DOI: 10.32615/ps.2019.147 PHOTOSYNTHETICA 58 (SI): 293-300, 2020

Special issue in honour of Prof. Reto J. Strasser

Chlorophyll *a* fluorescence parameters as indicators of a particular abiotic stress in rice

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Abstract

The aim of this study was to evaluate the validity of some chlorophyll (Chl) *a* fluorescence parameters as early indicators of a particular abiotic stress and also to characterize the effect of different abiotic stresses [high light, NaCl, polyethylene glycol (PEG)-induced osmotic stress, and heavy metals] on the electron transport chain of rice (*Oryza sativa* L.) seedlings. The results clearly revealed that Chl *a* fluorescence parameters differ between abiotic stress types and also allowed us to select some parameters which were specifically and intensively affected under different abiotic stresses. We observed that the performance index is a common sensitive parameter to evaluate the effect of above four different abiotic stresses in rice seedlings. Certain Chl *a* fluorescence parameters were significant for a specific stress. The ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules (F_V/F_0) was prominently decreasing and the maximum quantum yield of nonphotochemical deexcitation was prominently increasing upon exposure to high light stress. The maximum quantum yield of electron transport and the electron transport from PSII donor side to PSII reaction center was highly reduced under NaCl stress in rice seedlings. Moreover, F_V/F_0 and PSII structure function index were prominently decreasing and the dissipation per cross section was significantly enhancing under PEG stress. The pool size of reduced plastoquinone on the reducing side of PSII [total complementary area between the fluorescence induction curve and maximal chlorophyll fluorescence (F_M)], F_M, and the probability by which electrons move from PSII to PSI acceptor side were significantly decreasing under heavy metal stress.

Additional key words: JIP-test; photosynthesis; plant efficiency analyzer.

Introduction

Agricultural productivity is threatened by various biotic and abiotic stress factors which seriously impact the global food security. Abiotic stresses are major environmental problems which negatively influence the plant growth and development and ultimately cause reduction in agricultural production worldwide (Rodziewicz *et al*. 2014, Dresselhaus and Hückelhoven 2018). Various abiotic factors, such as salinity, drought, heavy metals, high light, and extremes of temperature, result in a range of adverse effects in plants and lead to the overproduction of reactive oxygen species (ROS), which are highly reactive, resulting in oxidative stress. Salinity stress in plants leads to growth inhibition, leaf chlorosis, malfunction of the chloroplasts, and photoinhibition (Yamane *et al*. 2004, Dąbrowski *et al*. 2016, Acosta-Motos *et al*. 2017, Dąbrowski *et al*. 2017). Osmotic stress reduces PSII activity, decreases the effective quantum yield of PSII, and causes degradation of D1 protein leading to the inactivation of PSII reaction center (Batra *et al*. 2014, Asrar *et al*. 2017). Cadmium (Cd) is a toxic heavy metal and it causes leaf chlorosis, growth

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Received 11 June 2019, *accepted* 5 November 2019.

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Abbreviations: 1 – V_I – the efficiency/probability with which a PSII trapped electron is transferred to PSI acceptor side; ABS/CS_0 – absorption flux per cross section at t = 0; Area – total complementary area between the fluorescence induction curve and F_M; DI_0/CS_0 – dissipated energy flux per cross section at t = 0; ET_0/CS_0 – electron transport flux per cross section at t = 0; F_M – maximal chlorophyll fluorescence; F_0 – minimal fluorescence; F_V – variable fluorescence; F_V/F_0 – the ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules; F_V/F_M – maximum quantum efficiency of PSII under dark adaptation; $PI_{(abs)}$ – performance index; RC/ABS – fraction of active PSII reaction centers; $SFI_{(abs)}$ – PSII structure-function-index; $T_{f(max)}$ – time to reach maximal fluorescence; TR_0/CS_0 – trapped energy flux per cross section at t = 0; V_I – the relative variable fluorescence at I-step; V_J – the relative variable fluorescence at J-step; V_K/V_J – the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms as an indicator of the PSII donor side limitation; Φ_{D0} – maximum quantum yield of nonphotochemical deexcitation; Φ_{E0} – the electron transport quantum yield.

