

## Performance of ETR and Y(II) of two Contrasting Rice (*Oryza sativa* L.) Genotypes under Water Stress

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**ABSTRACT:** Two rice (*Oryza sativa* L.) genotypes Parijata and Prasad were imposed to water stress (WS) to evaluate the performance of electron transport rate (ETR) and effective PSII quantum yield (Y (II) ). The experiment was performed in pots. The WS was imposed before 10 days of flowering by withdrawing irrigation and maintaining the pots at 50% field capacity. Under WS at flowering stage, the value of ETR, Y(II), Maximum quantum yield of PSII photochemistry (Fv/Fm), membrane stability index (MSI), relative water content (RWC), malondialdehyde (MDA) and total chlorophyll content were decreased in both the genotypes. However, the reduction was more prominent in Prasad. Reduction in Fv/Fm, ETR and Y (II) indicated balanced energy management in tolerant genotype Parijata. With higher ETR and Y (II) the tolerant genotype is associated with improving photosynthetic rate ( $P_n$ ). The studies showed that the genotype Parijata is tolerant and Prasad is susceptible to WS in nature. The experiment signifies the suitability of ETR and Y (II) for phenotyping of genotypes for tolerance to WS.

**Keywords:** Electron transport rate, quantum yield, water stress, Fv/Fm, MDA.

### INTRODUCTION

Rice (*Oryza sativa* L.) is the principal and dominant foods crop that contributing to feed nearly 50 % of the total world's population (Adhikari *et al.*, 2019). Water is the most essential input for growth and development of rice. With the commencement of climate change, WS envisaged an increase in most of the rice growing areas and posing the primary constraints for rice production (Liu *et al.*, 2018; Danakumara *et al.*, 2021). It alters multiple physiological processes in which photosynthesis is the major physiological targets. Under WS, the photosynthetic machinery mainly photo-system (PS) II is damaged severely which leading to photoinhibition in rice plants (Sharma *et al.*, 2020; Yao *et al.*, 2018). The value of ETR, Y(II), Fv/Fm can accurately and rapidly evaluate the health status of PS II photochemistry under WS (Wang *et al.*, 2018). The value of Fv/Fm ranged from 0.75 to 0.85 and reduction in this value is a sign of photoinhibition (Parida *et al.*, 2021). It was observed that the value of MSI, RWC, total chlorophyll and MDA content is decreased under

WS conditions. However, the value is significantly increased in tolerant genotypes than susceptible genotypes (Panda *et al.*, 2021; Xu *et al.*, 2015).

The healthy PSII could facilitate genotypes to maintain minimum reduction in ETR and Y(II) (Pradhan *et al.*, 2019). In the present experiment, an attempt has been aimed towards the proper establishment of photosynthetic traits along with MSI, RWC, total chlorophyll and MDA to give proper fame between tolerant and susceptible genotypes under WS conditions. Thus, the objective of this experiment was to assess the proper phenotyping traits that could help in the identification of tolerant genotypes at the flowering stage with an aim towards further drought tolerance breeding programme (Piveta *et al.*, 2021; Swain *et al.*, 2017). Again, the findings suggest that the efficiency of PSII markedly increases in the tolerant genotypes under WS, which could facilitate the genotypes to maintain high photosynthetic performance (Xu *et al.*, 2020). Nonetheless, kinetic studies of light curves prepared through curve values of sensitive

parameters such as Y(II) and ETR at different light intensities could be facilitated for phenotyping on the basis of their tolerance level under WS conditions.

## MATERIALS AND METHODS

**Experimental site and plant growth.** The performance of two rice (*Oryza sativa* L.) genotypes i.e. Parijata and Prasad selected from the vegetative stage drought screening experiment conducted during the dry season-2018 were studied in this experiment. A pot experiment was conducted during dry season-2019 with six replications for each genotype at ICAR-National Rice Research Institute, Cuttack, India, under well-watered (WW) and WS conditions. The Pot was filled with 4 Kg mixture of dried dust farm soil with farm yard manure (3:1 ratio) in a completely randomized design. Thinning was done to maintain single plant per pot. Recommended doses of fertilizer (N, P &K) was maintained, pesticides were applied whenever necessary and standard agronomic practices were followed for the growth of healthy plants. WS was imposed before 10 days of flowering by withdrawing irrigation and maintaining at 50% field capacity. Another set was under WW condition for maintaining 100% field capacity. Sampling was done at flowering stage.

