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How to cite

MISRA, Amarendra N., SRIVASTAVA, Alaka, STRASSER, Reto. Utilization of fast chlorophyll *a* fluorescence technique in assessing the salt/ion sensitivity of mung bean and Brassica seedlings. In: Journal of plant physiology, 2001, vol. 158, n° 9, p. 1173–1181. doi: 10.1078/S0176-1617(04)70144-3

This publication URL: https://archive-ouverte.unige.ch/unige:162089
Publication DOI: 10.1078/S0176-1617(04)70144-3

J. Plant Physiol. 158. 1173–1181 (2001)
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http://www.urbanfischer.de/journals/jpp

JOURNAL OF
PLANT PHYSIOLOGY

Utilization of fast chlorophyll *a* fluorescence technique in assessing the salt/ion sensitivity of mung bean and Brassica seedlings

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Received January 17, 2001 · Accepted April 17, 2001

Summary

Effects of NaCl and KCl alone or in combination with $CaCl_2$, Na_2SO_4 or K_2SO_4 on fast chlorophyll *a* (Chl *a*) fluorescence kinetics of mung bean (*Vigna radiata* L.) and Brassica (*Brassica juncea* Coss.) were studied. The concentrations of NaCl and KCl used were 100 mmol/L to 300 mmol/L and the concentration of $CaCl_2$ was 5 mmol/L. Na_2SO_4 and K_2SO_4 used were in the range of 50 to 200 mmol/L. Salt treatments were given to 6 d old seedlings and Chl *a* measurements were taken subsequently for 3 d after the salt treatment.

The fast Chl *a* fluorescence kinetics of mung bean and Brassica seedlings showed no significant change within 3 d of experiments without the addition of salts/ions. The salt/ion treatments significantly affected the Chl *a* fluorescence kinetics. The initial Chl *a* fluorescence level (F_0) increased after 3 d with NaCl, which was enhanced by the addition of $CaCl_2$. KCl and Na_2SO_4 or K_2SO_4 induced an increase in F_0 after 1 d of salt treatments. $CaCl_2$ alone and with NaCl or KCl enhanced F_M after 1 d of treatment. KCl alone increased F_M values after 1 d. However, the maximum Chl *a* fluorescence level (F_M), quantum yield of the primary photochemistry of PS II (ϕ_{Po}), quantum yield of electron transport beyond Q_A^- (ϕ_{Eo}), efficiency that a trapped electron can move further ahead of Q_A^- (ψ_o) and flux ratio of electron transport (E_{To}/RC) decreased significantly with NaCl treatments after 3 d of treatment. $CaCl_2$ ameliorated these effects. ABS/RC and DI_0/RC was enhanced several folds by NaCl treatments and $CaCl_2$ augmented these effects. Other salts and their combinations also showed enhancement in DI_0/RC. However, Na salts showed amplified effects at equimolar concentrations compared to K salts. The most prominent difference in the susceptibility of a system or genotype to different salts or ions is due to the changes of the abundance of active reaction centers per leaf cross-section (RC_0/CS_0).

Key words: Brassica – *Brassica juncea* – $CaCl_2$ – Chl *a* fluorescence – ions – mung bean – salinity – *Vigna radiata*

Abbreviations: ABS absorbance. – CS_0 cross section. – d days. – DI_0 dissipation of absorbed quanta. – ET electron transport from Q_A^- onwards. – ET_0/RC, ABS/RC and DI_0/RC are flux ratio of electron transport, absorbance and dissipation per reaction center, respectively. – F_0 initial Chl *a* fluo-

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rescence level. – F_M maximum Chl *a* fluorescence level. – F_V variable Chl *a* fluorescence measured as $F_M - F_0$. – J and I intermediate steps of Chl *a* fluorescence rise between F_0 = O level and F_M = P levels of Chl *a* fluorescence induction curve. – PS photosystem. – RC reaction center. – TR_0 excitation energy trapping. – ϕ_{Eo} Quantum yield of electron transport beyond Q_A^-. – ϕ_{Po} quantum yield of the primary photochemistry of PS II. – ψ_o Efficiency that a trapped electron can move further ahead of Q_A^-

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Introduction

The problem of soil salinity is a major limiting factor for crop productivity in the arable land all over the world (Misra et al. 1997). Soil salinity is due to the accumulation of excess amounts of Cl^-, SO_4^{-2}, CO_3^{-2} and HCO_3^- salts of Na^+, K^+, Mg^{+2} and Ca^{+2} (Greenway and Munns 1990). These salts affect plant growth and development. The most widely occurring problem of soil salinity is the abundance of NaCl in soils (Misra et al. 1995). Salt affected areas get low precipitation, which facilitate the accumulation of soluble salts in the root growth zone of crops (Misra et al. 1997). Even the irrigation practices in salt-affected areas increase the agricultural problems by remobilizing salts from deeper soil layers.

Salinity affects plant growth, metabolism and photosynthetic efficiency of crop plants (Misra et al. 1996, 1997, 1999). Mung bean is a salt-sensitive and Brassica is a salt-tolerant crop (Misra et al. 1995, 1996, 1999). Therefore, these crop plants are most suitable for study of the impact of salinity or ion effects on crops to assess the genetic basis of salinity resistance in plants. Misra et al. (1999) have reported that NaCl salinity affects PS II photochemical efficiency, charge separation of primary charge pairs in PS II and pigment-protein complexes of thylakoid membranes. Besides NaCl, salts like KCl, Na_2SO_4 and K_2SO_4 also occur in salt-affected soils. Salinity stress effects are reported to be ameliorated by $CaCl_2$ (Jimenez et al. 1997).

