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Physiological and Biochemical Responses of Different Rice (*Oryza sativa* L.) Genotypes under Terminal Heat Stress

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Abstract

Rice is the staple food of more than 60% of the world's population. It is very sensitive to heat stress during flowering and grain filling stages. Therefore, heat stress is an important constraint for rice productivity under changing climatic conditions. To evaluate the effect of terminal heat stress in different indica rice genotypes a field experiment was conducted in Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar during kharif season 2010. These genotypes were transplanted in two blocks, one for control and another block for imposing heat stress by covering the block with low density polythene (LDPE) sheet supported by bamboo sticks. Both the entry ends were open for sufficient ventilation. The heat stress was given during flowering. Daily maximum and minimum temperature was recorded with the help of automatic thermometer inside the LDPE sheet tunnel. In the present investigation, it was found that flag leaf area, total plant dry matter, chlorophyll content, chlorophyll fluorescence and NR activity decreased under heat stress, while proline level significantly increased in all rice genotypes. A significant reduction in grain yield, 1000 grain weight, number of filled spikelet and percent spikelet fertility was recorded. It is evident from the present study that the terminal heat stress may pose severe threat to rice productivity under globally changing climatic conditions.

Keywords: Rice, heat stress, leaf area, NR activity, proline, spikelet fertility

1. Introduction

Rice (*Oryza sativa* L.) is a globally important cereal crop and as a primary source of food, it accounts for 35–75% of the calorie intake of more than 3 billion humans. With the likely growth of world's population toward 10 billion by 2050, the demand for rice will grow faster than for other crops. There are many challenges in achieving higher productivity of rice. In future, the new challenges will include climate change and its consequences. The expected climate change includes the rise in the global average surface air temperature [14]. According to fifth assessment report (AR5) of the intergovernmental panel on climatic change (IPCC) the global surface temperature is likely to rise a further 0.3 to 1.7 °C during the 21st century. This increase in global temperature is the main cause of global warming, which is due to population explosion, the development of industries emitting greenhouse gases and excessive deforestation. It is increasing the world's average ambient temperature and exacerbating the problem of heat stress. Rising temperatures may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for the start of the season and crop maturity to reach earlier [6, 11, 17].

Heat stress is detrimental to many plant species in terms of growth and productivity, especially in the summer months and in warm and temperate climatic regions. Rice is very sensitive to high temperature stress during critical stages of growth, such as flowering and seed development [29] causing a serious reduction in grain yield due to reduced spikelet fertility, decreased grain weight, reduced grain filling, and higher percentage of white chalky grains. In addition, increased temperature causes serious reduction in grain size and amylose content. Other physiological and biochemical parameters such as chlorophyll content, proline content, and nitrate reductase activity were also severely affected [28].

Materials and Methods

Plant materials and experimental site

A field experiment was conducted during kharif season 2010 in rice physiology A1 block, of Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar (Uttrakhand) India. The seeds of ten rice genotypes, *viz.* IET 20114, IET 20734, IET 20735, IET 20894, IET 20907, IET 20915, IET 20923, IET 20924, IET 20926 and

IET 20944 obtained from the Directorate of Rice Research, Rajendranagar, Hyderabad were sown on 14th June and the seedlings were transplanted on 6th July in two separate blocks one for control and other for heat stress treatment with randomized block design.

Heat stress treatment

During flowering, the elevated heat stress condition was maintained by covering one block with transparent low density polythene (LDPE) sheet supported by bamboo sticks. Both the entry ends were open for sufficient ventilation. Another block which was not covered with LDPE sheet was considered as control. The daily maximum and minimum temperature was recorded with the help of automatic thermometer (Zel, England) inside the LDPE sheet tunnel from 5 Oct 2010 to 5 Nov 2010 [Fig.1]. Nitrogen (100 kg/ha), phosphorus (45 kg/ha) and potassium (60 kg/ha) were applied in field in form of urea, single super phosphate and muriate of potash respectively. Potassium and phosphorous applied as basal dose and nitrogen top dressed in three splits with 50% at 15 day after transplanting, 25% at active tillering and 25% at panicle initiation (Pi) stages.