Acknowledgements: We thank Director, RARS, Pattambi, Kerala, India, for providing seeds of rice varieties. P. Faseela acknowledges Department of Science and Technology, India for providing financial support under INSPIRE Fellowship scheme (IF130020).

inhibition, and alters photosynthesis in plants (Nagajyoti *et al*. 2010, Paunov *et al*. 2018). Even though light has an important role in photosynthesis, high intensity light in plants can cause photoinhibition of PSII resulting in a decrease of quantum yield and photosynthetic rate (Kasajima *et al*. 2009, Fiorucci and Fankhauser 2017).

The effects of different environmental stresses on photosynthesis can be effectively studied using chlorophyll *a* (Chl *a*) fluorescence kinetics as it is a noninvasive tool for the detection and quantification of the changes in the photosynthetic apparatus. Analysis of Chl *a* fluorescence parameters can be used as a precise tool to test direct response of adverse environmental factors, such as NaCl (Hniličková *et al*. 2017), high light (Faseela and Puthur 2017), drought (Krasteva *et al*. 2013), temperature (Martinazzo *et al*. 2013, Feng *et al*. 2014), nutrient deficiency (Živčák *et al*. 2014, Kalaji *et al*. 2017), heavy metals (Kalaji and Loboda 2007), herbicides or air pollutants toxicity (Hassan *et al*. 2013), ultraviolet radiation (Šprtová *et al*. 2000, Faseela and Puthur 2018), long-term exposure to shade (Dąbrowski *et al*. 2015), and the indirect assessment of their impact on plants. The measurement of photochemical processes by Chl *a* fluorescence gives us a clear idea of the intensity of the stress encountered by the plants (Murchie and Lawson 2013). The relationship between light-phase reactions of photosynthesis and Chl fluorescence induction transient from PSII can be analyzed using JIP approach (OJIP). This approach allows obtaining extensive information about the PSII photochemical activity, electron transport events, and other regulatory processes of photosynthetic process (Strasser *et al*. 2004, Stirbet and Govindjee 2011). A part of the calculated parameters within the JIP-test is related to energy fluxes of absorption of light energy (ABS), trapping of excitation energy (TR), and conversion of excitation energy to electron transport (ET) per reaction center (RC) on measured area of samples (CS).

Rice, wheat, and maize are the three leading food crops for world human population and with increase in population, the production of these crops also has to be increased. There are different abiotic stress factors that affect rice production, such as salinity, drought, temperature, *etc*. (Grover and Minhas 2000). In the present study, we determined changes in PSII performance as analyzed through Chl *a* fluorescence in response to different abiotic stresses in rice leaves. Measurement of Chl *a* fluorescence is widely used to measure photosynthetic performance in rice leaves and the function of the photosynthetic apparatus upon exposure to various environmental stresses (Yamane *et al*. 2008, Puteh *et al*. 2013, Kasajima 2017). In this study, we made an attempt to identify some Chl *a* fluorescence parameters as a specific indicator(s) to characterize and evaluate the effect of high light, NaCl, PEG, and heavy metal stresses on the responses of photosynthetic apparatus in rice seedlings.

Materials and methods

Plant material and growth conditions: Rice (*Oryza sativa* L.) seeds of Mangalamahsuri variety were obtained from regional agricultural research station, Pattambi, Kerala, India. Seeds were surface sterilized with 0.1% $HgC1_2$ solution for 4 min and the seedlings were grown in a plant growth chamber set at 14/10-h light/dark cycles at 300 µmol(photon) m^{-2} s^{-1}, temperature of 24 ± 2°C, and relative humidity of RH 55 ± 5%.