**Chlorophyll a fluorescence parameters.** The second leaves from the top of the plants were taken and incubated in dark condition for 40 minutes. The ChlF parameters were measured using an imaging fluorimeter instrument (Imaging PAM—MAXI version, Heinz Walz, Effeltrich, Germany). The different fluorescence parameters were analyzed with the imaging Win v2.46i software provided with the instrument. Fully expanded healthy second leaf from top of the plants were collected and incubated in dark for 40 minutes. The images were taken using an Imaging fluorimeter instrument (PAM—MAXI version, Heinz Walz, Effeltrich, Germany) and the different fluorescence parameters were analyzed using Imaging win software provided with the instrument, as explained by Pradhan *et al.*, (2019). Light curve was initiated with ascending order of light intensities of different photosynthetic active radiation (PAR) i.e. 0, 0, 1, 21, 56, 111, 186, 281, 336, 396, 461, 531, 611 and 701  $\mu\text{mol}/\text{m}^2/\text{s}$  with blue light pulse in 20 second interval to get ETR and Y (II).

**Total chlorophyll.** Total chlorophyll content of leaf tissue during flowering stage was determined according to the procedure of Arnon, 1949. Fifty milligrams of fresh leaf tissue was finely chopped and placed in 10 ml of 80% acetone. The samples were incubated at dark for 48 hours at 4°C. Absorbance was measured at 645 and 663 nm using spectrophotometer (UV-2600, SHIMADZU, Europe).

Total chlorophyll ( $\text{mg g}^{-1}$  fw) =  $20.2 (\text{OD } 645) + 8.02 (\text{OD } 663) \times V/W \times 1000$

The unit is expressed as  $\text{mg g}^{-1}$  fresh tissue weight.

Where, OD: Optical density

V: Final volume of solution (10 ml)

W: Weight of sample (50 mg)

### Membrane stability index (MSI)

The second leaves of the plants were collected, weighed and incubated at 30°C in water bath for 4 hours. Initial electrical conductivity (EC) reading by an EC meter was taken, then the samples were again incubated at 100°C for 15 minutes, and 2<sup>nd</sup> EC reading was taken. MSI was calculated following the procedure of Sairam, (1994).

$$\text{MSI (\%)} = 1 - \frac{(1 - (T_1/T_2))}{(1 - (C_1/C_2))} \times 100$$

Where:

T<sub>1</sub> and T<sub>2</sub>: 1<sup>st</sup> and 2<sup>nd</sup> EC reading of stressed plant's leaves

C<sub>1</sub> and C<sub>2</sub>: 1<sup>st</sup> and 2<sup>nd</sup> EC reading of control plant's leaves

**Relative Water Content (RWC).** Healthy, fully matured leaves were collected at mid day and cut into small pieces. RWC of fresh leaves was calculated according to method of Bhusan *et al.*, (2007).

RWC (%) = [(Fresh weight- Oven dry weight) / (turgid weight- Oven dry weight)]  $\times$  100

**Lipid peroxidation.** Malondialdehyde (MDA) is a decomposition product of lipid peroxidation, which was estimated according to the method of Yagi, 1998. Five hundred milligrams of freshly chopped leaf tissues were homogenized in 2 millilitre (ml) of 0.1% (W/V) trichloroacetic acid (TCA). At 4°C the homogenate was centrifuged at 10,000 rpm for 5 minutes, and collected supernatant was used for MDA estimation. One ml of the reaction mixture (0.5% of TBA + 4% of TCA) was added with 0.5 ml of supernatant. Mixture was boiled in a water bath for 1 hour and reaction was terminated at room temperature. Then samples were centrifuged at 10,000 rpm for 10 minutes and absorbance was taken at 520 nm and 600 nm using spectrophotometer (Genesys 200).

Amount of MDA =

$$\frac{\text{Absorbance at } 532 \text{ nm} - \text{Absorbance at } 600 \text{ nm}}{155} \times 1000$$

**Photosynthetic rate.** The photosynthetic rate ( $P_n$ ) was taken between 8.00 AM to 12 PM with the help of LI-6400 (LI-COR, Lincoln, Nebraska, USA) under bright sunny days. Second leaf of the plant was placed in the leaf chamber to take observations at a PFD of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , leaf temperature was 28°C, ambient CO<sub>2</sub> concentration was 410  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  air and vapour pressure deficit was 2.0 kPa.