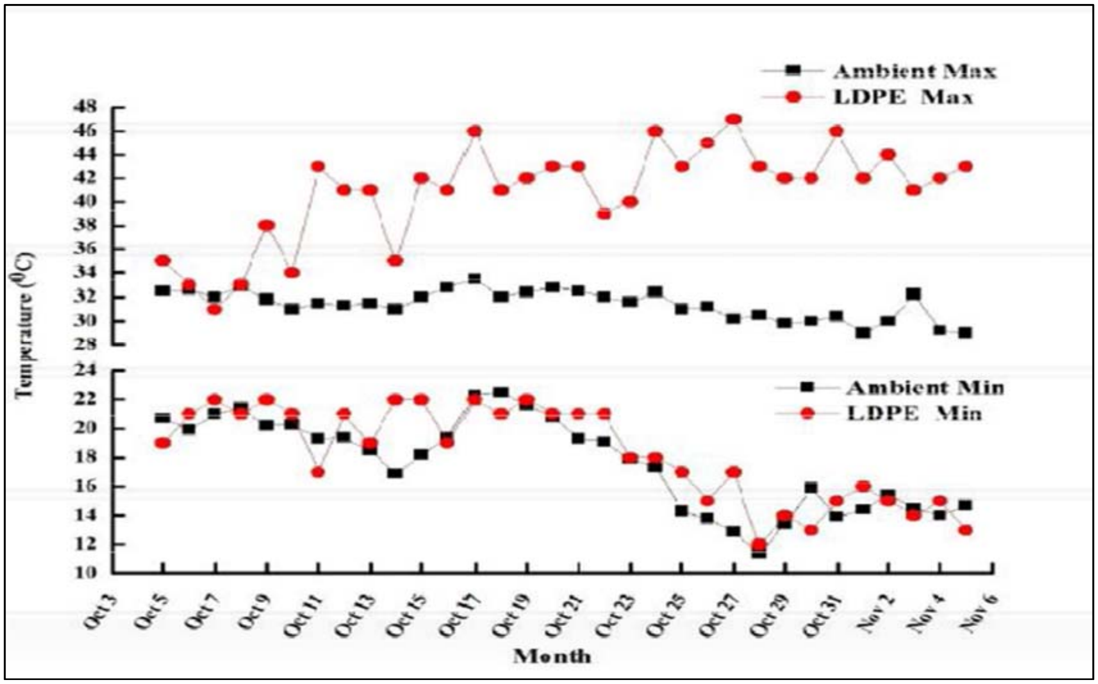

Fig 1: Maximum and minimum ambient and LDPE sheet temperature from October, 5 to November, 5 in year 2010.,

Flag leaf area: The effect of heat stress on flag leaf area of rice genotypes was determined on 10th day after treatment (10th DAT) and 25th day after treatment (25th DAT). The flag leaf area was calculated using the following equation of Palaniswany and Gomez (1974) [16]. The upper most fully expanded leaf of the mother tiller was selected for the estimation of flag leaf area. A factor of 0.75 was used to calculate the flag leaf area. It was expressed as cm2.

Flag leaf area = Length × Width × K (Factor 0.75)

Total dry matter (TDM): The total plant dry matter per plant was calculated at flowering stage by uprooting the complete plant and then placing the plant sample in the oven at 650C for three days.

Chlorophyll content: Chlorophyll content was determined in fresh leaves at flowering stage by using a method described by Hiscox and Isralesham 1979 [10].

Chlorophyll fluorescence: Chlorophyll fluorescence of rice flag leaves was measured at flowering stage with the help of Handy PEA (Hansatech, UK).

Nitrate reductase (NR) activity: It was observed in freshly harvested flag leaves at flowering by the method described by Hageman and Hucklesby 1971 [5].

Proline content: Proline content in young and old leaves of rice collected from both control and treated blocks at flowering were determined by the colorimetric method adopted from Bates *et al*. 1973 [2]. The standard curve was used for proline estimation and free proline content was expressed as µ moles proline g-1fr. weight.

Yield and its components: Grain yield was determined from both control and treated blocks and expressed in g/plant. The yield components, such as 1000-grain weight, and percent spikelet fertility per panicle also measured.

