Original Research Article

Assessment of heat-tolerance potential in QTL introgressed lines of hybrid rice restorer, KMR-3R through PS-II efficiency

.
ABSTRACT

Aims: To assess heat-induced PSII damage and efficiency in eight promising backcross introgression lines (BC_2F_6) of KMR-3R/N22 possessing qHTSF1.1 and qHTSF4.1 during *Kharif* 2018.

Study design: Randomized Complete Block Design (RCBD) with three replications.
Place and Duration of Study: ICAR-Indian Institute of Rice Research, Hyderabad India during wet/rainy (*Kharif*) season 2018.

Methodology: Eight ILs (BC_2F_6) and parents were evaluated for heat tolerance. The high-temperature stress was imposed by enclosing the crop with a poly cover tent (Polyhouse) just before the anthesis stage. The fluorescence parameters *viz.,* maximum efficiency of PSII photochemistry (Fv/Fm), Electron transport rate (ETR), effective PSII quantum yield (ΦPSII), coefficient of photochemical quenching (qP) and coefficient of non-photochemical quenching (qN) were measured under ambient and high-temperature stress.

Results: The heat-tolerance potential of ILs was assessed in terms of PSII activity. The results indicated that significant differences were observed between treatments (T), genotypes (G) and the interaction between T × G. The physiological basis of introgressed QTLs controls the spikelet fertility by maintaining the productive and adaptive strategies in heat-tolerant QTL introgressed lines with stable photosynthetic apparatus (PSII) under high-temperature stress.

Conclusion: The Fv/Fm ratio denotes the maximum quantum yield of PSII. The heat-tolerant QTL introgressed lines exhibited stable photosynthetic apparatus (PSII) and noted better performance under high-temperature stress. They may be used as donors for fluorescence traits in breeding rice for high-temperature tolerance.

Keywords: Hybrid rice, KMR-3R, High-temperature stress, Chlorophyll fluorescence

1. INTRODUCTION

Rice (*Oryza sativa* L) is a staple cereal for more than half of the global population and provides 35-80 % of total calorie uptake [1]. It was cultivated in a wide range of agro-climatic zones across the globe. In the context of climate change, the agriculture segment witnesses the impacts of high-temperature stress on crop productivity. The episodes of high-temperature and altered precipitation levels affect the global food security [2]. An increase

of 1°C of global mean temperature can reduce the yields of maize (7.4%), wheat (6%), rice (3.2%) and soybean (3.1%) which affords two-thirds of human caloric intake globally [3]. The global mean temperature was expected to rise by 2.5°C-5.8°C by the end of the present century, and the frequency of high-temperature episodes was anticipated more in the future [2]. In order to sustain global rice production and to meet the estimated demand of the growing population, breeding rice for high-temperature stress is remains a major priority. With increasing population and climate change, hybrid rice technology could be a great opportunity to increase food grain production with its yield heterosis and sustainability. Hybrid rice technology has proven its identity by contributing a major share in global rice production. In India, hybrid rice occupies an area of 3 million hectares and extends 3–4 million tons of rice production [4]. Rice hybrids have reliably noted a yield advantage of 10-13% over conventional varieties though capitulated to various biotic and abiotic stresses [5,6,7]. Madan [8] observed that the yield advantage of hybrids was affected in terms of reduction in seed setting rate under high-temperatures (38°C) when compared to normal temperatures (29°C - 35°C). Similarly, Zhou [9] and Hu [10] also reported the susceptibility of hybrid rice towards high-temperature stress. Usually, in India, rice hybrid seed production was followed in dry (summer) season. The high-temperature at the anthesis stage affects the pollen viability [11, 12] anther dehiscence [13,14], pollen tube elongation and stigma receptivity which eventually results in low spikelet fertility rate and reduced grain yield [15]. In order to develop heat tolerant rice hybrids, thus both the parental lines should possess high level of temperature tolerance [16]. The heat tolerance in the three-line hybrid rice system significantly associates with their parental lines [17, 18]. Moreover, the association between rice hybrids and their parental lines with regard to heat tolerance is still to be understood further because of its complexity of involving various biochemical and physiological pathways [16]. Thus, to sustain the heterosis of rice hybrids under high-temperature stress, the emphasis should be given to the improvement of heat tolerant hybrid rice parental lines. To achieve this, the restore line KMR-3R was introgressed with qHTSF1.1 and qHTSF4.1 for heat tolerance through the Marker-Assisted Backcross Breeding (MABB) approach. In this study, the heat tolerant QTL introgressed ILs and their parental lines were evaluated for chlorophyll fluorescence which is used to evaluate the photosynthetic performance of plants under abiotic stress conditions like high-temperature.