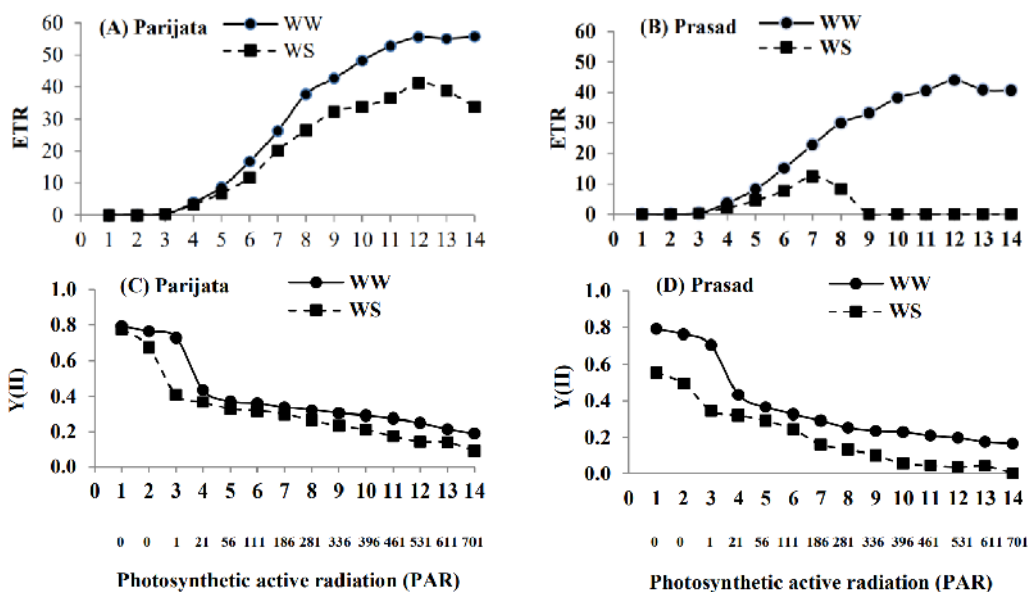
**Statistical Analysis.** All the data were taken from six replications. Pearson's correlation coefficient and p-value of the parameters were analyzed using Microsoft Excel. The statistical data analysis was done using Crop

Stat 7.2 software (IRRI, 2009) and XLSTAT-2014 statistical software. ANOVA was carried out at 5% level of significance for treatments and genotypes.

## RESULTS AND DISCUSSION

**Light response curve and variation in different chlorophyll fluorescence parameters.** The range of different PAR was used to measure the ETR and Y(II) for better characterization of PSII activity. The value of ETR of the studied rice genotypes was gradually increased with increasing light intensities (Fig. 1A, 1B). The reduction value of ETR at different PAR was clearly discriminating between tolerant and susceptible genotypes under WS conditions. The performance of ETR at various light intensities was better in the genotype Parijata while, Prasad could not perform better and the value was zero at 336  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Higher ETR at different PAR could have better photochemical efficiency with less damage in photosystem (PS) II and considered as the tolerant genotype (Wang *et al.*, 2016). The sharp reduction of ETR was more pronounced in Prasad signifying the destruction of PSII at higher light intensities (Joshi *et al.*, 2020). The value of Y (II) was decreased with increasing light intensity (Fig. 1C, 1D). Maintaining

higher Y(II) value at higher PAR signifies more reaction centre hence better photochemistry in PSII (Baker, 2008). It was noticed that Parijata maintained higher Y(II) value throughout the different light intensities whereas Y(II) was markedly reduced in Prasad under WS. Maxwell and Johnson, (2000) and Piveta *et al.*, (2021) stated that the genotypes continue with higher Y(II) and ETR value absorb more quanta and convert it into more chemical energy, and considered as tolerant genotype under WS conditions. Significant ( $p < 0.05$ ) reduction of Fv/Fm value was noticed in the studied genotypes under WS over WW condition (Fig. 2A). Under WW, the mean value of Fv/Fm is 0.791 however; under WS the mean value was significantly reduced to 0.664. The genotypes Parijata (0.774) was able to maintained higher value of Fv/Fm while, sharp reduction was noticed in Prasad (0.555). The reduction in Fv/Fm might be due to photo-inhibition and the poor performance of PS II activity. Maintenance of higher value of Fv/Fm signified better photochemistry and could be considered as tolerant genotypes under WS. The observed changes under WS are in agreement with the findings of Alharbi *et al.*, (2021).



**Fig. 1.** Variations in electron transport rate (ETR) (A & B) and effective PSII quantum yield (Y (II)) (C & D) of two rice genotypes i.e. Parijata and Prasad with changing light intensities under well water (WW) and water stress (WS) conditions. Values are expressed as means  $\pm$ SE (n = 6).