Statistical analysis: The analysis was carried out with the STPR2 statistical software developed by the Department of Mathematics, Statistics and Computer Science, G. B. Pant University of Agriculture and Technology, Pantnagar.

Results

Flag leaf area: Heat stress adversely affected flag leaf area. On 10th day after treatment (10th DAT) the highest flag leaf area was observed in IET 20926 (62.89 cm2) under control and (59.29 cm2) under treatment, whereas lowest in IET 20907 (41.63 cm2) under control and (38.37 cm2) under treatment [Fig.2 (A)]. On 25th day after treatment (25th DAT) the highest flag leaf area was observed in IET 20926 (70.00 cm2) under control and (65.00 cm2) under treatment, whereas lowest in IET 20907 (53.00 cm2) under control and (48.00 cm2) under treatment. The reduction in flag leaf area significantly increased as the days of heat stress treatment increased [Fig. 2(B)]. The percent reduction was recorded highest in IET 20944 (15.43% and 19.05%) and lowest in IET 20734 (2.59% and 4.33%) on 10th DAT and 25th DAT, respectively.

Total dry matter (TDM)

The total dry matter per plant was affected during heat stress. However, in this field experiment heat stress treatment had little effect on total dry matter production compared to control. The maximum TDM found in rice genotype IET 20734 (28.44 g) under control and (28.78 g) under treatment condition, whereas minimum in IET 20923 (21.26 g) under control and IET 20894 (19.00 g) under treatment condition at flowering [Fig.2 (C)].

Chlorophyll content: The total chlorophyll content was maximum in rice genotype IET 20114 (2.65 mg/g fr. wt.) under control and in IET 20926 (2.55 mg/g fr.wt.) under treatment, while minimum in IET 20923 (1.71 mg/g fr. wt.) under control and in IET 20944 (1.52 mg/g fr.wt.) under treatment condition. The average total chlorophyll content was found to be higher in control (2.17 mg/g fr. wt.) as compared to treatment (2.04 mg/g fr. wt.). The overall genotypic difference (Gn) and interaction (TxGn) were found to be statistically significant [Fig.2 (D)].