Abiotic stress treatments: The seeds were germinated in plastic bottles (22 × 12 cm) containing absorbent cotton soaked with half strength Hoagland medium (control), stress-inducing concentrations of NaCl (100 mM), polyethylene glycol (PEG)-6000 (20%) (the pressure exerted by PEG was –4.89 MPa), and Cd (2 mM) solutions prepared in half strength Hoagland medium. The concentrations of stress-inducing solutions of NaCl/PEG/Cd were selected from the different concentrations of NaCl (25, 50, 75, 100, 125, and 150 mM), PEG (5, 10, 15, 20, 25, and 30%) and Cd (0.5, 1, 1.5, 2, 2.5, and 3 mM). The concentrations, which caused ~ 50% growth retardation in terms of shoot length, fresh and dry mass of seedlings, were selected as the stress-inducing concentration (data not shown). Chl *a* fluorescence parameters were analyzed after rice seedlings were exposed to salt, heavy metal, and PEG stress for 10 d right from germination. For high-light intensity treatment, after 10 d of growth in half strength Hoagland medium, rice seedlings were exposed to high light [2,000 µmol(photon) m^{-2} s^{-1}] for 2 h, provided by 1,000 W *PAR64* (*Philips*) metal halide lamps, and Chl *a* fluorescence was measured. Light intensity at the surface of the leaves was measured by a solar radiation monitor (*EMCON*, Cochin, India). To avoid direct heating of the leaf surface, a 20-cm deep glass chamber filled with water was placed between the lamp and the plants.

Chl *a* fluorescence measurements: The polyphasic Chl *a* fluorescence rise was measured at 10 d after germination in the leaves of rice seedlings exposed to different abiotic stresses. Chl *a* fluorescence transients were measured with the plant efficiency analyzer (*Handy PEA*, *Hansatech Ltd*., Norfolk, UK). All measurements were performed on the upper surfaces (middle portion) of the first formed leaves, following a dark-adaptation period of 20 min, using the leaf clips. Maximal fluorescence was induced by a 1-s pulse of white light [3,000 µmol(photon) m^{-2} s^{-1}] with the gain adjusted to 0.7 to avoid scaling problems. Thereafter, Chl *a* fluorescence signals were analyzed with the *Biolyzer HP3* software (Strasser *et al*. 2000). Various fluorescence parameters, such as the maximal fluorescence (F_M), the ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules (F_V/F_0), the maximum quantum yield of nonphotochemical deexcitation (Φ_{D0}), the electron transport quantum yield (Φ_{E0}), PSII structure-function-index [$SFI_{(abs)}$], performance index [$PI_{(abs)}$], and the total complementary area between the fluorescence induction curve and F_M (Area), minimal fluorescence (F_0), variable fluorescence (F_V), maximum quantum efficiency of PSII under dark adaptation (F_V/F_M), the relative variable fluorescence at I-step (V_I), the probability by which electrons move from PSII to PSI acceptor side ($1 - V_I$), the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms as an indicator of the PSII donor side limitation (V_K/V_J), time to reach maximal fluorescence [$T_{f(max)}$] were determined and the phenomenological leaf model was constructed in response to different abiotic stresses in rice seedlings. Phenomenological leaf models are derived based on the calculation of parameters per excited leaf cross section. The thickness of each arrow represents the value of absorbance (ABS/CS_0), trapping flux (TR/CS_0), electron transport (ET/CS_0) or heat dissipation of excess light (DI/CS_0), expressed per leaf cross section. The black points represent the fraction of inactive reaction centers. (Fig. 2).

Statistical analysis: The average values from 30 measurements, recorded on second leaves from top of the plant for each treatment, are shown. Statistical analysis was carried out according to *Duncan*'s multiple range test at 5% probability level using *SPSS* software (*version 16.0*, *SPSS Inc*., Chicago, USA).

Results

After exposure of rice seedlings to four different types of abiotic stress, the relative values (relative to the control) of Chl *a* fluorescence parameters were plotted as shown in spider plots and energy pipeline leaf models (Figs. 1, 2). We found that PI calculated on absorption basis was highly reduced when exposed to four different abiotic stresses. $PI_{(abs)}$ is an integrative parameter, which considers different phenomena related to PSII photochemical activity. The decrease of $PI_{(abs)}$ was 86, 78, 81, and 94% after exposing to high light, NaCl, PEG, and heavy metal stress, respectively. Maximal fluorescence and the Area also declined in rice seedlings and it was shown that heavy metal stress lead to an enhanced reduction in F_M (59%) and Area (74%), whereas the other three abiotic stresses resulted in the reduction of these two parameters by less than 30% only, compared with the control plants. Likewise, F_V and F_V/F_M decreased in all the stress treatments as compared with control seedlings. However, the rate of decrease in F_V/F_M was not significant in rice seedlings after exposing to high light, NaCl, PEG, and heavy metal stress (Fig. 1, Table 1).