2. MATERIAL AND METHODS

2.1 Plant material

Eight ILs (BC_2F_6) derived from the KMR-3R/Nagina22 were used in this study. KMR-3R is a promising restorer line deployed in the development of a public bred popular hybrid, KRH-2. Nagina22 (N22) is a potential genetic resource for heat tolerance and is widely used in heat-tolerance studies [19, 20, 21].

2.2 Development of ILs from KMR-3R/Nagina22

The two heat tolerant conferring QTLs- qHTSF1.1 and qHTSF4.1 from N22 were introgressed into KMR-3R through the MABB approach. Two successive backcrosses and pedigree method was followed for further advancement of generations up to BC_2F_6. The SSR markers associated with QTLs - RM431, RM11943 and RM12091 for qHTSF1.1 on chromosome 1 and RM5757 for qHTSF4.1 on chromosome 4 [19] were employed for genotypic confirmation of QTL positive plants. As the KMR-3R is a restorer, the derived ILs were also screened for fertility restoration with DDRM-Rf3-10 [22] and RMS SF 21-5 [23] for the *Rf3* gene; RM6100 [24] and RMS PRR 9-1 [23] for *Rf*4 gene. The markers used for foreground selection and QTL harbouring status of ILs were represented in Table 1.

2.3 High-temperature treatment

The present study was carried out at the Indian Institute of Rice Research farm, Hyderabad, India (17.53° 19'N latitude and 78.27° 29' E longitude, 542.7 MSL, with a mean temperature

of 31.2°C and mean annual precipitation of 988.3 mm) during wet (*Kharif*/rainy) season 2018. Rice seeds were sown as nursery and 25 days old seedlings were manually uprooted and transplanted at the main field in a square meter area per genotype at the rate of one seedling per hill. A spacing of 10 × 20 cm was adopted uniformly. Gap filling was done wherever seedlings failed to establish within 5-7 days after transplantation. The experiments were replicated thrice in a randomized complete block design (RCBD) with same sets of genotypes in two experiments under normal and high-temperature stress. Standard agronomic practices and integrated pest management was followed throughout the experiment. The high-temperature stress was imposed by enclosing the crop with a poly cover tent (Polyhouse) just before the anthesis stage [25, 26, 27]. It was assembled with a transparent polythene sheet (>92% transmittance) of 1 mm thickness (~19 gauge) and supported over a metal framework. An increase in the temperature (>5°C) over the ambient conditions was noticed inside the polyhouse. A standard thermo-hygrometer was employed to record the minimum and maximum temperature and relative humidity (RH) inside the polyhouse throughout the experiment period (Table 2).

2.4 Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured at the reproductive stage (1 week after anthesis) using a portable fluorometer (PAM-210, Walz, Effeltrich, Germany). The fluorescence parameters *viz.,* maximum efficiency of PSII photochemistry (Fv/Fm), Electron transport rate (ETR), effective PSII quantum yield (ΦPSII), coefficient of photochemical quenching (qP) and coefficient of non-photochemical quenching (qN) were measured in dark adapted (30-45 min.) leaves collected from genotypes under ambient and high-temperature stress.

2.5 Statistical analysis

The data collected on chlorophyll fluorescence was analysed statistically using two-way analysis of variance (ANOVA) [28] in software *Statistix 8.1* [29]. The derived data from the ANOVA, represented with standard errors of mean (SE) and the honest significant difference (HSD) ($P = 0.05$) between treatments and genotypes.