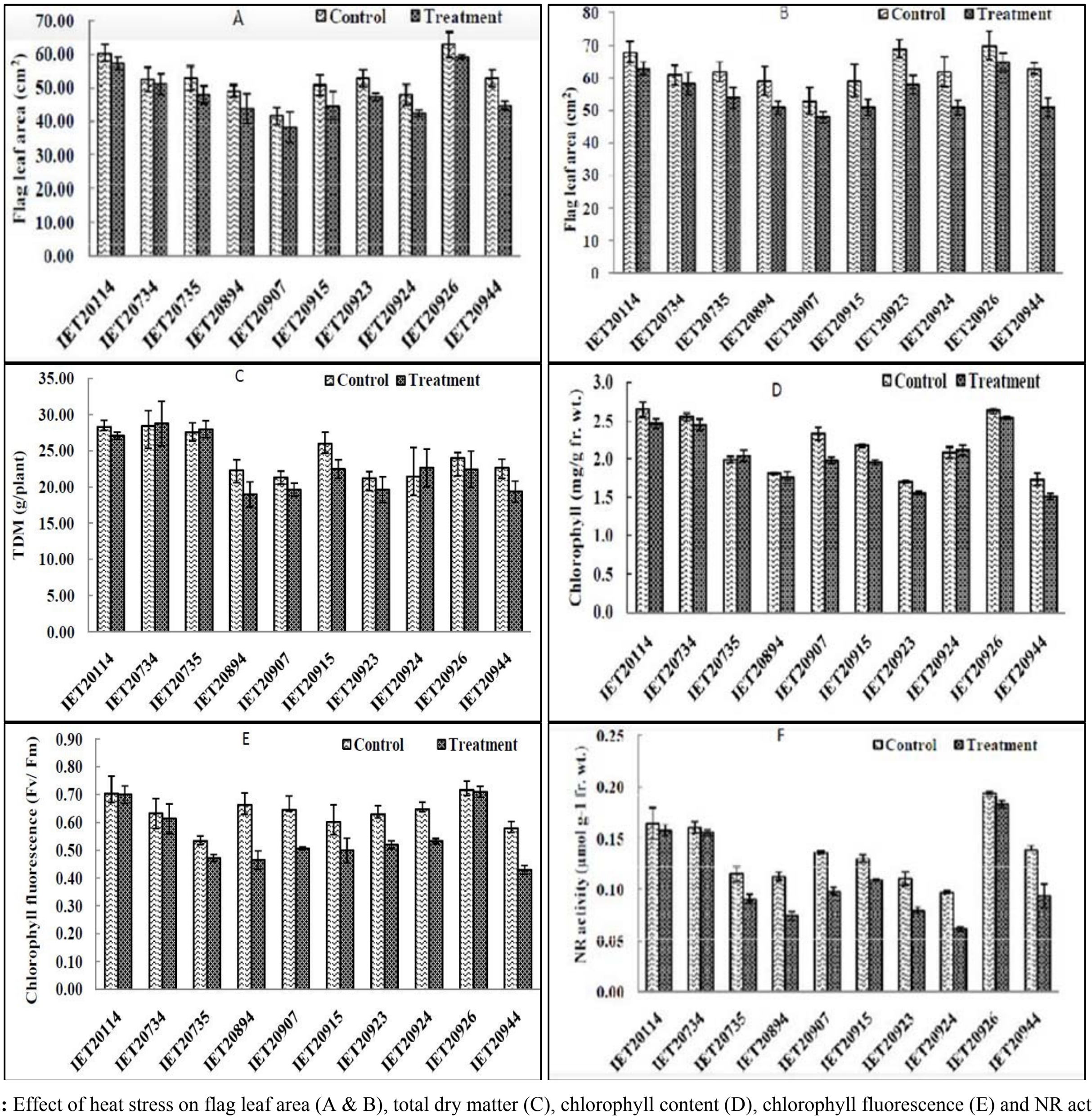

Fig 2: Effect of heat stress on flag leaf area (A & B), total dry matter (C), chlorophyll content (D), chlorophyll fluorescence (E) and NR activity (F) in different rice genotypes

Chlorophyll fluorescence: The highest chlorophyll fluorescence was found in IET 20926 (0.718) under control and (0.712) under treatment, whereas lowest in IET 20735 (0.534) under control and in IET 20944 (0.431) under treatment. The average chlorophyll fluorescence was found to be higher in control (0.636) as compared to treatment (0.546). The overall treatment (T), genotypes (Gn) and TxGn interaction were found to be statistically significant [Fig.2 (E)].

Proline content: The proline content significantly increased in all rice genotypes under heat stress as compared to control. This increase is more in new and younger leaves compared to old. The genotype IET 20926 accumulates highest proline (72.55 µg g-1fr. wt.) under control and (113.97 µg g-1fr. wt.) under heat treatment condition, while genotype IET 20924 accumulates lowest proline (30.89 µg g-1fr. wt.) under control and (44.43 µg g-1fr. wt.) under treatment in young leaves, respectively. Similarly, in old leaves proline content was maximum in IET 20926 (48.15 µg g-1fr. wt.) under control and (70.00 µg g-1fr. wt.) under treatment condition, whereas minimum proline in IET 20924 (20.39 µg g-1fr. wt) under control and in IET 20735 (28.00 µg g- 1fr. wt) under treatment condition [See table 1].

Table 1: Effect of heat stress on proline content in young and old leaves of different rice genotypes at flowering stage.

Proline content (µg g-1 fr.wt.)						
Genotypes	**Control**	**Young leaves Treatment**	**Change (%)**	**Control**	**Old leaves Treatment**	**Change (%)**
IET 20114	65.110	105.430	0.619	43.050	67.000	0.556
IET 20734	64.050	92.880	0.450	42.370	58.000	0.369
IET 20735	32.710	44.450	0.359	21.510	28.000	0.302
IET 20894	49.790	57.840	0.162	33.030	37.000	0.120
IET 20907	35.920	50.000	0.392	23.750	31.000	0.305
IET 20915	34.390	49.340	0.435	22.820	32.000	0.402
IET 20923	33.810	49.320	0.459	22.240	31.000	0.394
IET 20924	30.890	44.430	0.438	20.390	29.000	0.422
IET 20926	72.550	113.970	0.571	48.150	70.000	0.454
IET 20944	50.150	66.570	0.327	36.000	44.020	0.223
Mean	46.937	67.423	0.421	31.331	42.702	0.355
	T	Gn	T× Gn	T	Gn	T× Gn
S.Em±	1.09	2.45	3.47	0.355	0.794	1.12
CD (5%)	NS	7.02	9.93	NS	2.27	3.21