Chlorophyll *a* fluorescence is widely used as a tool to screen the alternations in photosynthetic apparatus and its functional efficiency (Havoux et al. 1988, Belkhodja et al. 1994, Jimenez et al. 1997). This could be used as a tool for detecting the salt effects on plants (Smillie and Nott 1982, Bongi and Loreto 1989). However, Jimenez et al. (1997) suggested that this tool can only be used as an indicator of salt stress in rose plants when additional stress of high light is added. These authors have suggested that the evaluation of fluorescence parameters are not clear-cut for salt stress, as it is for other stress factors like water stress, chilling, freezing and air pollution. However, changes in fluorescence parameters have been used as an early warning system for salinity stresses (Smillie and Nott 1982, Bongi and Loreto 1989), although these changes were less specific and not as dramatic. Even quantum yield of the primary photochemistry of PS II (F_V/F_M ratio) was reported to be unaffected by salinity (Brugnoli and Lauteri 1991, Brugnoli and Bjorkman 1992) or was affected when superimposed by high irradiance (Sharma and Hall 1992) and temperature stress (Larcher et al. 1990). As the fluorescence parameters are suggested to be less affected by salt stress alone, it is imperative that the studies of ion effects would have practical limitations using fluorescence measurements. However, in the present study we suggest emphatically that this technique can be used as a highly sensitive method for salt and ion effects and their interactions in plants. Also, the genotype differences in salt or ion sensitivity can be evaluated using fast fluorescence kinetic measurements.

In the present study, we verify whether Chl *a* fluorescence parameters can be used for screening salinity stress. We report the changes in Chl *a* fluorescence parameters of mung bean and Brassica leaves affected by NaCl and KCl alone or in combination with $CaCl_2$. The effect of different SO_4^{-2} salts is compared with those of Cl^- salts.

Materials and Methods

Plant material

Mung bean (*Vigna radiata* L. cv. Sujata) and Brassica (*Brassica juncea* Coss. cv. Pusa bold) were grown on nylon mess floated over distilled water at 25 ± 1 °C under continuous illumination of 125 µmol m^{-2} s^{-1}. Six-day-old seedlings were transferred to distilled water containing NaCl or KCl (0 mmol/L, 100 mmol/L, 200 mmol/L and 300 mmol/L) alone, or in combination with 5 mmol/L $CaCl_2$. The Na_2SO_4 and K_2SO_4 salt treatments were given at 0 mmol/L, 50 mmol/L, 100 mmol/L and 200 mmol/L concentrations. Each treatment had minimum 3 replicates with at least 5 plants in each replicate.

Fluorescence measurements

Plant Efficiency Analyzer (PEA, Hansatech Ltd., UK) measures fast fluorescence kinetics of leaves at room temperature. The leaf samples were dark-adapted for 5 min before the fluorescence measurements. Dark-adapted leaves were illuminated homogeneously over an area of 4 mm dia with an array of 6 LED (λ max. 650 nm, 600 W m^{-2}) and fluorescence signals were detected using a PIN photodiode after passing through a long-pass filter (50 % transmission at 720 nm). Fluorescence transients were recorded from 10 µsec to 1 sec with the data acquisition rate of 10 µsec for first 2 msec and then at the rate of 1 msec with a resolution of 12 bits. The fluorescence signal at 40 µsec after the onset of illumination was considered as F_0 (Srivastava et al. 1995, Strasser and Strasser 1995, Strasser et al. 1999, 2000).

Theoretical considerations for the OJIP transients

The fast fluorescence kinetics of all oxygenic photosynthetic materials show a transient fluorescence rise known as O, J, I and P (Strasser and Strasser 1995). The maximal fluorescence intensity F_P denotes the F_M values where all the reaction centers (RCs) are physiologically closed. The F_0 state indicates the physiological state when all the RCs are open.

Energy fluxes per RC and per CS, and flux ratios or yield ratios have been derived using the theory of energy fluxes in biomembranes (Sironval et al. 1981) and the experimental values of the OJIP-fluorescence transient values. The constellation of their values at any instant is considered as an expression of the function of the system (Kruger et al. 1997).

Absorbance (ABS) refers to the photon absorbed by the antenna pigment molecules. A part of the absorbed energy is trapped (TR) by the RC, and the other portions dissipated as heat and fluorescence. The trapped energy is converted to redox energy by electron transport (ET) to Q_A and Q_B. The different yields of absorbed energy and the specific fluxes at time zero can be derived according to the JIP-test (Strasser et al. 2000) as follows:

$TR_0/ABS = \phi_{Po} = (1 - F_0)/F_M = F_V/F_M$ $TR_0/RC = M_0 \cdot (1/V_J)$

$ET_0/TR_0 = \psi_o = 1 - V_J$ $ABS/RC = M_0 \cdot (1/V_J) \cdot (1/\phi_{Po})$

$ET_0/RC = M_0 \cdot (1/V_J) \cdot \psi_o$ $ET_0/ABS = \phi_{Eo} = \phi_{Po} \cdot \psi_o$

Where:

$V_J = (F_{2ms} - F_0)/(F_M - F_0)$ $M_0 = (dV/dt)_0 = 4.(F_{300\mu s} - F_0)/(F_M - {}_0)$

The total absorption and active RC per CS has been calculated as follows:

$RC/CS_0 = \phi_{Po} \cdot (V_J/M_0) \cdot F_0$ $ABS/CS_0 = F_0$.

Performance index

The product of the specific fluxes, the performance indices (PI) can be calculated using the JIP test as suggested by Srivastava et al. (1999) and Strasser et al. (2000).

$PI_{ABS} = (RC/CS)\ [\phi_{Po} / (1 - \phi_{Po})]\ [\psi_o/(1 - \psi_o)]$

By using the Nernst's equation the log PI can be defined as a driving force for photosynthesis.

Driving force $(DF_{ABS}) = \log (PI_{ABS})$

Results and Discussion

The fluorescence characteristics of mung bean and Brassica seedlings changed with the concentration and nature of salt/ion treatments (Fig. 1). There was no significant change in the fluorescence kinetics within 3 d of experiments without the addition of salts/ions in mung bean seedlings (Fig. 1A). Since the salt/ion-induced changes in fluorescence characteristics were not significantly different from each other after 1 d of treatment, only the data after 3 d are shown and the differences are interpreted in terms of salt or ion basis. NaCl salt induced an increase in F_0 and a decrease in F_M of mung bean seedlings (Fig. 1D). The F_0 value of 200 mmol/L NaCl-treated seedlings were enhanced by 16 % of that of the control seedlings. The decrease in F_M values was much more drastic than the changes in F_0 values. Compared to the effects of NaCl, KCl treatment showed a decrease in the F_M values only at

