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Field Phenotyping of Rice Genotypes for Heat Stress Tolerance based on Morpho-physiological Parameters at Reproductive Stage

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ABSTRACT: Global warming poses a severe threat to agricultural ecosystems worldwide. Rice is staple food for almost half of world population. Heat stress is harmful for rice crop at reproductive stage. Under such scenario, for maintaining rice productivity and further enhancing it, it is necessary to study the morpho-physiological aspects of the heat stress tolerance in rice at reproductive stage. 22 rice genotypes were phenotyped on the basis morpho-physiological traits in field for heat stress tolerance. Exposure to heat stress at reproductive stage resulted in reduction in the number of productive tillers, number of filled grains and unfilled grains, panicle length, MSI, total dry matter and harvest index and increase in number of unfilled grains. However, some of the genotypes such as Rasi, RNR15048, KNM 1638 has performed on par with the control under heat stress. Hence, these genotypes can be considered as heat stress tolerant genotypes.

Keywords: Heat Stress, Rice, Abiotic stress, Physiology, Reproductive stage.

INTRODUCTION

Rice (*Oryza sativa* L.), stands second among world's cereal crops and nearly half of the population prefers rice as a stable food for their energy requirement. During the years 2021-22, India cultivated rice over an area of 463.79 lakh ha with a production of 130.29 million tones and its yield potential was about 2809 kg ha^{-1} (Department of Agriculture and Farmers Welfare, Annual Report 2022-23). Climate change lead by global warming is playing havoc with rice ecosystems worldwide by way of frequent occurrence of various abiotic stress such as drought stress, heat stress, flooding etc. Among all the abiotic stresses heat stress at reproductive stage is one of the main reasons of sudden rice crop loss in many parts of India. The ideal temperature for growing rice plants is about 25–35°C (Hussain *et al*., 2019). Beyond that temperature, the rice plants face a cascade of morphological, physiological, and biochemical alternations. The plant's response towards temperature stress is influenced by duration, severity, timing (during the day or at night) and developmental stages. Rice is vulnerable to high temperature at all stages (Aghamolki *et al.*, 2014) particularly at reproductive stage. High temperature at reproductive stage is a major threat to rice production across rice growing ecosystems. During reproductive stage, high temperature is leading to pollen mortality, poor yield and poor quality of grain as well (Lawas *et al*., 2019).

For every 1°C rise in global mean temperature, there is a 3.2% reduction in rice yield (Zhao *et al.*, 2017). Elevated temperature hinders the process of pollination (Jagadish *et al*., 2007), shortens the grain filling period and shows a negative influence on spikelet fertility and grain quality (Cheabu *et al.*, 2018). The primary cause of spikelet sterility is poor anther dehiscence, low pollen shedding on the stigma and pollen mortality which results in poor fertilization at a temperature of >35°C (Jagadish *et al.*, 2010). Due to poor fertilization, the rate of seed set is reduced. Furthermore, high temperature reduces the period of grain filling (Kumar *et al*., 2021) especially high night temperature leading to not only poor grain yield. High temperature interferes with vital physiological process like photosynthesis and respiration leading to the production of highly reactive oxygen species. Moreover, the integrity of the cell membrane is reduced due to high temperature causing an electrolyte leakage (Halford, 2009). Thus, high temperature adversely affecting the productivity of rice has become bottleneck for enhancing rice production further. With this brief background an experiment was conducted to screen 22 rice genotypes for heat stress tolerance on the basis of morpho-physiological parameters at reproductive stage during *Rabi* season of the year 2022-2023 in the experimental fields of ICAR-IIRR, Hyderabad.

MATERIAL AND METHODS

The field experiment is conducted to screen 22 genotypes for heat stress tolerance in ICAR-IIRR during the years of 2022-2023. The seeds of 22 genotypes were collected from ICAR-IIRR, Hyderabad and PJTSAU Hyderabad. The 2 sets seeds of 22 genotypes were prepared, one set was sown as timely sown control treatment and the second set was sown after 30 days as heat treatment. After 21 days, the seedlings were pulled out from nursery and transplanted in field with spacing of 20 × 15 cm in a factorial RBD with three replications and two treatments. For heat stress treatment, one set was sown on normal sowing time and another set was sown after a gap of 30 days in order to coincide the reproductive stage of the 2nd set of genotypes with the natural heat of the summer months of April and May. To monitor daily temperature, the data loggers were installed in the field.

