the fluorescence measurements were performed in the middle portion on the abaxial

surface of the leaves. Three measurements were taken for each plantlet.

### c) Chl a fluorescence measurements

Chl a fluorescence was measured at room temperature with the Plant Efficiency Analyzer (HandyPEA, Hansatech Instruments Ltd, King's Lynn, UK). The fluorescence activating light was provided by an array of three light-emitting diodes which were focused onto the leaf surface to provide homogenous illumination. All measurements were performed on the upper surface of the fully expanded leaves by using the leaf clips. All samples were dark-adapted for 30 min prior the measurements. The leaves exhibit the Chl a fluorescence rise during the first second of illumination after dark adaptation period. The fluorescence signals were detected by a high performance PIN photodiodes detector and were received by the sensor head during recording and digitized in the control unit using a fast Analogue/digital converter. Every measurement records 118 data points (Handy PEA manual user's guide). These data were analyzed and conducted using the software Biolyzer 4HP (The fluorescence analyzing program by Bioenergetics laboratory, University of Geneva, Switzerland).

### d) Statistical analysis

Results are presented as means  $\pm$  standard errors. Statistical difference between the means of various groups were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test. A value of  $P < 0.05$  was used as the criterion of significance. Calculations were made with computer assisted analysis using the SPSS software.

In the present study, among the various parameters which were calculated according to OJIP-test (Table 1), our focus was on the parameters that were significantly affected by salt stress.

## 3. Results and discussion

The effect of salinity levels on the shape of Chl a fluorescence transient is shown in Fig. 1. The OJIP transient revealed a conspicuous difference between salinity treatments. The fluorescence induction curves demonstrated a distinct reduction with increase in the NaCl concentration, especially at phases J, I and P. Hence, the OJIP transients were normalized at original fluorescence ( $F_0$ ) in order to reveal changes in the shape of curves more clearly.

**Table 1.** Summary of the JIP-test formulae using data extracted from the OJIP chlorophyll a fluorescence transient**Extracted and technical fluorescence parameters**

$F_0$  = F50 $\mu$ s, fluorescence intensity at 50  $\mu$ s  
 $F_j$  = fluorescence intensity at the J-step (at 2ms)  
 $F_I$  = fluorescence intensity at the I-step (at 30 ms)  
 $F_M$  = maximal fluorescence intensity  
 $T_{FM}$  = time to reach Fm (ms)  
 $V_j$  = relative variable at the J-step =  $(F_{2ms} - F_0) / (F_M - F_0)$   
 Area = area between fluorescence curve and  $F_M$   
 $F_v/F_0$  = activity of the water-splitting complex  
 $dV/dt_0 = M_0 = 4 \cdot (F_{300} - F_0) / (F_M - F_0)$   
 $S_m = \text{Area} / (F_M - F_0)$   
 $N$  = turnover number of  $Q_A$

**Quantum efficiencies or flux ratios or yields**

$\phi_{p_0} = \text{TR}_0/\text{ABS} = [1 - (F_0 / F_M)] = F_v / F_M$   
 $\phi_{E_0} = \text{ET}_0/\text{ABS} = [1 - (F_0 / F_M)] \cdot \psi_0$   
 $\psi_0 = \text{ET}_0/\text{TR}_0 = (1 - V_j)$

**Specific fluxes or specific activities**

$\text{ABS}/\text{RC} = M_0 \cdot (1/V_j) \cdot (1 - \phi_{p_0})$   
 $\text{TR}_0/\text{RC} = M_0 \cdot (1/V_j)$   
 $\text{ET}_0/\text{RC} = M_0 \cdot (1/V_j) \cdot \psi_0$   
 $\text{DI}_0/\text{RC} = (\text{ABS}/\text{RC}) - (\text{TR}_0/\text{RC})$

**Phenomenological fluxes or phenomenological activities**

$\text{ABS}/\text{CS} = \text{ABS}/\text{CS}_{\text{chl}} = \text{Chl}/\text{CS}$  or  $\text{ABS}/\text{CS}_0 = F_0$  or  $\text{ABS}/\text{CS}_M = F_M$   
 $\text{TR}_0/\text{CS} = \phi_{p_0} \cdot (\text{ABS}/\text{CS})$   
 $\text{ET}_0/\text{CS} = \phi_{p_0} \cdot \psi_0 \cdot (\text{ABS}/\text{CS})$   
 $\text{DI}_0/\text{CS} = (\text{ABS}/\text{CS}) - (\text{TR}_0/\text{CS})$