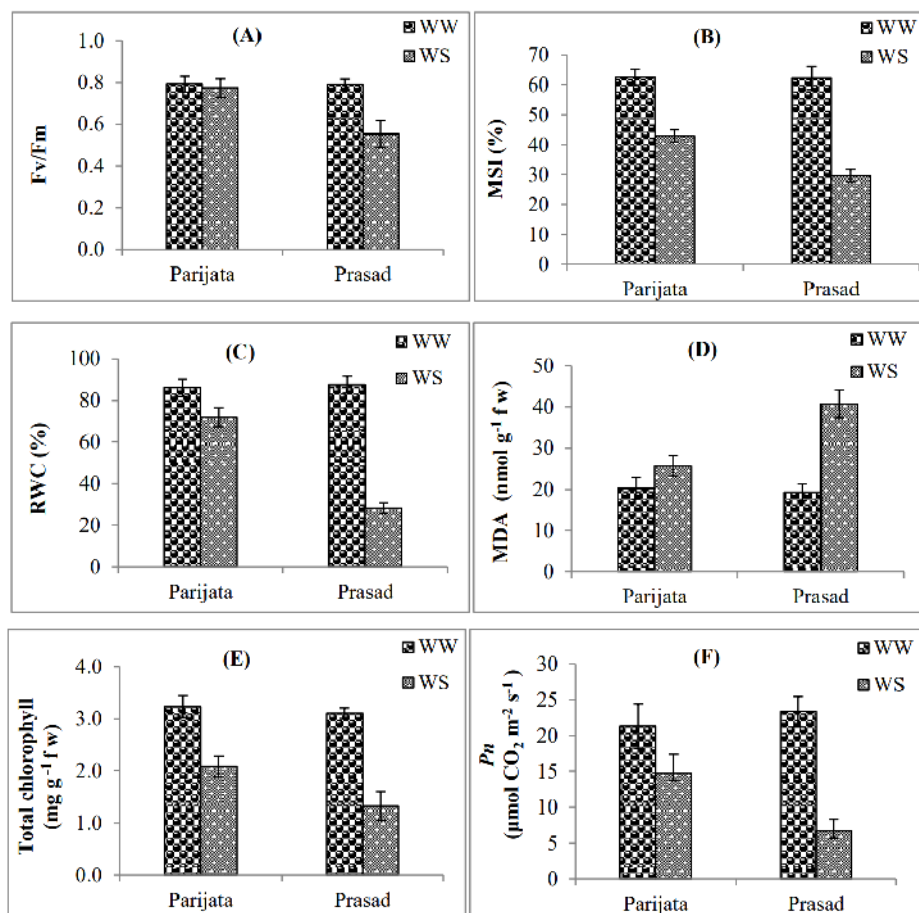
The value of MSI was significant ( $p < 0.05$ ) reduced under WS over WW condition in the studied genotypes (Fig. 2B). The mean value of MSI was 62.33 % under WW and 36.2 % under WS condition. The genotypes Parijata (42.94%) was recorded with the highest value of MSI whereas, Prasad (29.62%) possessed lower MSI under WS. The MSI value was sharply reduced in Prasad as compared with Parijata. The reduction in MSI

suggests that WS damaged the membrane of the cell and leakage of electrolyte which disturbs metabolic processes, as reported earlier (Bhattacharjee and Dey, 2018; Mishra *et al.*, 2018). Significant ( $p < 0.05$ ) reduction of RWC was also noticed under WS in both the genotypes (Fig. 2C). The mean value of RWC was 86.74% under WW and 50.11% under WS conditions. The genotypes Parijata (71.93%) was recorded highest

value whereas, Prasad (28.28%), possessed lower RWC value under WS. The RWC value was sharply reduced in susceptible genotypes as compared with the tolerant genotypes. Maintaining the higher value of RWC signifies better protoplast hydration (Dash *et al.*, 2017; Yang *et al.*, 2019).