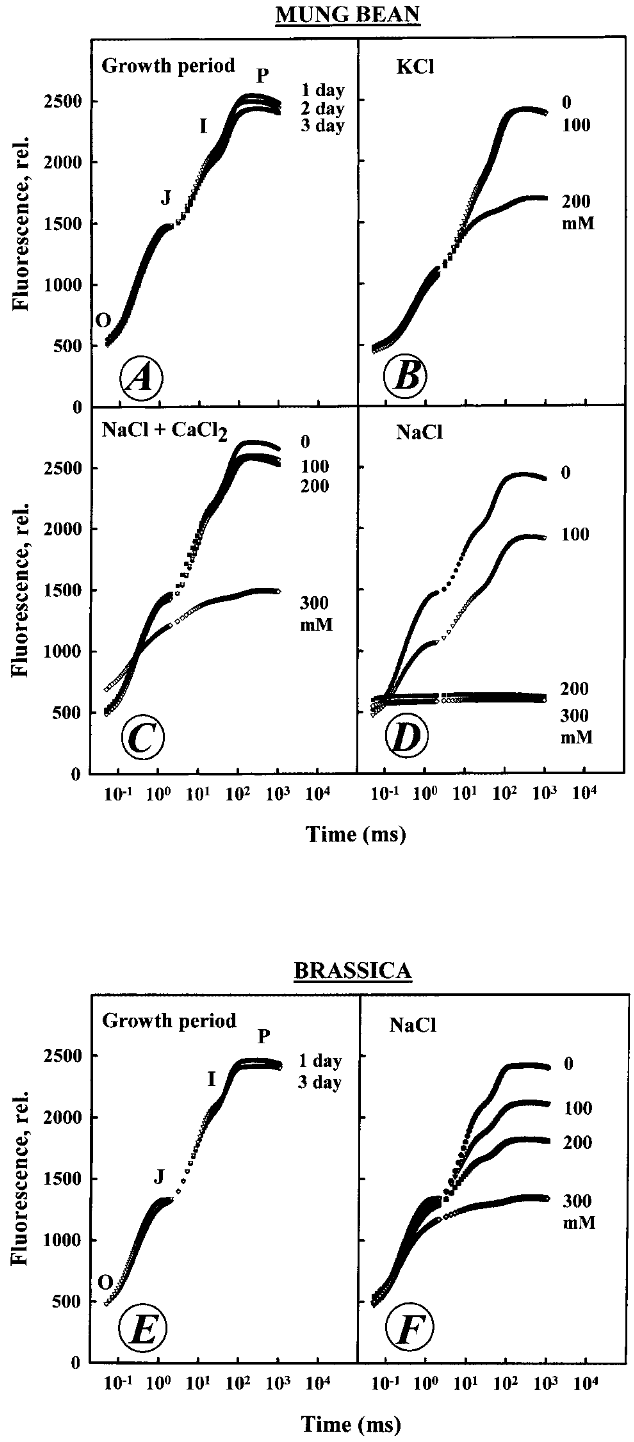

Figure 1. Effect of salts on the fluorescence induction kinetics of mung bean [A–D] and Brassica [E, F] seedlings. Six day old seedlings were treated with different salts and measurements were done after 1, 2 and 3 d. Flourescence kinetics shown in A and E are control seedlings after 1, 2 and 3 d of treatment. Transients shown in B–D and F are obtained after 3 d of NaCl [D, F], NaCl + $CaCl_2$ [C] and KCl [B] treatments.

200 mmol/L and no significant effect on fluorescence parameters were observed at 100 mmol/L (Fig. 1 B). Na_2SO_4 treatment was also less detrimental for mung bean seedlings at equimolar concentrations of the ions (fluorescence curves not shown). Treatment of $CaCl_2$ with NaCl salt treatments retarded the decrease in F_M and enhancement in F_0 up to 200 mmol/L NaCl (Fig. 1 C). The seedlings treated with NaCl along with $CaCl_2$ showed a F_0 rise of 28 % and F_M decrease of 46 % over control values (Fig. 1 C).

As that of the mung bean seedlings there were no changes in the fluorescence kinetics of Brassica seedlings during the growth period from 1 d to 3 d (Fig. 1 E). However, the F_0 values increased and F_M values decreased gradually with an increasing concentration of NaCl (Fig. 1 F). The F_0 rise and F_M decrease indicates a block in the electron transport to Q_A^- (Krause and Weis 1991). NaCl salinity is reported to affect water oxidation (PS II photochemical efficiency) and charge separation of primary charge pairs in PS II of mung bean and Brassica seedlings (Misra et al. 1999). Both Q_A^- and Q_B^- charge recombination with S states were affected by NaCl salinity.

Figure 2 shows the effect of different salts on the quantum yield of the primary photochemistry of PS II (ϕ_{Po}), the efficiency that a trapped electron can move further ahead of Q_A^- (ψ_o) and the probability that an absorbed photon will move an electron into the electron transport chain (ϕ_{Eo}). The changes in fluorescence intensities by salinity is in sharp contrast to the earlier reports that F_V/F_M ratio was unaffected by salinity (Brugnoli and Lauteri 1991, Brugnoli and Bjorkman 1992). In the present study, there was no need to superimpose high irradiance (Sharma and Hall 1992) or temperature stress (Larcher et al. 1990) to bring about the changes in Chl fluorescence parameters. The discrepancy by earlier authors could have been due to the use of resistant genotypes, where the effect of salinity was not manifested unless the system was severely stressed either by imposing another stress or by high concentrations of salts. However, earlier workers used the changes in fluorescence parameters as an early warning system for salinity stresses (Smillie and Nott 1982, Bongi and Loreto 1989).