It was observed that F_V/F_0 decreased with the application of high light, NaCl, PEG, and heavy metal stress in rice seedlings. On exposure to high light and PEG-induced osmotic stress, there was the enhanced reduction in F_V/F_0 (78 and 72%, respectively), but when treated with NaCl and heavy metal stress, the reduction was only 29% as compared with the control. V_J (the relative variable fluorescence at J-step) increased, when rice seedlings were treated with different abiotic stresses and the increase was up to 30–41% when exposed to high light, PEG, and NaCl stresses as compared with control. But the treatment with heavy metal did not bring about any change in relative value of V_J in rice seedlings. Even though the parameter V_I (the relative variable fluorescence at I-step) did not show any significant variation among treatments (only < 20%), $1 – V_I$ (the probability by which electrons move from PSII to PSI acceptor side) exhibited an enhanced reduction (70%) on exposure to heavy metal stress in rice seedlings. However, when treated with other three abiotic stresses (NaCl, PEG, and high light stress), the reduction was only 10–30% as compared with the control plants. Even though, high light, NaCl, and PEG stress treatments in rice leaves resulted in an increase of the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms (V_K/V_J) and it was more pronounced upon exposure to NaCl stress, *i.e.*, 41% increase as compared with control leaves. Moreover, the other parameters like F_0 (minimal fluorescence) and $T_{f(max)}$ (time to reach maximal fluorescence) also did not change considerably in rice seedlings when treated with various abiotic stresses as compared with the control (Fig. 1, Table 1).

The quantum yield of electron transport (Φ_{E0}) decreased in rice seedlings when exposed to various abiotic stress treatments; the decrease of Φ_{E0} in rice on exposure to NaCl was the highest (72%) than that of other three stress treatments (35, 31, and 11% during high light, PEG, and heavy metal, respectively), as compared with the control

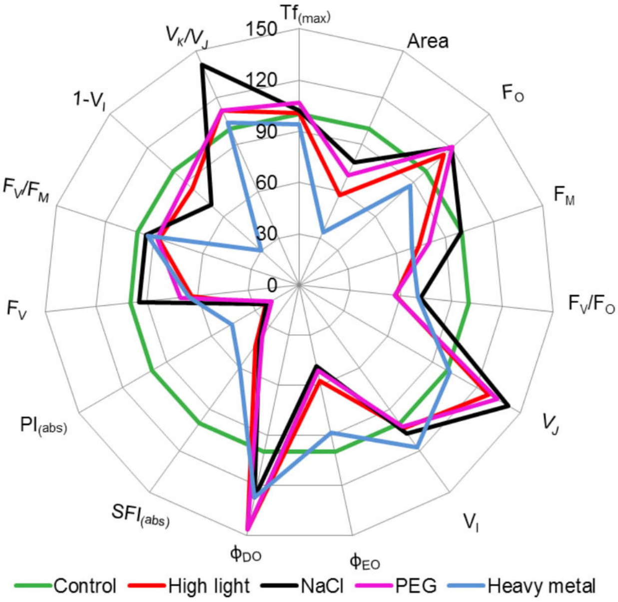

Fig. 1. Spider plot of selected Chl *a* fluorescence parameters characterizing PSII of rice seedlings when subjected to high light, NaCl, heavy metal, and PEG-induced osmotic stress. The data are shown as percentage of control. F_0 – minimal fluorescence, F_M – maximal fluorescence, Area – total complementary area between the fluorescence induction curve and F_M, F_V – variable fluorescence, F_V/F_M – maximum quantum efficiency of PSII under dark adaptation, F_V/F_0 – the ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules, $PI_{(abs)}$ – performance index, $SFI_{(abs)}$ – PSII structure-function-index, $T_{f(max)}$ – time to reach maximal fluorescence, V_I – the relative variable fluorescence at I-step, V_J – the relative variable fluorescence at J-step, V_K/V_J – the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms as an indicator of the PSII donor side limitation, $1 – V_I$ – the efficiency/probability with which a PSII trapped electron is transferred to PSI acceptor side, Φ_{D0} – maximum quantum yield of nonphotochemical deexcitation, Φ_{E0} – the electron transport quantum yield.