3. RESULTS AND DISCUSSION

3.1 Introgression of QTLs from N22 into KMR-3R

The QTLs governing spikelet fertility under high-temperature stress *i.e.,* qHTSF1.1 (Chromosome 1) and qHTSF4.1 (Chromosome 4) reported in Nagina22 [19, 20, 21] were targeted to introgress into KMR-3R using MABB. Among the eight ILs, IL2 and IL3 were introgressed with qHTSF1.1; IL4 and IL5 were with qHTSF4.1; IL6, IL7 and IL8 were with both the qHTSF1.1 and qHTSF4.1 QTLs. IL1 has no introgressed QTLs and considered as negative control.

3.2 Chlorophyll fluorescence

To understand the effect of high-temperature on chlorophyll fluorescence traits, the heat tolerance governing QTL introgressed lines were evaluated in this study. Measuring the chlorophyll fluorescence in abiotic stress tolerance studies is a quick and non-invasive *invivo* method. It helps to evaluate the genotypes for their physiological capacity of stress tolerance [30, 31]. Among the fluorescence parameters, the maximum efficiency of PSII photochemistry (Fv/Fm) was used as a stress tolerance indicator for various abiotic stresses [31]. The data on chlorophyll fluorescence under ambient and high-temperature stress was represented in Figure 1.

3.3 Maximum quantum yield of PSII (Fv/Fm)

The maximum quantum yield of PSII (Fv/Fm) recorded a significant reduction under high-temperature stress. Significant differences were observed between treatments (T), genotypes (G) and the interaction between T × G (Table 3). The mean of Fv/Fm was reduced from 0.782 in control to 0.722 under high-temperature (7.7% reduction over stress). All the QTL positive ILs noted with higher Fv/Fm value (IL8 is on par) than N22 (0.736) under high-temperature except QTL negative line, IL1 (0.588). This is due to the effect of introgressed QTLs, which not only controls spikelet fertility under high-temperature, also the photosynthetic traits. The similar results were identified in the introgressed lines of improved white ponni (IWP) with qHTSF1.1 and qHTSF4.1 [32]. The ratio of variable fluorescence to maximum fluorescence (Fv/Fm), and the base fluorescence (F0) correlate with heat tolerance [33]. The percent reduction in Fv/Fm was also higher in IL1 (25%) and the remaining ILs were noted with less percent reduction than N22. The reduction of Fv/Fm under high-temperature stress was reported previously [32, 34]. Han [35] also observed that Fv/Fm values decreases upon gradual increase of temperature. At 26°C it was 0.836; 0.817 at 35 °C; 0.782 at 40 °C and 0.62 at 45 °C. It indicates the inhibition of PSII activity under high-temperature stress. Sailaja [36] also observed that the marginal reduction of Fv/Fm values in the all the genotypes studied at both vegetative and reproductive stage under high-temperature stress. Poli [25] in their study on rice reported no reduction in Fv/Fm in NH219 whereas it reduced in N22 (5.8) and IR64 (12.02) under high-temperature stress. It indicates that there was no damage to the PSII complex hence it was considered as heat tolerant. Similarly, studies on sorghum also reported the decreased activity of PSII photochemistry indicated with lower Fv/Fm values at high-temperature stress [37].

3.4 Electron transport rate (ETR)

The mean electron transport rate (ETR) for all the genotypes was decreased by 16% under high-temperature stress. Among the genotypes, QTL positive ILs and N22 were observed with higher ETR than recurrent parent, KMR-3R (14.55) and QTL negative IL1 (21.65) under high-temperature stress. The IL8 (27.3) and IL4 (27.08) were observed with higher ETR under high-temperature stress. The electron transport machinery in photosynthetic membranes is identified to be very sensitive to the high-temperature stress. Previously, Ma [38] and Pshybytko [39] also reported the reduction in ETR under high-temperature studies. The reduction in ETR by high-temperature stress and attributed the reduction to an impediment of electron flow from QA- to acceptor pool. Reduction and collapse of linear photosynthetic ETR due to high-temperature stress primarily results from enhanced thylakoid membrane leakiness and damage at PSII [40]. Sailaja [36] observed the mean reduction in ETR of all the genotypes studied by 16.7% and 19.0% at vegetative and reproductive stages respectively.