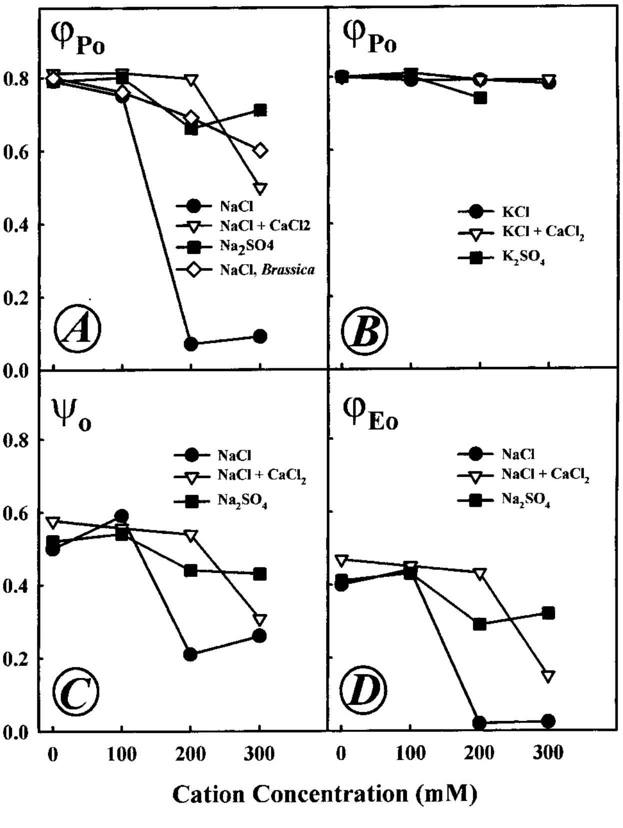

Figure 2. The effect of different concentrations of Na- (A, C, D) and K-salts (B) on the quantum efficiency ϕ_{Po} (A, B) or flux ratios ψ_o (C) and ϕ_{Eo} (D) of mung bean seedlings after 3 d of treatment. Note the changes in ϕ_{Po} of Brassica seedlings treated with NaCl (A).

Na_2SO_4 treatment affected ϕ_{Po} in mung bean seedling only by 20 % of control value compared to 90 % in NaCl treatments at equimolar concentrations of Na^+ ion (Fig. 2). $CaCl_2$ treatment augmented the quantum yield to the control value only up to 200 mmol/L NaCl treatment. NaCl treatment (at 300 mmol/L) with $CaCl_2$ caused a decrease of ϕ_{Po} to 42 % of the control value (Fig. 2). This suggests that $CaCl_2$ could only retard the detrimental effects of NaCl by counter-ion effects and defer the toxic effects of NaCl to a higher concentration. Compared to that of mung bean seedlings, the changes in ϕ_{Po} in NaCl salt-treated seedlings of Brassica showed a slow and gradual decrease with increasing salt concentrations (Fig. 2). The decrease at 300 mmol/L NaCl was 26 % of control values. Salts of K^+ alone or in combination with $CaCl_2$ showed no significant change in ϕ_{Po} of mung bean (Fig. 2) and Brassica (data not shown). These results corroborate the report on amelioration of NaCl salinity stress effects by $CaCl_2$ (Jimenez et al. 1997).

The electron transport per trapped photon molecule (ψ_o) was enhanced at 100 mmol/L NaCl but decreased to 42 % and 49 % of control values, respectively at 200 mmol/L and 300 mmol/L concentrations (Fig. 2). Addition of $CaCl_2$ enhanced the ψ_o of control seedlings and retarded the NaCl-induced decrease in ψ_o of mung bean seedlings. However, the ψ_o value of seedlings treated with 300 mmol/L NaCl and $CaCl_2$ decreased relatively to same value as that without $CaCl_2$ (Fig. 2). Na_2SO_4 treatments decreased ψ_o value only up to 16 % at 300 mmol/L NaCl.

The quantum yield for electron transport beyond Q_A^-($\phi_{Eo} = ET_0/ABS$) which is a product of ϕ_{Po} and ψ_o is also depicted in Figure 2. ϕ_{Eo} was retarded to less than 8 % at 200 mmol/L and 300 mmol/L NaCl and $CaCl_2$ ameliorated the effect up to 200 mmol/L NaCl. Na_2SO_4 treatments also affected ϕ_{Eo}. However, the decrease in ϕ_{Eo} values of Na_2SO_4-treated seedlings were only 22 % of control value (Fig. 2).

The performance index (PI) of the salt-stressed seedlings showed an exponential relationship with ϕ_{Eo} (Fig. 3). Brassica seedlings treated with Na^+ salts and mung bean seedlings with K^+ salts were grouped in clusters. However, mung bean

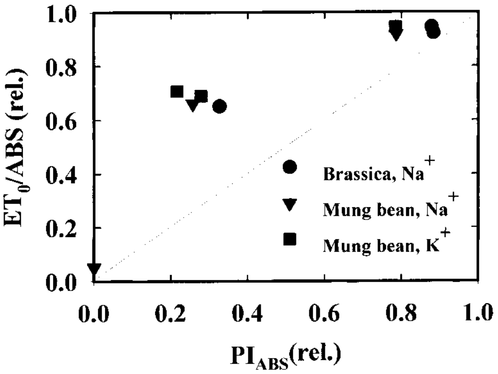

Figure 3. The relationship in the probability that an absorbed photon will move an electron into the electron transport chain (ϕ_{Eo} = (ET_0/ABS) rel.)) and the performance index (PI_{ABS} (rel.)) of Na^+ or K^+-treated mung bean and Brassica seedlings.

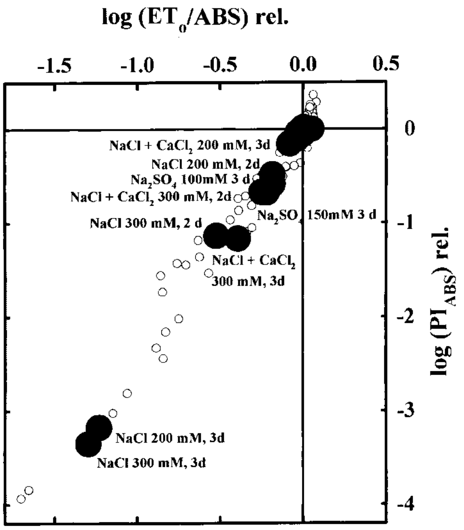

Figure 4. The logarithmic relationship between ϕ_{Eo} (= (ET_0/ABS)rel.), and the performance index (PI_{ABS})rel. of mung bean seedlings treated with different concentrations of Na^+ salts with or without the addition of $CaCl_2$. The bold closed circles denote the average values of each treatment (n = 5–7) and the small open circles represent the individual values for the treatments. Note the linear relationship between the two log functions and the salt concentration dependence of these functions. The four clusters show the different salts or their combined effects to the similar extent.

seedlings treated with NaCl salts showed minimum values for PI and ϕ_{Eo}. The log (ET_0/ABS) rel. as a function of log (PI_{ABS}) rel. for different Na-salt treatments are shown as a straight line in a double logarithmic plot in Figure 4. The two log functions have a linear relationship suggesting that the effects of different salts are through their ionic interactions with the photosynthetic systems. The relative effects of salts or ions can be detected from such double log plots. The dose responses and gradual time course of action of salts can also be determined from the linearity of this curve. It is very clear that NaCl salts are more detrimental to photosynthetic systems than Na_2SO_4. Also equimolar concentrations of Cl^- is more toxic than SO_4^{-2} ions (Fig. 4). The scatter plots for individual data points (open circles) also suggest that the variations in NaCl-treated seedlings were bigger compared to that of Na_2SO_4 or in combination with $CaCl_2$. Such an analysis suggests that stress effects for ions or salts and their concentrations can be analyzed through such double logarithmic plots.