All values represent mean of triplicates

Nitrate reductase (NR) activity: The leaf nitrate reductase activity declined under heat stress. The percent reduction in NR activity was observed maximum in genotype IET 20924 (36%) and minimum in IET 20734 (3.00%). The maximum nitrate reductase activity was found in rice genotype IET 20926 (0.194 µ mol g-1 fr. wt) under control and (0.184 µ mol g-1 fr. wt) under treatment condition, whereas minimum in IET 20924 (0.097 µ mol g-1 fr. wt) under control and (0.062 µ mol g-1 fr. wt) under treatment [Fig.2 (F)].

Rice yield & its attributes

Terminal heat stress sharply reduced grain yield and thousand grain weight in rice genotypes. Reduction in yield and thousand grain weight vary with genotypes. The rice genotype IET 20926 had highest grain yield (33.97 g) in control and IET 20734 (29.00 g) had highest grain yield in treatment condition, whereas IET 20944 had lowest grain yield (19.01 g) in control and (16.76 g) in treatment. Although, some genotypes gave better yield under heat stress condition, their thousand grain weight was found to be very low. Rice genotype IET 20894 had maximum thousand grain weight (48.19 g) in control and IET 20926 had (32.67 g) in treatment, whereas IET 20923 had minimum (21.35 g) in control and (7.67 g) in treatment. The percent spikelet fertility was maximum in rice genotype IET 20894 (86%) under control and in IET 20734 (64%) under treatment, while minimum in IET 20944 (64%) under control and in IET 20915 (25%) under treatment (See table 2).

Table 2: Effect of heat stress on grain yield, thousand grain weight and spikelet fertility (%) of rice genotypes.

	Grain yield (g/plant)			**Thousand grain weight (gm)**			**Spikelet fertility (%)**		
Genotypes	**Control**	**Treatment**	**Reduction (%)**	**Control**	**Treatment**	**Reduction (%)**	**Control**	**Treatment**	**Reduction (%)**
IET 20114	31.88	27.08	14.92	34.63	28	19.15	76	58	42.38
IET 20734	30.30	29	4.29	33.54	25.33	24.46	78	64	35.58
IET 20735	32.67	27.34	16.31	34.78	14.33	58.8	78	46	54.08
IET 20894	30.10	26.31	12.59	48.19	11.67	75.79	86	40	59.70
IET 20907	29.91	27.1	9.39	26.86	12.33	54.09	84	46	53.62
IET 20915	19.96	17.88	10.42	23.26	10.67	54.14	76	25	75.07
IET 20923	26.2	17.32	33.89	21.35	7.67	64.08	67	28	71.80
IET 20924	32.42	23.13	28.66	45.67	11.67	74.46	75	27	72.67
IET 20926	33.97	28	17.57	38.7	32.67	15.59	67	62	38.45
IET 20944	19.01	16.76	11.84	25	13.33	46.66	64	43	57.04
Mean	28.64	23.99	15.98	33.19	16.767	48.722	75	44	56.04
	T	Gn	T× Gn	T	Gn	T× Gn	T	Gn	T× Gn
S.Em±	0.492	1.1	1.55	1.42	3.17	4.49	2.07	4.64	6.56
CD (5%)	NS	3.15	4.46	4.07	9.1	12.87	NS	13.29	18.8

All values represent mean of triplicates.

Discussion

Being sessile organism, plants are frequently exposed to various environmental stresses, such as drought, salinity, cold and hot temperatures. At present heat stress is a major environmental stress that limits plant growth, metabolism, and productivity worldwide [9]. The negative effect of high temperature stress on plant morphological, biochemical, and physiological processes were well documented in previous research [12, 24]. However, most of these studies were confined to pot experiment and few attempts have been made to evaluate the effect of heat stress under field condition.