3.5 Effective PSII quantum yield (ΦPSII)

The mean effective PSII quantum yield (ΦPSII) was reduced significantly under high-temperature stress by 18.4% over the control. All the QTL positive ILs were observed with higher values after the N22 (0.335) under heat-stress. Conversely, KMR-3R (0.156) and QTL negative IL1 (0.229) were observed with lower values. Moreover, IL6 (10%) and IL8 (11%) were noted with less percent reduction than N22 over control. Previously, Tahir [41] reported the reduction in ΦPSII under high-temperature stress in wheat. Sailaja [36] observed a reduction in PSII quantum yield by 21% at reproductive stage.

3.6 Co-efficient of photochemical quenching (qP)

Under high-temperature stress, there was a reduction in mean co-efficient of photochemical quenching (qP) in all the genotypes. The mean qP under control was 0.431 which reduced to

0.337 under high-temperature stress (21.8% reduction over stress). N22 was observed with maximum qP for both the experimental conditions (0.483 in control and 0.460 in high-temperature stress). Among the ILs, IL7 (0.353) was observed with highest qP value next to the N22. QTL negative IL1 was observed with the qP value of 0.340. Sailaja [36] observed a reduction in qP under high-temperature stress by 12% at vegetative stage and 15% during the reproductive stage.

3.7 Co-efficient of non-photochemical quenching (qN)

The mean Co-efficient of non-photochemical quenching (qN) of all the genotypes was reduced under high-temperature stress (0.674) when compared to control (0.744). Among the genotypes, N22 (0.801) and IL4 (0.705) were observed with high qN values under high-temperature stress. IL1 (0.652) was observed with lower qN value among the ILs in stress environment. Sailaja [36] observed an increase in qN under high-temperature stress in the genotypes studied.

4. CONCLUSION

The Fv/Fm ratio denotes the maximum quantum yield of PSII. The heat tolerant QTL introgressed lines exhibited stable photosynthetic apparatus (PSII) and noted better performance under high-temperature stress. They may be used as donors for fluorescence traits in breeding rice for high-temperature tolerance. Among the eight introgressed lines, IL7 and IL8 were identified as heat-tolerant with reference to the chlorophyll fluorescence traits.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R et al. Climate Change Affecting Rice Production: The Physiological and Agronomic Basis for Possible Adaptation Strategies. 2009; 101:59-122.
2. IPCC. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds R. K. Pachauri and L. A. Meyer 2014. (Geneva: IPCC).

3. Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, et al. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences. 2017; 114(35): 9326-9331.

4. Senguttuvel P et al. Rice Hybrids Released in India. Compendium No. 103/2019. ICAR-IIRR, Rajendranagar, Hyderabad-500 030. India. 2019; 127.

5. Virmani SS, Mao CX, and Hardy B. (Eds.). Hybrid rice for food security, poverty alleviation, and environmental protection. 2003. Int. Rice Res. Inst.

6. Serraj R, Kumar A, McNally KL, Slamet-Loedin I, Bruskiewich R, Mauleon R et al. Improvement of drought resistance in rice. In Advances in agronomy. 2009; 103:41-99. Academic Press.

7. Villa JE, Henry A, Xie F, Serraj R. Hybrid rice performance in environments of increasing drought severity. Field Crops Research. 2012; 125:14-24.

8. Madan P, Jagadish SVK, Craufurd PQ, Fitzgerald M, Lafarge T, Wheeler TR. Effect of elevated CO_2 and high-temperature on seed-set and grain quality of rice. J Exp Bot. 2012; 63:3843–3852.

9. Zhou YW, Gong HB, Li C, Hu CM, Lin TZ, Sheng SL. Influence of thermal damage on seed-setting rate of 67 indica hybrid rice combinations. Acta Agric Jiangxi, 2009; 21: 23–26. (in Chinese with English abstract).

10. Hu SB, Zhang YP, Zhu DF, Lin XQ, Xiang J. Evaluation of heat resistance in hybrid rice. Chin J Rice Sci, 2012; 26:751–756. (in Chinese with English abstract).

11. Song ZP, Lu BR, Chen KJ. A study of pollen viability and longevity in *Oryza rufipogon*, *O. sativa* and their hybrids. Int Rice Res Notes, 2001; 26:31–32.

12. Wassmann R, Dobermann A. Climate change adaptation through rice production in regions with high poverty levels. J Semi-Arid Tropical Agric Res, 2007; 4(1): 1–24.