A comparison of the slope for log PI_{ABS} versus log ϕ_{Eo} = log ET_0/ABS shows a distinct pattern of salt effects for NaCl and KCl (Fig. 5). The slope (m) for KCl-treated seedlings was 4.05 and that for NaCl salt was 2.58. Thisanalysis depicts that as log (PI_{ABS}) is positively correlated to log (ϕ_{Eo} = ET_0/ABS), the lower ‹m› values would represent a smaller decrease in the photosynthetic performance, when the yield for electron transport decreases.

The slope analysis for different salts and their ions were analyzed by combining the salts of the similar ions (Table 1). This analysis confirms our hypothesis that the ‹m› value (where $m = \Delta \log PI/\Delta \log \phi_{Eo}$) derived from the fast Chl *a* fluorescence kinetics can be taken as an indicator for salt or ion effects on photosynthetic efficiency analysis during salt stress. Also the tolerance and susceptibility of the genetic systems can be determined through this analysis. So, we propose that this analysis can be used for a wide range of applications from agronomy to biotechnological analysis of susceptibility of genotypes or photosynthetic systems to salts or ions. The differences in ‹m› values for mung bean seedlings under salt stress are clearly in an increasing order starting with NaCl, NaCl+$CaCl_2$, Na_2SO_4, KCl, KCl+$CaCl_2$, K_2SO_4 (Table 1). The ionic effects compared by this analysis depicts

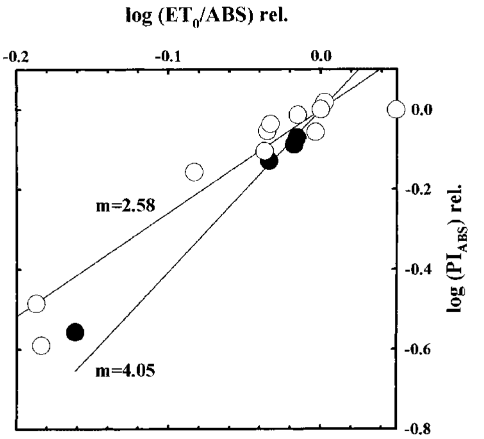

Figure 5. Analysis of the slope angles (m) for the linear realtionship between log ϕ_{Eo} (= log (ET_0/ABS)rel.) and log performance index (= log (PI_{ABS})rel.) of mung bean seedlings treated with different concentrations of K^+ (closed circles) and Na^+ (open circles) salts. The relative values of ‹m› can be taken as an index for effectiveness of the salts on the photosynthetic systems. The lower the value of ‹m› the lesser the inhibitory effect of the salts on the quantum efficiency of photosynthetic electron transport (ϕ_{Eo}) and the performance index (PI_{ABS})rel.

that the order of ion toxicity on photosynthetic efficiency of mung bean seedlings are Cl^- > Na^+ > SO_4^{-2} > K^+ (Table 1). The ‹m› values for NaCl salt-stressed mung bean and Brassica seedlings were 2.58 and 2.2, respectively (Table 1). The ‹m› values for interactions of ions and plants are also shown in Table 1 that can be used in the crop efficiency analysis.

We have analyzed the relative performance index of the salt-stressed plants (Table 2). The -log values (pPI) or the driving force for electron transport, in terms of the absorbed quanta and the cross section of the leaf, showed that the relative performance index [pPI_{ABS}(rel.) and pPI_{CSo}(rel.)] was maximum for NaCl treatment in mung bean. $CaCl_2$ ameliorated the effect of NaCl in such a way that pPI_{ABS}(rel.) and pPI_{CSo}(rel.) values approached 0.1. Similar effects were seen for Brassica seedlings with NaCl treatment, although the pPI_{ABS}(rel.) and pPI_{CSo}(rel.) values were less than a seventh of that of mung bean seedlings (Table 2). The pPI_{ABS}(rel.) values for Na_2SO_4, K_2SO_4 and KCl treatments were nearly 0.5 to 0.6. $CaCl_2$ salt also showed countering action to KCl. The pPI_{ABS}(rel.) and pPI_{CSo}(rel.) values were similar to those of NaCl. These observations along with our earlier analysis of the slope values, clearly suggests that in terms of counter-ion effects $CaCl_2$ acts with Cl^- ions (comparing the values of NaCl and KCl in Table 2). These effects were shown to be due to an enhancement in the electron transport efficiency in the salt-stressed seedlings. This analysis also showed that the relative susceptibility of mung bean seedlings to NaCl salinity, compared to that of Brassica seedlings or other salts, was very high. This can be depicted by the analysis of pPI(rel.) values and/or relative electron transport efficiency of salt-stressed seedlings (Table 2). Differential effects of ions can also be assessed from this analysis.

Table 1. Regression analysis for log [(PI) rel.] and log [(ET_o/ABS) rel.] for ion effects on mung bean and Brassica seedlings. The slope is given as «m». The regression equation is log [PI (rel.)] = m * log [ET_o/ABS (rel.)].

R^2 and n denote the Correlation coefficient and number of samples, respectively.