**Density of reaction centres**

$\text{RC}/\text{CS} = \phi_{p_0} \cdot (V_j/M_0) \cdot \text{ABS}/\text{CS}$

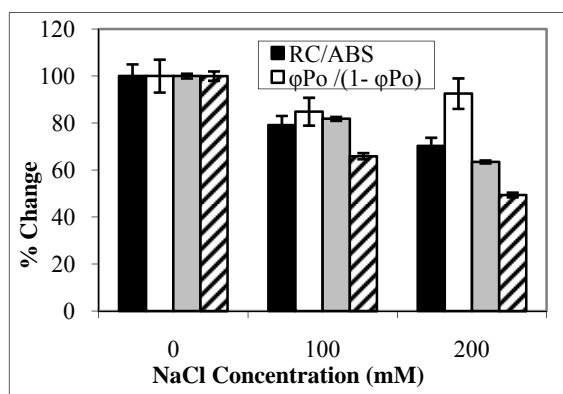
**Performance indexes**

$\text{PI}_{\text{ABS}} = (\text{RC}/\text{ABS}) \cdot [\phi_{p_0}/(1 - \phi_{p_0})] \cdot [\psi_0/(1 - \psi_0)]$   
 $\text{PI}_{\text{CS}} = (\text{RC}/\text{CS}) \cdot [\phi_{p_0}/(1 - \phi_{p_0})] \cdot [\psi_0/(1 - \psi_0)]$

**Driving forces**

$\text{DF}_{\text{ABS}} = \log(\text{PI}_{\text{ABS}})$   
 $\text{DF}_{\text{CS}} = \log(\text{PI}_{\text{CS}}) = \log(\text{PI}_{\text{ABS}}) + \log(\text{ABS}/\text{CS})$

ABS, absorption energy flux; CS, excited energy cross-section of leaf sample; DI, dissipation energy flux at the level of the antenna chlorophyll; ET, flux of electron from  $Q_A^-$  into the electron transport chain;  $\phi_{D_0}$ , quantum yield of dissipation;  $\phi_{E_0}$ , probability that an absorbed photon will move an electron into electron transport further than  $Q_A^-$ ;  $\phi_{p_0}$ , maximum quantum yield of primary photochemistry;  $\text{PI}_{\text{ABS}}$ , performance index;  $\psi_0$ , efficiency by which a trapped excitation, having triggered the reduction of  $Q_A$  to  $Q_A^-$ , can move an electron further than  $Q_A^-$  into the electron transport chain; RC, reaction center of PSII; RC/CS, fraction of active reaction centers per excited cross-section of leaf; TR, excitation energy flux trapped by a RC and utilized for the reduction of  $Q_A$  to  $Q_A^-$ .



**Fig.1.** The OJIP Chl a fluorescence transient curve in canola exposed to various concentration of NaCl after 7 days

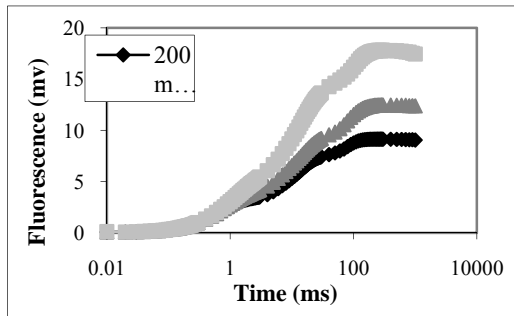
A decrease in the fluorescence yield at phases J, I and P may be due to the inhibition of electron transport at the donor site of PSII to reaction centers or due to a decrease in the pool size of the electron acceptors in reducing the PSII ( $Q_A$ ,  $Q_B$  and PQ pool) site [9, 14]. From the data calculated by JIP-test and shown in Table 2,  $\phi_{Po}$ , the efficiency quantum yield of primary photochemistry and  $F_v/F_0$ , the activity of water-splitting complex at the donor site of PSII, were unaffected by salinity. These observations suggest that the salinity stress has no effects on the electron transfer rates at the donor site of PSII.