13. Matsui T, OMASA K, Horie T. High-temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). Plant Prod Sci. 2000; 3:430–434.

14. Matsui T, Kobayasi K, Kagata H, Horie T. Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot and humid condition. Plant Prod Sci. 2005; 8:109–114.

15. Satake T and Yoshida S. High-temperature-induced sterility in indica rices at flowering. Jpn J Crop Sci, 1978; 47:6-17.

16. Guan-fu F, Cai-xia Z, Yong-jie Y, Jie X, Xue-qin Y, Xiu-fu, Z et al. Male parent plays more important role in heat tolerance in three-line hybrid rice. Rice Science, 2015; 22(3): 116-122.

17. Kuang HC, Wen SS, Liu GM. Studies on the heat tolerance of Luhui 17 and its cross Iiyou 7 at head sprouting. South west China J Agric Sci. 2002; 1:106–108. (in Chinese).

18. Gong HB, Zhou YW, Li C, Hu CM, Sheng SL, Diu LP et al. Effect of heat stress on the seed-setting rate of indica hybrid rice combinations widely planted in China. Jiangsu Agric Sci. 2008; 2: 23–25. (in Chinese).

19. Ye C, Argayoso MA, Redona ED, Sierra SN, Laza MA, Dilla, CJ et al. Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. Plant Breeding. 2012; 13(1): 33-41.

20. Ye C, Tenorio FA, Argayoso MA, Laza MA, Koh HJ, Redona ED et al. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. BMC Genetics. 2015a. 16; 41.

21. Ye C, Tenorio FA, Redona ED. Fine-mapping and validating qHTSF4.1 to increase spikelet fertility under heat stress at flowering in rice. Theor Appl Genet. 2015b; 128(8):1507–1517.

22. Balaji Suresh P, Srikanth B, Hemanth Kishore V, Subhakara Rao I, Vemireddy L, Dharika N, et al. Fine mapping of Rf3 and Rf4 fertility restorer loci of WA-CMS of rice (Oryza sativa L.) and validation of the developed marker system for identification of restorer line. Euphytica. 2012; 187(3):421-435.

23. Pranathi K, Viraktamath BC, Neeraja CN, Balachandran SM, Rao PK, Revathi P et al. Development and validation of candidate gene-specific markers for the major fertility restorer genes, Rf4 and Rf3 in rice. Molecular breeding. 2016; 36(10):145.

24. Singh AK et al. Application of molecular markers in rice breeding: progress at IARI. In: Advances in marker assisted selection workshop. Trainee's manual, Handouts and references. 2005.

25. Poli Y, Basava RK, Panigrahy M, Vinukonda VP, Dokula NR, Voleti SR et al. Characterization of a Nagina22 rice mutant for heat tolerance and mapping of yield traits. Rice. 2013; 6(1): 36.

26. Prasanth VV, Basava KR, Babu MS, VGN VT, Devi SR, Mangrauthia SK et al. Field level evaluation of rice introgression lines for heat tolerance and validation of markers linked to spikelet fertility. Physiology and molecular biology of plants. 2016; 22(2): 179-192.

27. Senguttuvel P, Jaldhani V, Raju NS, Balakrishnan D, Beulah P, Bhadana VP et al. Breeding rice for heat tolerance and climate change scenario; possibilities and way forward. A review. Archives of Agronomy and Soil Science. 2020:1-18.

28. Gomez KA and Gomez AA. Statistical procedures for agricultural research (2nd ed.). New York: Wiley. 2010.

29. Statistix 8.1 User's Manual. Analytical Software, Tallahassee. 2003.

30. Maxwell K and Johnson GN. Chlorophyll fluorescence—a practical guide. Journal of experimental botany. 2000; 51(345):659-668.

31. Baker NR and Rosenqvist E. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal of experimental botany, 2004; 55(403):1607-1621.

32. Vivitha P, Raveendran M, Vijayalakshmi C and Vijayalakshmi D. Genetic dissection of high-temperature stress tolerance using photosynthesis parameters in QTL introgressed lines of rice cv. Improved White Ponni. Indian Journal of Plant Physiology, 2018; 23(4): 741-747.

33. Yamada M, Hidaka T, and Fukamachi H. Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. Scientia Horticulturae. 1996; 67(1-2):39-48.