Plant	Salt or iron	m	R^2	n
Mung bean	NaCl	2.582	0.998	68
	NaCl + $CaCl_2$	2.960	0.999	75
	Na_2SO_4	3.116	0.988	80
	KCl	3.477	0.986	65
	KCl + $CaCl_2$	3.869	0.998	60
	K_2SO_4	4.375	0.999	50
	Na + ion	2.612	0.996	223
	K^+ ion	3.894	0.978	175
	Cl^-	2.589	0.997	143
	Ca^{++} + Cl^-	3.000	0.994	267
	SO_4^-	3.392	0.952	130
	Combined salts	2.633	0.991	398
Brassica	NaCl	2.208	0.992	70
	NaCl + $CaCl_2$	2.676	0.993	70
	Na + ion	2.283	0.985	140
Mung bean and Brassica combined	NaCl	2.5487	0.996	138
	NaCl + $CaCl_2$	2.878	0.995	145
	Na_2SO_4	3.093	0.985	64
	Plant x Na^+	2.800	0.994	363

Table 2. Ion sensitivity of performance index and activities of mung beean and Brassica seedlings after 3 d of treatment with different salts and their combinations. The relative values are calculated from the ratio between the treated seedlings and the untreated (control) seedlings.

pPI_{ABS} (rel.) = –log [PI_{ABS} (Treatment) / PI_{ABS} (Control)].

pPI_{CS} (rel.) = –log [PI_{CSo} (Treatment) / PI_{CSo} (Control)].

where $PI_{Cso} = F_0 * PI_{ABS}$.

Treatments	pPI_{ABS} (rel.)	ET_0/ABS (rel.)	pPI_{CSo} (rel.)	ET_0/CS_0 (rel.)
Mung bean				
NaCl	3.352	0.051	3.289	0.058
NaCl + $CaCl_2$	0.105	0.919	0.092	0.946
Na_2SO_4	0.590	0.655	0.516	0.782
Mung bean				
KCl	0.556	0.689	0.569	0.669
KCl + $CaCl_2$	0.106	0.945	0.121	0.913
K_2SO_4	0.666	0.706	0.653	0.727
Brassica				
NaCl	0.485	0.650	0.523	0.734
NaCl + $CaCl_2$	0.056	0.992	0.110	0.835

The performance index in the leaves is the cumulative effects of the functions of ψ_o, ϕ_{Po}, CS_0/ABS and RC_0/CS_0 (Srivastava et al. 1999). Figure 6 shows the component analysis for the changes in performance index (PI) of the salt-stressed mung bean and Brassica seedlings. The components which were most affected by salt stress in mung bean are ϕ_{Po}, RC_0/CS_0 and the electron transport (ψ_o) per trapped electrons. The significant difference in Brassica and mung bean seedlings was the increase in RC_0/CS_0 of Brassica seedlings treated with Na^+ salts (Fig. 6). The susceptibility of photosynthetic systems at the ϕ_{Po} level to different stress factors are well documented (Kruger et al. 1997, Srivastava and Strasser 1997, Ouzounidou et al. 1997). In this study we suggest that there are differences in the changes in ϕ_{Po} in the susceptible and tolerant systems, as well with different salt or ion treatments. However, the most prominent differences in the susceptibility of a system or genotype to different salts or ions are due to damage of active RC, which results in decreased RC_0/CS_0 and the photochemical efficiency of PS II. Although the electron transport efficiency beyond Q_A^- decreased along with the number of reaction centers and electron donation to Q_A, the severity of the decrease in ϕ_{Po} suggests that the donor side is relatively more affected than the acceptor side. Salt-induced acceptor side inhibition of charge transfer was reported earlier (Misra et al. 1999, Sahu et al. 1998). The salt-

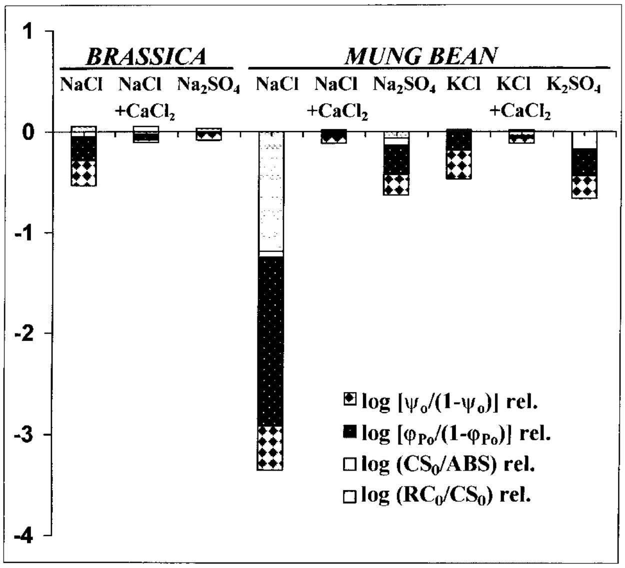

Figure 6. Component analysis for the decrease in the log performance index (driving force) of salt-affected seedlings. The variations in salt treatments and genotypes are characterized by the differences between their photosynthetic components. A several-fold difference between the functions of ϕ_{Po} and RC_0/CS_0 can be taken into account as the major contributory factor for the decreased photosynthetic performance of plant systems to salt treatments.

induced damage to the reaction center could be due to ion induced rapid turn-over. Further studies are needed to prove this hypothesis by evaluating the genetic variations in susceptibility of plant systems to various stress factors.

The salt/ion-induced changes in the phenomenological parameters of fluorescence kinetics are shown in a pipeline Model (Fig. 7A, B). In this model the OJIP values were taken to express PS II activities in terms of the cross section unit (Leaf model, right panel) and per unit reaction center (membrane model, left panel). NaCl salt treatment in mung bean seedlings inhibited the electron transport beyond Q_A^- per