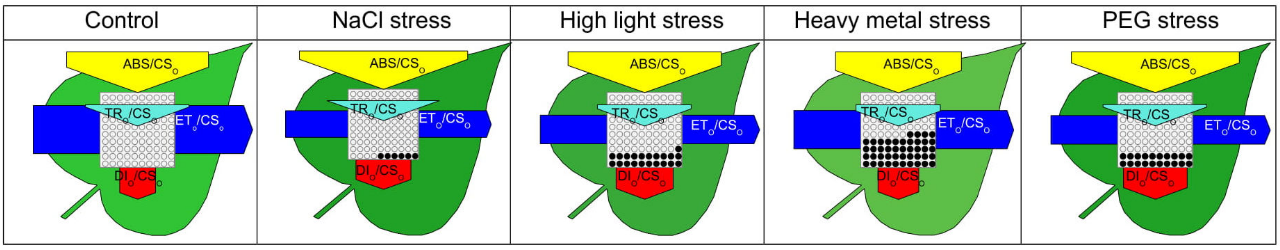

Fig. 2. Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in rice seedlings when subjected to high light, NaCl, heavy metal, and PEG-induced osmotic stress. The value of each parameter can be seen in relative changes in width of each *arrow*. Active reaction centers (RCs) are shown as *open circles* and inactive RCs are *closed circles*. ABS/CS$_0$ – absorption flux per cross section at t = 0, TR$_0$/CS$_0$ – trapped energy flux per cross section at t = 0, ET$_0$/CS$_0$ – electron transport flux per cross section at t = 0, DI$_0$/CS$_0$ – dissipated energy flux per cross section at t = 0.

seedlings. Maximum quantum yield of nonphotochemical deexcitation (Φ_{D0}) increased in stressed leaves, but this enhancement was highly pronounced during high-light exposure (125%). In the case of other three stress treatments, the increase of Φ_{D0} was very low. SFI$_{(abs)}$, 'structure-function-index' refers to the structural and functional PSII events leading to electron transport within photosynthesis; it was highly reduced in rice seedlings when they were exposed to different abiotic stresses. However, the decrease was found to be more significant in plants treated with PEG (92%) as compared to other treatments (40–56%) (Fig. 1, Table 1).

The phenomenological energy fluxes per cross section could be visualized using the energy pipeline leaf model (Fig. 2). An increase in energy absorbed per excited cross section (ABS/CS$_0$) was observed in rice seedlings under high light, PEG, and NaCl treatments (8, 19, and 27%, respectively). In contrast, it was showed that ABS/CS$_0$ was lower during heavy metal treatment (11%) as compared with the control. The electron transport flux per cross section (ET$_0$/CS$_0$) decreased and this decrease was to the extent of 20–39% in all stress treatments. The total dissipation to the cross section (DI$_0$/CS$_0$) increased in all treatments studied and it was more evident in plants treated with PEG (77%) as compared with the control. The percentage increase in DI$_0$/CS$_0$ in high light and NaCl-treated plants was about 60% and it was only 15% in heavy metal-stressed leaves as compared with those recorded for the control. Likewise, trapping flux to the cross section (TR$_0$/CS$_0$) was found to be enhanced upon NaCl treatment, but it was in a decreasing manner in the other stress treatments. The density of the active reaction centers (RC/CS$_0$) also decreased in plants exposed to different abiotic stresses than that recorded in control seedlings and it was about 21, 6, 19, and 43% in high light-, NaCl-, PEG-, and heavy metal-treated plants, respectively. RC/ABS, representing the fraction of active PSII reaction centers, decreased when rice seedlings were treated with different abiotic stresses and the decrease was up to 15–32% when exposed to high light, PEG, NaCl, and heavy metal stress as compared with control leaves (Fig. 2).

Discussion

Photosynthesis, the most important physiological process of plants, is highly susceptible to abiotic stresses and it can be studied to a great extent by measuring the PSII characteristics *via* analyzing the Chl *a* fluorescence parameters (Strasser *et al*. 2000). The effects of abiotic stress on target sites, such as components of photosynthetic electron transport, can be detected and analyzed through the JIP-test and its parameters (Stirbet and Govindjee 2011). With regard to the effect of high light, NaCl, heavy metal, and PEG stress on the photosynthetic electron transport and photosynthetic efficiency, the variation in Chl *a* fluorescence parameters may be due to the inhibition of electron transport or damage to the donor side or acceptor side of PSII.