Panda *et al.*, (2020) stated that lipid peroxidation is significantly associated with the production of MDA. The level of MDA was prominently increased under WS (Panda, 2007) (Fig. 2D). The accumulations of MDA in the cell ultimately increase the electrolyte leakage (Maryam *et al.*, 2012). In the study, MDA content was positively associated with WS and maximum accumulation of MDA was noticed in Prasad (40.68 nmol g<sup>-1</sup> f w) might be due to higher production of reactive oxygen species. The genotype Parijata (25.66 nmol g<sup>-1</sup> f w) observed minimum increment of MDA reflects less oxidative damage and higher MSI, thus improves the adaptive mechanism under WS (Khaleghi *et al.*, 2019). Lower production of MDA along higher Fv/Fm, RWC and MSI were well

associated with stress tolerance mechanism. It is also noted that total chlorophyll content was significantly ( $p < 0.05$ ) reduced in both the genotypes under WS compared to WW (Fig. 2E). The mean value of total chlorophyll content was 3.17 mg g<sup>-1</sup> fw under WW and 1.70 mg g<sup>-1</sup>fw under WS condition. The genotype Parijata (2.08 mg g<sup>-1</sup>fw) was able to uphold higher total chlorophyll content while, the value was sharply reduced in Prasad (1.33 mg g<sup>-1</sup>fw) under WS. The  $P_n$  was significantly reduced in both the genotypes and the genotype Parijata (14.75  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was maintained highest value whereas, Prasad (6.69  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), possessed lower value under WS (Fig. 2F). The reduction of chlorophyll and  $P_n$  is due to photo-oxidation of chlorophyll, chloroplast ultrastructure degradation, and our findings corroborate with the findings of Piveta *et al.*, (2021). In our study, the genotype Parijata was well associated with the tolerance mechanism and maintained higher ETR, Y(II), Fv/Fm, MSI, RWC, total chlorophyll content and  $P_n$  under WS.



**Fig. 2.** Variations in (A) maximum quantum yield of PSII photochemistry (Fv/Fm), (B) membrane stability index (MSI) (%), (C) relative water content (RWC) (%), (D) malondialdehyde (MDA) (nmol g<sup>-1</sup> f w) and (E) total chlorophyll content (mg g<sup>-1</sup> f w) (F) photosynthetic rate ( $P_n$ ) of two rice genotypes i.e. Parijata and Prasad under well water (WW) and water stress (WS) conditions. Values are expressed as means  $\pm$ SE (n = 6).

**Correlation among different traits.** The correlation matrix among different traits under WS (Table 1) illustrated that, Fv/Fm had highly positive significant association with MSI ( $p < 0.01$ ), RWC ( $p < 0.01$ ), total chlorophyll content ( $p < 0.01$ ), photosynthetic rate ( $p < 0.01$ ), and negative correlation with MDA ( $p < 0.01$ ). MSI showed highly positive significant relationship with RWC ( $p < 0.01$ ), chlorophyll content ( $p < 0.01$ ),

photosynthetic rate ( $p < 0.01$ ), and negative correlation with MDA ( $p < 0.01$ ). RWC was positively and significantly associated with chlorophyll content ( $p < 0.01$ ), photosynthetic rate ( $p < 0.01$ ), and negative correlation with MDA ( $p < 0.01$ ). MDA showed negative significant correlation with chlorophyll content ( $p < 0.01$ ) and photosynthetic rate ( $p < 0.01$ ) under WS conditions.

**Table 1: Correlation matrix of maximum quantum yield of PSII photochemistry (Fv/Fm), membrane stability index (MSI), relative water content (RWC), malondialdehyde (MDA), total chlorophyll content and photosynthetic rate ( $P_n$ ) of two rice genotypes i.e. Parijata and Prasad under water stress (WS) conditions.**

Parameters	Fv/Fm	MSI	RWC	MDA
MSI	0.838			
RWC	0.886	0.977		
MDA	-0.665	-0.948	-0.921	
Chlorophyll	0.577	0.726	0.756	-0.730
$P_n$	0.920	0.886	0.930	-0.768

Significance level  $p < 0.01$

## CONCLUSION

An attempt has been inclined to the recognition of suitable traits that can accurately distinguish between tolerant and susceptible genotypes under WS. The genotypes Parijata showed more tolerance to WS. Targeting ETR and Y (II) at different light intensities was found to provide significant phenotypic information and sensitivity levels of genotypes under WS. With higher ETR and Y (II) the tolerant genotype is associated with improving photosynthetic rate ( $P_n$ ). Strong correlations between Fv/Fm, MSI, RWC, MDA and total chlorophyll suggested that these parameters should be studied in the association while evaluating WS tolerance.

## FUTURE SCOPE

High throughput phenotyping techniques of the tolerant genotype are required to link plant photosynthetic traits to phenomes and its application to unravel the novel traits contributing to stress tolerance.

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**Conflict of interest.** The authors declare that they have no conflict of interest.

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