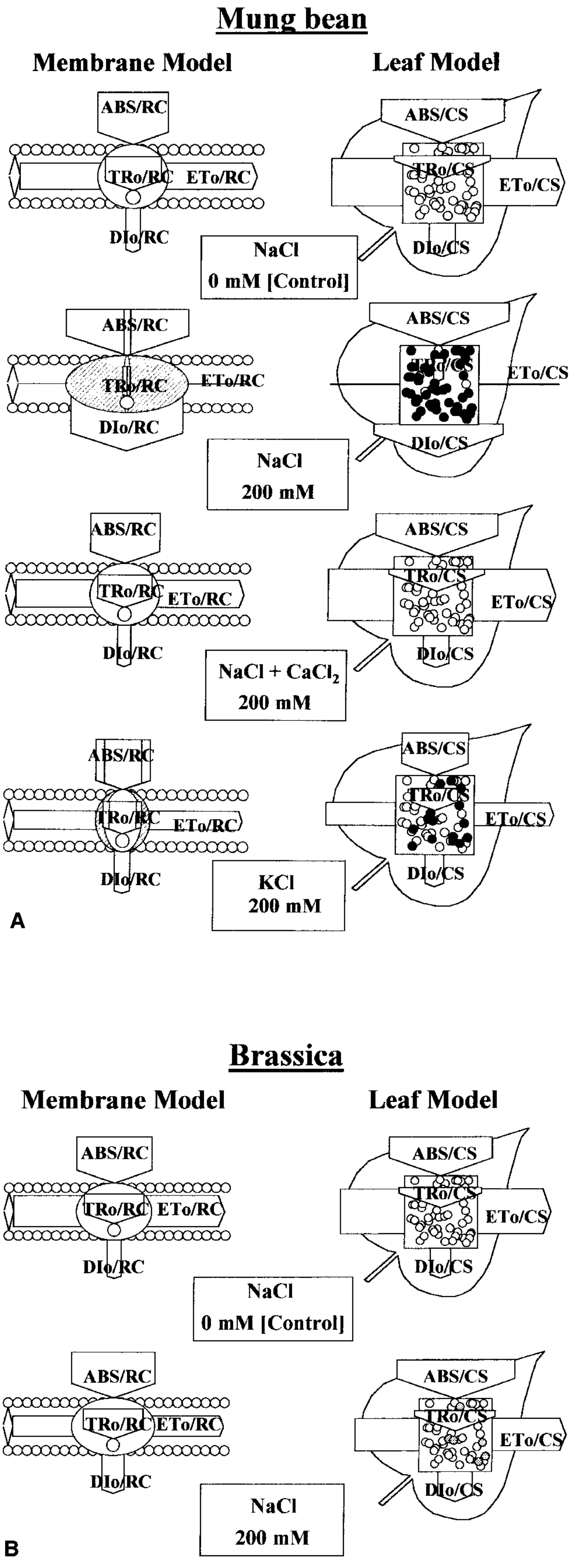

Figure 7. The pipeline model for the salt/ion-induced changes in the phenomenological parameters of fluorescence kinetics of mung bean (A) and Brassica (B). The leaf model (right panel) shows phenomenological fluxes or apparent activities per cross section (CS_0) and the membrane model (left panel) shows the specific activities per unit reaction center (RC). The density of active photosynthetic units involved in the reduction of Q_A, per cross section, is shown as small open circles in the leaf model. The small closed circles (A) demonstrate inactive photosynthetic units and the small hatched circles (B) represent newly synthesized units. The arrows indicate fluxes for light absorbance (ABS), excitation energy trapping (TR_0), energy dissipation (DI_0) and electron transport (ET_0) beyond Q_A^-. The width of each arrow denotes the relative size of the fluxes or the antenna. Note that the dimentions of the figure in the membrane model (left panel, A) for NaCl 2000 mmol/L is reduced 10× to fit into the plane of the paper.

cross section (ET_0/CS) due to the inactivation of the reaction centers (closed black circles). The trapping efficiency per cross section (TR_0/CS) also decreased. However, NaCl treatment increased the aborption (ABS/CS) and heat dissipation (DI_0/CS). These effects were several times greater in the membrane model (left panel) where because of the inactivation of the active reaction centers, the ABS, DI_0 and TR_0 values increased in terms of specific activity of active reaction centers (Fig. 7 A, left panel). Equimolar quantity of NaCl showed less detrimental effect on Brassica seedlings (Fig. 7 B), compared to the mung bean seedlings (Fig. 7 A). The reaction centers remained active and there was even synthesis of new reaction centers (hatched circles) in Brassica seedlings treated with 200 mmol/L NaCl, which was completely inhibitory to mung bean (compare Fig. 7 A with 7 B). Like mung beans, the electron transport (ET_0) of Brassica also decreased. However, absorbance (ABS) and dissipation (DI_0) per cross section or per reaction center increased with NaCl treatment, although the quantum of change in Brassica seedlings was less than 10 × than that of mung bean seedlings. This phenomenon is distinct depiction of genetic variability in the salt tolerance of these two crops (Misra et al. 1999). Addition of $CaCl_2$ along with NaCl, which completely blocked the electron transport in mung bean, could completely ameliorate the inhibitory effect of NaCl (Fig. 7 A). Comparison of the salt/ion effects on the photosynthetic efficiency of mung bean seedlings also showed that KCl was less inhibitory to electron transport processes compared to equimolar concentrations of NaCl (Fig. 7 A). The ABS, TR_0 and DI_0 values of KCl-treated seedlings decreased compared to the control (no salt) seedlings. The pigment content of the leaf also decreased (data not shown). This resulted in lesser damage to the reaction center complexes (black circles). The degradation of leaf pigments was induced by KCl and therefore, diluting the Chl per cross section leads to a decrease in the absorbance and trapping of photons per cross section. The lesser damaging effect of KCl in mung bean, as compared to NaCl, appears to be through its effect on pigment metabolism. NaCl treatment might have a direct effect of Na^+ on the reaction center complex itself leading to severe salt stress effects. The augmentation of photosynthetic efficiency by $CaCl_2$ in salt-stressed mung bean seedlings to the control values could be due to repletion of Ca^+ in the NaCl-treated seedlings which could have been replaced from the photosynthetic systems by high concentrations of Na^+ ion (Shen and Katoh 1991). In this model we have also conformed that the most prominent differences in the susceptibility of a system or genotype to different salts or ions are due to the variations in the active reaction centers.

Conclusion

It is concluded from the present study that the fast Chl *a* fluorescence technique can be utilized for assessment of salt/ion effect of plants. Comparison of the salt/ion effects on the photosynthetic efficiency of mung bean seedlings also showed that KCl was less inhibitory to electron transport processes compared to equimolar concentrations of NaCl. Addition of $CaCl_2$ along with NaCl, which completely blocked the electron transport in mung bean, could completely ameliorate the inhibitory effect of NaCl. Also it is possible to differentiate between the susceptible and tolerant genotypes for salt susceptibility.

The most prominent differences in the susceptibility of a system or genotype to different salts or ions are due to damage of active RC, which results in decreased RC_0/CS_0 and the photochemical efficiency of PS II. Although the electron transport efficiency beyond Q_A^- decreased along with the number of reaction centers and electron donation to Q_A, the severity of the decrease in ϕ_{Po} suggests that the donor side is relatively more affected than the acceptor side.