Leaf is a principal plant organ that undergoes photosynthesis. The photosynthate from leaves is the main source of grain-filling materials in rice. In rice, 90% of grain yield originates from the photosynthetic production of leaves after heading, especially from flag leaf. So flag leaf area is an important factor for grain yield in rice. High temperature stress seriously limits photosynthetic production capability of rice and this is an important factor for reduction of grain yield [14]. The high temperature has positive effect on biomass production at early stages of growth which might be due to enhanced tillering, leaf expansion and nutrient uptake. Whereas, positive effect of higher temperature on biomass is negligible or becomes negative in later stages due to larger burden from increased maintenance respiration associated with excessive growth, faster senescence and shortened growth period [21]. In plants, chlorophyll content is an important indicator of photosynthetic activity, stress and nutritional status. Generally, healthy plants are expected to have higher chlorophyll content than unhealthy plants growing in the same growth period. Therefore, studies on leaf chlorophyll content and its relationships with plant stress and nutrition are important for agricultural field management and for optimizing agricultural practices. Heat stress altered total chlorophyll content and leaf photochemical efficiency in the stressed plants. The total chlorophyll content fell in the heat stressed cultivars in relation to the control plants [15, 26]. Chlorophyll fluorescence is an effective and non damaging tool for elucidating various aspects of the photosynthetic apparatus in intact leaves of higher plants. It can be used to estimate the rate of photosynthetic electron transport and photosynthetic quantum yield and capacity of thermal energy dissipation under high light stress [3, 18]. High temperatures reduce chlorophyll fluroscence (Fv) in attached leaves, protoplasts, chloroplasts, or thylakoids of rice. Injury to PSII in photosynthetic organelles and thylakoids and the match between these profiles and Fv, an indicator of damage to PSII and the kinetics of injury over time suggest that the photosystem is susceptible to high-temperature damage in rice [1]. The Fv/Fm ratio decreased significantly under high temperature stress, when rice seedlings were treated at 260C, 350C, 400C and 450C for 48 h, respectively [7]. The accumulation of compatible solutes is one of the adaptive strategies of plants in response to abiotic environmental stresses. Accumulation of these solutes like proline, glycine betaine and sucrose contribute to osmotic adjustment, prevention of protein denaturation, preservation of enzyme structure and activity and protection of membranes from damage by reactive oxygen species (ROS). The heat injury during floral development of sensitive genotypes may be due to the decline in proline concentration during the early floral bud development stage and its transportation is inhibited from anther walls to pollen [8, 20, 22]. Nitrate reductase (NR) catalyzes the first enzymatic step in nitrate assimilation in higher plants, involving reduction of nitrate to nitrite. NR is regulated by many environmental factors as well as endogenous factors that affect plant growth and metabolism [4, 19]. In perennial grass (*Leymus chinensis* L.) the activities of NR and GS involved in nitrogen assimilation significantly decrease, when subjected to moderate, sever and extreme drought and high temperature [27]. At flowering and later stages, temperature directly exert negative influence on rice yield by causing pollen sterility, empty or unfilled grains, low grain weight, poor seed setting, large decrease in percent spikelet fertility per panicle as well as alteration in various enzymatic activity, such as sucrose synthase and starch synthase. The loss of rice grain yield due to heat stress has been reported earlier by various workers [13, 23, 28].

Conclusion

Global warming is likely to increase the frequency of heat episodes. The global agriculture system is drastically affected by increasing climate variability, especially high temperature, leading to adverse effects on crop growth and development. The present study has shown that terminal heat stress is an important constraint for rice productivity under changing climate condition in terms of reduction in chlorophyll content, chlorophyll fluorescence, NR activity, flag area, total dry matter production and yield related parameters, like grain yield, thousand grain weight, spikelet fertility. Accumulation of stress related compounds are well documented under various type of stresses among which proline is most common accumulated compound. The increasing proline level protects plant's enzyme form destruction, which is necessary to achieve optimum yield under heat stress. This can be used for screening tools to select heat tolerant genotype. In future, there is further need to develop high yielding thermo-tolerant crop plants by better understanding of physiological and biochemical processes to overcome terminal heat stress.

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