Our results showed that a significant decrease occurs in PI on absorption basis in rice leaves after exposure to all four abiotic stresses (high light, NaCl, PEG, and heavy metal). It is one of the most powerful and comprehensive parameters, which is related to the plant vitality, and it was drastically reduced when rice seedlings were subjected to these different abiotic stresses due to inactive reaction centers and reduction in the electron transfer from Q_A^-. PI is the product of three independent characteristics: concentration of active reaction centers per Chl, a parameter related to primary photochemistry, and a parameter related to electron transport (Strasser *et al*. 2004, Stirbet *et al*. 2018). Many authors have reported that it is the best parameter to investigate the photosynthetic efficiency and the effects of abiotic stresses on PSII in different plants, *e.g.*, NaCl stress in wheat (Mehta *et al*. 2010) and barley (Kalaji *et al*. 2011a), heavy metals and nutrient deficiency in barley (Kalaji *et al*. 2018), drought stress in wheat, ryegrass (Živčák *et al*. 2008b, Dąbrowski *et al*. 2019), and maize leaves (Lepeduš *et al*. 2012), urban pollution (Swoczyna *et al*. 2010), and also to screen genotypes with better performance and grain production in *Lathyrus* under drought stress (Silvestre *et al*. 2014).

During high-light treatment, very low F_V/F_0 value and very high value of Φ_{D0} were recorded in rice seedlings. The reduction in F_V/F_0 indicates the decrease in the ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules (Strasser *et al*. 2000, 2010; Kalaji *et al*. 2018). Moreover, the increase in Φ_{D0}, maximum quantum yield of nonphotochemical deexcitation, suggested the enhancement in the dissipation of absorbed light as heat or fluorescence. It was shown that maximal fluorescence

Table 1. Chl *a* fluorescence parameters of *Oryza sativa* var. 'Mangalamahsuri' after exposure to different abiotic stress factors [high light (2 h), NaCl (100 mM), PEG (20%) and heavy metal (2 mM Cd)]. *Different letters* indicate statistically different means ± SE at $p<0.05$. $T_{f(max)}$ – time to reach maximal fluorescence, Area – total complementary area between the fluorescence induction curve and F_M, F_0 – minimal fluorescence, F_M – maximal fluorescence, F_V/F_0 – the ratio between the rate constants of photochemical and nonphotochemical deactivation of excited Chl molecules, V_J – the relative variable fluorescence at J-step, V_I – the relative variable fluorescence at I-step, $1 - V_I$ – the efficiency/probability with which a PSII trapped electron is transferred to PSI acceptor side, V_K/V_J – the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms as an indicator of the PSII donor side limitation, F_V – variable fluorescence, F_V/F_M – maximum quantum efficiency of PSII under dark adaptation, Φ_{E0} – the electron transport quantum yield, Φ_{D0} – maximum quantum yield of nonphotochemical deexcitation, $SFI_{(abs)}$ – PSII structure-function-index, $PI_{(abs)}$ – performance index.