34. Kumar AN, Vijayalakshmi C and Vijayalakshmi D. Chlorophyll and chlorophyll fluorescence as influenced by combined heat and drought stress in rice. Biosciences, 2014; 1461.

35. Han F, Chen H, Li XJ, Yang MF, Liu GS and Shen SH. A comparative proteomic analysis of rice seedlings under various high-temperature stresses. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2009; 1794(11): 1625-1634.

36. Sailaja B, Subrahmanyam D, Neelamraju S, Vishnukiran T, Rao YV, Vijayalakshmi, P et al. Integrated physiological, biochemical, and molecular analysis identifies important traits and mechanisms associated with differential response of rice genotypes to elevated temperature. Frontiers in plant science. 2015; 6:1044.

37. Mohammed AR and Tarpley L. Effects of high night temperature and spikelet position on yield-related parameters of rice (Oryza sativa L.) plants. European journal of agronomy. 2010; 33(2): 117-123.

38. Ma W, Wei L and Wang Q. The response of electron transport mediated by active NADPH dehydrogenase complexes to heat stress in the cyanobacterium Synechocystis 6803. Science in China Series C: Life Sciences. 2008; 51(12):1082-1087.

39. Pshybytko NL, Kruk J, Kabashnikova LF and Strzalka K. Function of plastoquinone in heat stress reactions of plants. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2008; 1777(11):1393-1399.

40. Schrader SM, Wise RR, Wacholtz WF, Ort DR and Sharkey TD. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. Plant, Cell & Environment. 2004; 27(6):725-735.

41. Tahir ISA, Nakata N, Yamaguchi T, Nakano J and Ali AM. Physiological response of three wheat cultivars to high shoot and root temperatures during early growth stages. Plant Production Science. 2009; 12(4):409-419.

Table 1. ILs with different combinations of QTLs related to high-temperature tolerance

S. No.	QTL	Marker	Plant numbers
1	qHTSF1.1 Positive	RM431	IL2, IL3
2	qHTSF4.1 Positive	RM5757	IL4, IL5
3	qHTSF1.1 & qHTSF4.1 Positive	RM431 and RM5757	IL6, IL7, IL8
4	qHTSF1.1 & qHTSF4.1 Negative	RM431 and RM5757	IL1

Table 2. Monthly meteorological data recorded during *Kharif* 2018

Season / Month	Ambient conditions				Polyhouse				Ambient conditions			
	TEMPERATURE (°C)		Relative Humidity (%)		TEMPERATURE (°C)		Relative Humidity (%)		RAINFALL (mm)	RAINY DAYS	SUN SHINE (hours)	WIND SPEED (Km h^{-1})
Kharif 2018	MAX	MIN	I	II	MAX	MIN	I	II				
JULY	30.6	21.4	89	70	-	-	-	-	87.6	6	3.3	11.2
AUGUST	29.4	20.8	88	71	-	-	-	-	159.6	10	3.8	10.2
SEPTEMBER	31.4	19.9	90	58	-	-	-	-	43.4	3	5.9	2.9
OCTOBER	32.4	16.5	86	39	39.9	23.1	87.9	53.6	43.2	2	7.4	2.9
NOVEMBER	31.3	13.7	87	38	40.5	21.7	87.3	54.6	0.0	0	8.2	2.8
Mean	31.02	18.46	88	55.2	40.2	22.4	87.6	54.1	-	-	5.72	6
Total	-	-	-	-	-	-	-	-	333.8	21	-	-

Table 3. Effect of high-temperature stress on Chlorophyll fluorescence traits.