The effect of individual components of salt/ion(s) and their combined effects can be deciphered using the analysis of performance index and the slope (m) analysis. This analysis confirms our hypothesis that the ‹m› value (where $m = \Delta \log PI/\Delta \log \phi_{Eo}$) derived from the fast Chl *a* fluorescence kinetics can be taken as an indicator for salt or ion effects on photosynthetic efficiency analysis during salt stress. Also the tolerance and susceptibility of the genetic systems can be determined through this analysis. So, we propose that this analysis has a wide range of applications from agronomy to biotechnological analysis of susceptibility of genotypes or photosynthetic systems to salts or ions. The differences in ‹m› values for mung bean seedlings under salt stress showed that the detrimental effects of different salts are in the decreasing order starting with NaCl, NaCl+$CaCl_2$, Na_2SO_4, KCl, KCl+$CaCl_2$, K_2SO_4. The ionic effects compared by this analysis depicts that the order of ion toxicity on photosynthetic efficiency of mung bean seedlings are $Cl^- > Na^+ > SO_4^{-2} > K^+$. Mung bean seedlings were found to be more susceptible to NaCl salt stress compared to the Brassica seedlings. Also, the ‹m› values for interactions of ions and plants can be used in the crop efficiency analysis.

Acknowledgements. ANM is thankful to University of Geneva for support during the Summer of 1998 and 2000.

References

Belkhodja B, Morales F, Abadia A, Gomez-Apaprisi J, Abadia J (1994) Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). Plant Physiol 104: 667–673

Bongi G, Loreto F (1989) Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. Plant Physiol 90: 1408–1416

Brugnoli E, Bjorkman O (1992) Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta 187: 335–347

Brugnoli E, Lauteri M (1991) Effect of salinity on stomatal conductance, photosynthetic capacity and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. Plant Physiol 95: 628–635

Greenway H, Munns R (1990) Mechanism of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31: 149–190

Havoux M, Ernez M, Lannoye R (1988) Selection de varietes de blé dur (*Triticum durum* Desf.) et de blé tendre (*Triticum aestivum* L.) adaptees a la secfheresse par la mesure de l'extinction de la fluorescence de la chlorophylle *in vivo*. Agronomie 8: 193–199

Jimenez MS, Gonzalez-Rodriguez AM, Morales D, Cid MC, Socorro AR, Cabarello M (1997) Evaluation of chlorophyll fluorescence as a tool for salt stress detection in roses. Photosynthetica 33: 291–301

Krause G, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42: 313–349

Kruger GHJ, Tsimilli-Michael M, Strasser RJ (1997) Light stress evokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. Physiol Plant 101: 265–277

Larcher W, Wagner V, Thammathaworn A (1990) Effect of superimposed temperature stress on *in vivo* chlorophyll fluorescence of *Vigna unguiculata* under saline stress. J Plant Physiol 136: 92–102

Misra AN, Sahu SM, Misra M (1995) Soil salinity-induced changes in pigment and protein contents in cotyledons and leaves of Indian mustard (*Brassica juncea* Coss.). Acta Physiol Plant 17: 375–380

Misra AN, Murmu B, Singh P, Misra M (1996) Growth and proline accumulation in mung bean seedlings as affected by sodium chloride. Biol Plant 38: 531–536

Misra AN, Sahu SM, Meera I, Mohapatra P, Das N, Misra M (1997) Root growth of salt susceptible and a salt resistant rice (*Oryza sativa* L.) during seedling establishment under NaCl salinity. J Agron Crop Sci 178: 9–14

Misra AN, Sahu SM, Misra M, Ramaswamy NK, Desai TS (1999) Sodium chloride salt stress-induced changes in thylakoid pigment protein complexes, photosystem II activity and thermoluminescence glow peaks. Z Naturforsch Sect C 54: 640–644

Ouzounidou G, Moustakas M, Strasser RJ (1997) Sites of action of copper in the photosynthetic apparatus of maize leaves: Kinetic analysis of chlorophyll fluorescence, oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals. Australian J Plant Physiol 24: 81–90

Sahu SM, Misra AN, Misra M, Ramaswamy NK, Desai TS (1998) Sodium chloride salt stress-induced changes in thylakoid pigment-protein complexes, PS II activity of mungbean (*Vigna radiata* L.) seedlings. In: Garab G (ed) Photosynthesis: mechanism and effects. Vol IV. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 2625–2628

Sharma PK, Hall DO (1992) Changes in carotenoid composition and photosynthesis in sorghum under high light and salt stresses. J Plant Physiol 140: 661–666

Shen J-R, Katoh S (1991) Inactivation and calcium-dependent reactivation of oxygen evolution in photosystem II preparations treated at pH 3.0 or with high concentrations of NaCl. Plant Cell Physiol 32: 439–446

Sironval C, Strasser RJ, Brouers M (1981) Equivalence entre la theorie des flux et la theorie des relations enter proportions de pigments pour la description de la repartition de lenergie lumineuse absorbee par les membranes photoactives. Bull Acad R Belg 67: 248–259

Smillie R, Nott R (1982) Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo.* Plant Physiol 70: 1049–1054

Srivastava A, Strasser RJ (1997) Constructive and estructive action of light on the photosynthetic apparatus. J Sci Ind Res 56: 133–148

Srivastava A, Strasser RJ, Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61: 32–42

Srivastava A, Strasser RJ, Govindjee (1999) Greening of peas: parallel measurements of 77 K emission spectra, OJIP chlorophyll *a* fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. Photosynthetica 37: 365–392

Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: The JIP test. In: Mathis P (ed) Photosynthesis: from light to biosphere. Vol V. Kluwer Acad Publ, Dordrecht, The Netherlands, pp 977–980

Strasser RJ, Srivastava A, Tsimilli-Michael M (1999) Screening the vitality and photosynthetic activity of plants by the fluorescence transient. In: Behl RK, Punia MS, Lather BPS (eds) Crop improvement for food security. SSARM, Hissar, India, pp 72–115

Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (eds) Probing photosynthesis: Mechanism, regulation and adaptation. Taylor and Francis, New York London, pp 445–483