Parameter	Control	High light	NaCl	PEG	Heavy metal
$T_{f(max)}$	176 ± 4^{ab}	177 ± 4^{ab}	180 ± 5^{a}	188 ± 5^{a}	166 ± 3^{b}
Area	$26{,}306 \pm 752^{a}$	$15{,}189 \pm 302^{d}$	$20{,}624 \pm 351^{b}$	$18{,}433 \pm 466^{c}$	$8{,}941 \pm 163^{e}$
F_0	398 ± 3^{b}	454 ± 14^{a}	478 ± 10^{a}	482 ± 10^{a}	347 ± 10^{c}
F_M	$2{,}068 \pm 60^{a}$	$1{,}519 \pm 34^{bc}$	$2{,}060 \pm 46^{a}$	$1{,}656 \pm 42^{b}$	$1{,}439 \pm 24^{c}$
F_V/F_0	2.89 ± 0.06^{a}	1.64 ± 0.05^{c}	2.06 ± 0.06^{b}	1.62 ± 0.04^{c}	2.02 ± 0.06^{b}
V_J	0.53 ± 0.01^{c}	0.68 ± 0.02^{b}	0.75 ± 0.02^{a}	0.71 ± 0.01^{b}	0.54 ± 0.01^{c}
V_I	0.80 ± 0.06^{b}	0.83 ± 0.06^{b}	0.86 ± 0.06^{ab}	0.82 ± 0.06^{b}	0.94 ± 0.06^{a}
$1 - V_I$	0.20 ± 0.01^{a}	0.17 ± 0.01^{b}	0.14 ± 0.01^{c}	0.18 ± 0.01^{b}	0.06 ± 0.00^{d}
V_K/V_J	0.31 ± 0.01^{c}	0.34 ± 0.01^{b}	0.43 ± 0.02^{a}	0.34 ± 0.02^{b}	0.31 ± 0.01^{c}
F_V	$1{,}670 \pm 43^{a}$	$1{,}065 \pm 28^{d}$	$1{,}582 \pm 34^{b}$	$1{,}174 \pm 13^{c}$	$1{,}092 \pm 22^{d}$
F_V/F_M	0.81 ± 0.04^{a}	0.71 ± 0.02^{b}	0.77 ± 0.04^{a}	0.71 ± 0.02^{b}	0.76 ± 0.04^{a}
Φ_{E0}	0.35 ± 0.01^{a}	0.20 ± 0.01^{c}	0.17 ± 0.00^{d}	0.18 ± 0.01^{cd}	0.31 ± 0.01^{b}
Φ_{D0}	0.26 ± 0.00^{c}	0.38 ± 0.00^{a}	0.33 ± 0.01^{b}	0.38 ± 0.01^{a}	0.33 ± 0.01^{b}
$SFI_{(abs)}$	1.02 ± 0.04^{a}	0.45 ± 0.01^{c}	0.41 ± 0.01^{cd}	0.38 ± 0.01^{d}	0.61 ± 0.01^{b}
$PI_{(abs)}$	7.47 ± 0.14^{a}	1.73 ± 0.05^{c}	1.67 ± 0.03^{c}	1.41 ± 0.03^{d}	3.44 ± 0.06^{b}

and the Area were tremendously decreased under heavy metal stress. The total complementary Area between the fluorescence induction curve and F_M is a tool to represent the pool size of reduced plastoquinone on the reducing side of PSII (Kalaji *et al.* 2017). The decrease in Area and F_M was due to the inhibition of electron transfer rates from reaction center to quinone pool that produces an excess of excitation energy which gets dissipated as heat. Our results were supported by the work of Żurek *et al.* (2014) who reported that the elevated content of various heavy metals in perennial grasses growing in heavy metal contaminated soils decreased the Area and F_M. Along with this, Mathur *et al.* (2016) reported that there was a gradual reduction in the Area in wheat plants when exposed to increasing concentrations of heavy metal; they suggested that this was due to blockage of electron transfer from the reaction center to quinone pool. Likewise, the decrease in F_M was observed (Żurek *et al.* 2014). The parameter $1 - V_I$ is interpreted as the efficiency/probability by which electrons move from PSII to PSI acceptor side, which was drastically retarded upon heavy metal treatment. The similar effect was observed by Rastogi *et al.* (2019), who suggested that the decrease of $1 - V_I$ was associated with the significant decrease of PSI activity due to metal treatment. Moreover, Küpper *et al.* (2019) discussed that the decline in $1 - V_I$ in leaves under heavy metal treatment related to the decrease in the efficiency or probability with which a PSII trapped electron is transferred to PSI. Therefore, we can conclude that these parameters can be considered as the sensitive parameters for evaluating the effect of heavy metals in rice seedlings. Also considering the drastic reduction in F_V/F_0 and increase in Φ_{D0} at high light, these parameters can be taken as parameters for evaluating the effect of high light stress in rice seedlings.