| Trait	Mean		% reduction over High-temperature stress	Tukey HSD (P<0.05)			
	Control	High-temperature		Treatment (T)	Genotype (G)	T × G	CV (%)
Maximum quantum yield of PSII (Fv/Fm)	0.782	0.722	7.7	0.007	0.026	0.042	1.79
Effective PSII quantum yield (ΦPSII)	0.337	0.275	18.4	0.010	0.038	0.061	6.44
Electron transport rate (ETR)	27.689	23.662	14.5	0.760	2.820	4.512	5.65
Co-efficient of photochemical quenching (qP)	0.431	0.337	21.8	0.011	0.040	0.064	5.35
Co-efficient of non-photochemical quenching (qN)	0.744	0.674	9.4	0.013	0.049	0.079	3.58
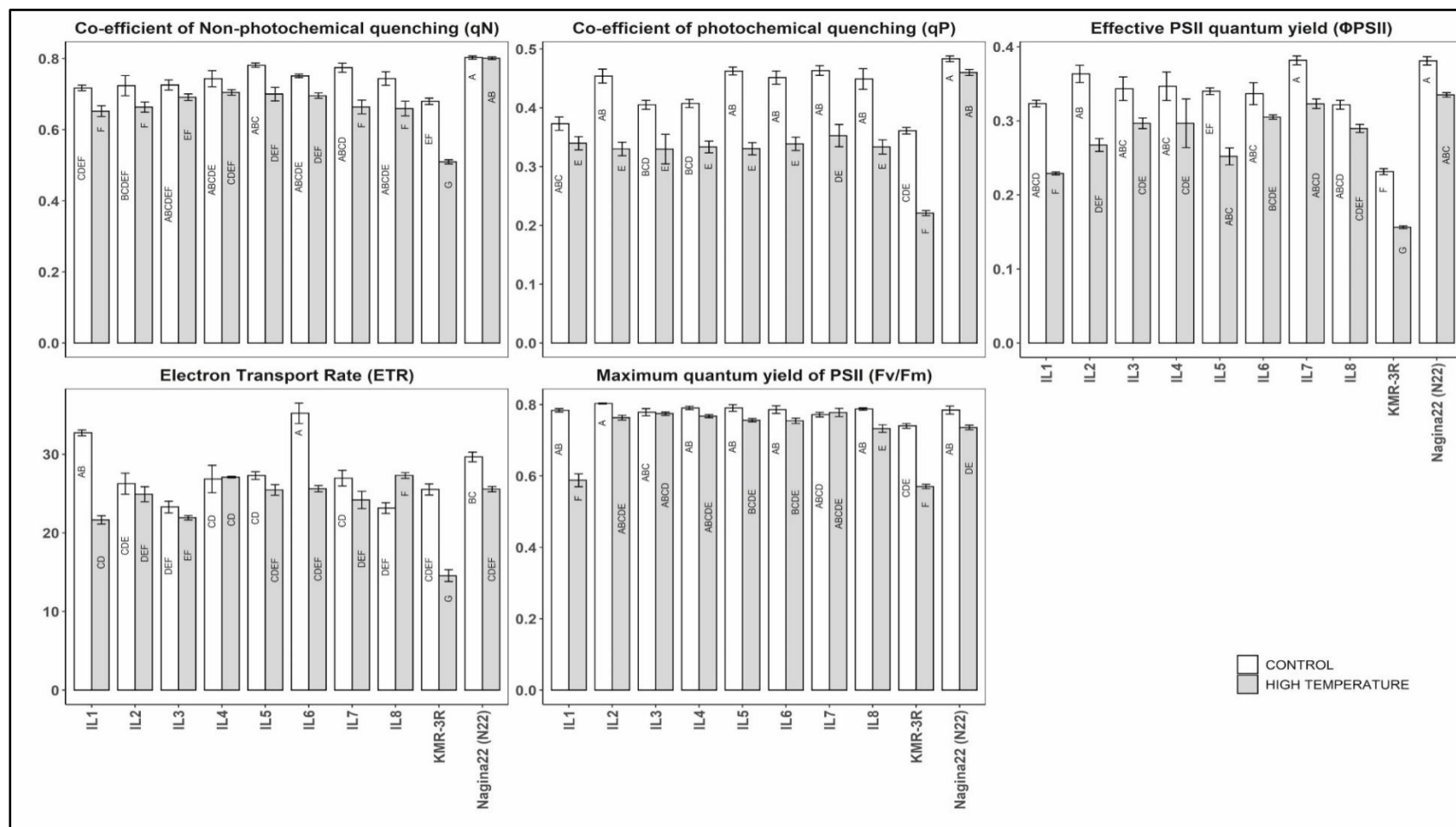
Figure 1. Effect of high-temperature stress on Chlorophyll fluorescence traits in ILs and parents.