To localize the target of salt stress in the electron transport chain of PSII, some parameters from Table 2 can be useful. The first one is  $V_J$ , the relative variable fluorescence at J step.  $V_J$  reflects a measure of the fraction of the primary quinone electron acceptor of PSII in reduced state [ $Q_A^-/Q_{A(total)}$ ] [9]. The data indicated that  $V_J$  was increased by 7% and 21% with an increase in salinity levels (Table 2). The next measured parameter is Area, i.e. the area over the fluorescence curve between  $F_0$  and  $F_m$  which is proportional to the pool size of the electron acceptors of PSII ( $Q_A$ ,  $Q_B$  and PQ pool) [10, 14, 15, 16]. The area was decreased during exposure to different salinities by 6% and 15% with increase in the NaCl concentration as compared to control (Table 2). The parameter N, the number of  $Q_A$  redox turnovers until  $F_m$  is reached [14, 16, 19], was decreased about 20% and 41% with an increase in the salt concentration in the medium, respectively (Table 2). These results demonstrated that  $Q_A^-$  cannot reoxidized efficiently to  $Q_A$  in the presence of NaCl, and had lower value in higher salinity treatments. Above all, the rates of electrons which reached to  $Q_A$  ( $\phi_{Po}$ ) were constant in different salinity treatments and the pool size of

quinones ( $Q_A$ ,  $Q_B$  and PQ pool) and the number of  $Q_A$  turnovers were decreased with the increase in the salinity levels. Therefore,  $V_J$  increased in higher salt treatments due to a reduction of total  $Q_A$  and the lower value of reoxidation of the  $Q_A^-$  with increase in the NaCl concentration. Hence, the ratio [ $Q_A^-/Q_{A(total)}$ ] enhanced at higher salinity levels.  $\psi_o$  is the efficiency of electron transfer from  $Q_A^-$  to  $Q_B$ . Our observations showed that as the salinity increased in the medium, the amount of this parameter was decreased (Table 2). These findings suggest that the reduction of  $\psi_o$  was possibly due to the decrease in the  $Q_A^-$  reoxidation resulting in the decrease in the rates of electron transport beyond  $Q_A^-$  at high salinity levels. Similar reductions were obtained in the quantum yield of electron transport ( $\phi_{Eo}$ ). The I and P steps of fluorescence induction curve had been suggested to reflect an accumulation of the  $Q_B^-$  and  $Q_B^{2-}$ , respectively [9, 16]. We had a distinct downfall in the shape of Chl a fluorescence transient at phases I and P with an increase in the salt concentration (Fig. 1). These findings are in accordance with the data of  $\psi_o$ . The inhibition of electron transfer from  $Q_A^-$  to  $Q_B$  results in the reduction of electron transfer from  $Q_B^-$  to electron transport chain beyond  $Q_B^-$  at high salinity levels. The decrease in the efficiency of electron transfer from  $Q_A^-$  to  $Q_B$  ( $\psi_o$ ) and the quantum yield of electron transport ( $\phi_{Eo}$ ) at high salinities indicate that possibly the acceptor site in the electron transport of PSII is a major target site of salinity stress in canola plant.

**Table 2.** Chlorophyll a fluorescence parameters of canola grown under different concentration of NaCl in the hydroponic medium, Numbers are given as percentage of control after 7 days of salt application. Values within one row followed by the same letter are not significantly different at  $P < 0.05$ .

200 mM NaCl	100 mM NaCl	Control	Parameter
121 <sup>a</sup>	107 <sup>b</sup>	100 <sup>c</sup>	$V_J$
75 <sup>c</sup>	94 <sup>b</sup>	100 <sup>a</sup>	Area
59 <sup>c</sup>	80 <sup>b</sup>	100 <sup>a</sup>	N
92 <sup>a</sup>	97 <sup>a</sup>	100 <sup>a</sup>	$F_v/F_0$
98 <sup>a</sup>	97 <sup>a</sup>	100 <sup>a</sup>	$\phi_{Po}$
86 <sup>c</sup>	92 <sup>b</sup>	100 <sup>a</sup>	$\phi_{Eo}$
88 <sup>c</sup>	94 <sup>b</sup>	100 <sup>a</sup>	$\psi_o$