The maximum quantum yield of electron transport (Φ_{E0}) was strongly decreased under NaCl stress in rice seedlings as reported earlier in *Brassica napus* L. (Bacarin *et al.* 2011) and *Spirulina platensis* (Zhang *et al.* 2010). The increase in the ratio of variable fluorescence in time 0.3 ms to variable fluorescence in time 2 ms (V_K/V_J) upon exposure to NaCl stress in rice seedlings indicated that oxygen-evolving complex was affected and thus the electron transport from PSII donor side to PSII reaction center was inhibited as reported earlier by Yan *et al.* (2013) and Kalaji *et al.* (2016). Under PEG stress, the values of F_V/F_0 and $SFI_{(abs)}$ greatly decreased, while DI_0/CS_0 significantly increased in rice seedlings. Reduction in $SFI_{(abs)}$, an indicator of PSII structure and function, and an increase in dissipated energy as heat and fluorescence may be due to the accumulation of inactive PSII reaction centers and a lower quantum yield of PSII photochemistry in plants (Stirbet *et al.* 2018). Chl *a* fluorescence analysis revealed that the drought stress caused the impaired electron transfer to PSII reaction center due to the variations in energy absorption, trapping, electron transport, and dissipation per cross section, which results in reduction of photosynthetic efficiency of PSII (Stirbet *et al.* 2018, Khatri and Rathore 2019). According to Živčák *et al.* (2013) drought stress gradually decreased PSII electron transport and nonphotochemical quenching increased in wheat leaves which supports the roles of alternative electron sinks (either from PSII or PSI) and cyclic electron flow for photoprotection of PSII and PSI, which also generates ATP needed for countering the drought stress conditions. Moreover, the drought stress moderately decreased absorption and electron transport rate as well as the number of functional reaction centers in crop plants (Brestič and Živčák 2013). Therefore, considering the decline in Φ_{E0} under NaCl stress and significant decrease in F_V/F_0 and $SFI_{(abs)}$ as well as the increase in DI_0/CS_0 under PEG stress, these parameters are found to be more reliable in assessing NaCl and PEG stresses, respectively.

The data obtained from Chl *a* fluorescence analysis revealed that some fluorescence parameters can be selectively used for identifying and evaluating the effect of high light, NaCl, PEG, and heavy metal stress in rice seedlings. The results from the present investigation strongly indicate that these chosen Chl *a* fluorescence parameters could be used as a tool for the identification of a particular abiotic stress even in the earlier growth stages of rice seedlings. Similar findings were reported under cadmium and lead stress (Kalaji and Loboda 2007), salt stress (Kalaji *et al.* 2011a), low and high light stress (Kalaji *et al.* 2012), temperature stress (Kalaji *et al.* 2011b) in barley cultivars, and nutrient deficiency in tomato and maize (Kalaji *et al.* 2014). Moreover, Kalaji *et al.* (2014, 2016) selected some Chl *a* fluorescence parameters as a species-specific approach to identify/predict the nutrient deficiency in tomato and maize plants and according to Živčák *et al.* (2014), some selected parameters are considered as more reliable and more useful to assess nitrogen deficiency in wheat. Under drought stress, different Chl *a* fluorescence parameters such as F_V/F_M vary according to the severity of drought and it was found that these reduced drastically after prolonged drought stress (Živčák *et al.* 2008a).

Conclusively, we found that performance index is a common sensitive parameter to evaluate the effect of high light, heavy metal, NaCl, and PEG stress in rice seedlings. Certain Chl *a* fluorescence parameters were significant for a specific stress. F_V/F_0 was prominently decreasing and the maximum quantum yield of nonphotochemical deexcitation (Φ_{D0}) was prominently increasing upon exposure to high light stress. The maximum quantum yield of electron transport (Φ_{E0}) and the electron transport from PSII donor side to PSII reaction center (V_K/V_J) was highly reduced under NaCl stress in rice seedlings. Moreover, F_V/F_0, and PSII structure function index [$SFI_{(abs)}$] were prominently decreasing and the dissipation per cross section (DI_0/CS_0) was significantly enhanced under PEG stress. The pool size of reduced plastoquinone on the reducing side of PSII, the maximal fluorescence (F_M), and the probability by which electrons move from PSII to PSI acceptor side ($1 - V_I$) were significantly decreasing under heavy metal stress. High light, heavy metal, NaCl, and PEG stresses reduced the photochemical efficiency of PSII; the most significantly changed Chl *a* fluorescence parameter(s) can be selectively used for evaluating and characterizing the consequence of a particular abiotic stress on the photochemistry in rice seedlings.

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