**Fig. 2.** Changes in the percentage of the density of reaction centers (RC/ABS), the efficiency of light reaction [ $\phi_{P_0} / (1 - \phi_{P_0})$ ], the efficiency of biochemical reaction [ $\psi_o / (1 - \psi_o)$ ] and performance index (PI<sub>ABS</sub>) in canola exposed to various concentration of NaCl after 7 days

In the present study we also calculated the density of reaction centers (RC/ABS), the efficiency of light reactions [ $\phi_{P_0} / (1 - \phi_{P_0})$ ] and the efficiency of biochemical reactions [ $\psi_o / (1 - \psi_o)$ ] by JIP-test, which are shown in Fig. 2. The density of the reaction centers (RC/ABS) was decreased by 15% and 30% at 100 mM and 200 mM NaCl concentration, respectively. It has been reported that after most environmental stresses, a part of the reaction centers (RCs) transformed to inactive reaction centers [16]. These inactive RCs act as efficient excitation traps but dissipate all their excitation energy as heat as a possible protective mechanism. Hence, these RCs cannot reduce  $Q_A^-$  and so they do not contribute to the variable fluorescence.

The inactivation of some RCs quickly occurs under the stress conditions and increases in larger value at higher levels of stresses [16]. The efficiency of light reactions [ $\phi_{P_0} / (1 - \phi_{P_0})$ ] referred to O to J steps of OJIP transient curve and expressed the photochemical phase of PSII [9, 14]. The results showed that this parameter was not affected by salinity treatments. Therefore, it seems that high salt stress did not influence light reactions of PSII, including the number of quanta absorption by the antenna pigments, the amount of tapping flux to the reaction centers and activity of water-splitting complex. In contrast, the efficiency of biochemical reactions [ $\psi_o / (1 - \psi_o)$ ] was markedly influenced by salt stress. Fig. 2 shows that during exposure to salinity levels, the ratio [ $\psi_o / (1 - \psi_o)$ ] decreased and became 75% and 63% of the control. The biochemical reactions of PSII referred to steps J to I to P of the OJIP transient induction curve [9, 14, 16]. These findings are in accordance with previous data of  $\psi_o$  and  $\phi_{E_0}$ . This indicated that salinity leads to significant decrease in electron transport rates as non-photochemical dependent phase. The above three parameters can be combined into a multi-parametric expression so-called

performance index (PI<sub>ABS</sub>) [15-19] and can be calculated as:

$$PI_{ABS} = RC/ABS \cdot \phi_{P_0} / (1 - \phi_{P_0}) \cdot \psi_o / (1 - \psi_o)$$

PI<sub>ABS</sub> is an indicator of plant vitality and provides useful and quantitative information about photosynthetic apparatus [9, 10, 14, 16, 18, 19, 21]. This index combined the three main functional steps of photosynthetic activity including light energy absorption, excitation energy trapping and conversion of excitation energy to electron transport. Our results (Table 2) show that this parameter is sensitive enough to detect the difference between different salt treatments. With an increase in NaCl concentration, a significant decrease in the value of PI<sub>ABS</sub> was revealed and its value became 72% and 42% of the control at 100 mM and 200 mM NaCl concentration, respectively. These results demonstrated that PI<sub>ABS</sub> is particularly useful in revealing differences in the response of PSII to salinity conditions and can be used as a sensitive detector for PSII activity of canola plant under variable conditions.

It is concluded from the present study that the Chl a fluorescence measurements can be used for assessment of salt effects on intact Canola plant. Through the measurement of OJIP fluorescence transients and analysis of these transients by the JIP-test, we understood that salt stress had different effects on various fractions of PSII and the acceptor site of electron transport is influenced more by salinity as compared to the donor site of electron transport. The highest differences were observed in acceptor site of electron transport, especially in the number of  $Q_A^-$  redox turnovers, which results in decreased efficiency of electron transfer from  $Q_A^-$  to  $Q_B$  ( $\psi_o$ ), the quantum yield of electron transport ( $\phi_{E_0}$ ) and the efficiency of biochemical reactions [ $\psi_o / (1 - \psi_o)$ ]. In conclusion, it seems the performance index (PI<sub>ABS</sub>) as a multi-parametric index is a suitable indicator for evaluation of salt effects on photosynthetic apparatus in Canola plant.

### Acknowledgment

This work was supported by Graduate Studies of University of Isfahan. Also authors wish to thank Plant Stress Center of Excellence (PSCE) in University of Isfahan.

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