A. Experimental Material

Twenty-two genotypes- MTU 1010, IR 64, RNR 15048, RNR 31479, RNR 21278, RNR 29325, JGL 33124, KNM 1638, BAM 181, IRGC 126084, Gaja-Baru, Sufal-Bhula, Suweon 311, IC 438644, Rasi, Bakal, JGL 27356, Maha-Chai, Moshi, BPT 5204, N22 and Vandana

B. Experimental observation

Membrane Stability Index. Membrane Stability Index was carried out according to Deshmukh *et al.* (1991). MSI was calculated by measuring the electrical conductivity of leaf leachates in double-distilled water at 40°C and 100°C. Weigh 0.1 g of leaf discs of uniform size and place them in test tubes containing 10 ml of double distilled water in two sets. One set was heated at 40°C for 30 minutes and another set was boiled at 100°C for 15 minutes in a boiling water bath and their electrical conductivities C_1 and C_2 were recorded by Conductivity meter.

$$\text{MSI (\%)} = 1 - \frac{C_1}{C_2} \text{ x } 100$$

Number of Productive Tillers. Panicle bearing tillers were counted during flowering stage and then expressed as productive tillers per plant.

Number of filled grains and Number of unfilled grains. The topmost three panicles were manually counted for filled and unfilled grains and the data of filled and unfilled grains were expressed as average of three panicles.

Panicle length. The topmost three panicles were measured with ruler and expressed as average of three panicles (cm).

Total dry matter. Plants were sampled from each treatment and genotype at maturity for estimating total dry matter. Samples were dried in an oven until the weight was reached to constant. Dried samples were weighed using an electronic balance and expressed in $gm^{-2.}$

Harvest Index. The harvest index was calculated by using the formula as given below

$$\text{Harvest Index (\%)} = \frac{\text{Economic yield (g/plant)}}{\text{Biological yield (g/plant)}} \text{ x } 100$$

RESULTS AND DISCUSSION

As per weather data shown in Table 1. The maximum daily temperature ranged from 30.4°C to 35.8°C in control plot whereas it ranged from 33.3°C to 44°C in treatment plot during the crop growing season. During flowering stage, the temperature in control about was about 35.8°C whereas in case of treatment plot it was about 41.9°C. It is clear that treatment plot receives heat stress treatment during flowering stages.

Table 1: Weather parameters during *Rabi* 2022-23 at ICAR-IIRR, Hyderabad.

Phenological stage	Control (1st Sowing)				Treatment (2nd Sowing)			
	Temperature (°C)		RH (%)		Temperature (°C)		RH (%)	
	Max	Min	I	II	Max	Min	I	II
Sowing to PI	30.4	14.4	85.1	37.4	33.3	15.5	82.2	30.2
PI to 50% Flowering	34.3	17.9	80.8	33.2	41.9	22.3	81.2	45.1
Flowering to Physiological Maturity	35.8	21.9	82.4	43.9	44.0	23.1	79.9	43.1
Mean	33.5	18.1	82.7	38.2	39.7	20.3	81.1	39.5

A. Membrane Stability Index

MSI is the most important and simple parameter to measure the stress levels of the plants. Under heat stress, the photosynthetic apparatus of the cell gets heats up which leads to production of highly reactive oxygen species. This highly reactive oxygen species interferes with various components of the cell membranes which leads to improper functioning of cell membranes. Hence the membrane leaks. This membrane leakage is measured as electrical conductivity during the measurement of MSI. The membrane stability index can be used as a simple screening protocol for heat stress tolerance (Veronica *et al.*, 2019). In the present study, the membrane stability index (%) of rice genotypes showed significant difference for genotypes, treatments, genotype and treatment interaction (G×T) at $p<0.05$. The MSI of rice genotypes showed wide variation under heat stress (60.2%) over control (70.5%). The percent reduction of MSI under heat stress was 14.61%. In control, the membrane stability index (%) of tested genotypes ranged between 50.3% (BAM181) and 82.7% (N22) and in heat stress treatment, it ranged between 31.3% (Moshi) and 77.2 (N22). When compared to control, Moshi (42.46%) had exhibited highest decrease in membrane stability index (%) with respect to heat stress while the least decrease was observed in Rasi (5.24%) (Fig. 1).

The diminishing membrane stability index might be due to the rate of lipid per-oxidation increased which was induced by the presence of reactive oxygen species and this leads to an increase in membrane leakage (Kumar *et al*., 2016). Prasertthai *et al*. (2022) also reported that the membrane was unstable due to increasing solute leakage, whereas in tolerant varieties like N22 and CN1 exhibited a smaller increase (7.15%) in solute leakage and maintained their membrane integrity under heat stress. The maintenance of membrane integrity under heat stress was due to increased antioxidant activity and minimum ROS accumulation was reported in N-L-44. The membrane stability of the N-L-44 is similar to that N22 a well-known heat stress tolerant donor (Umesh *et al*., 2016). Further Harshada *et al*. (2021) reported that the genotypes which showed minimum electrical conductivity under heat stress can be considered as heat-tolerant genotypes and the genotypes which showed maximum as thermo-sensitive genotypes. Some of the genotypes from the present study like N22, Rasi, RNR15048, KNM1638, JGL31479, Gaja-Baru, Bakal and JGL27356 had shown minimum leakage (non-significant reduction) as compared to control counterparts that means they can be considered as heat tolerant genotypes on the basis of MSI performance. The genotypes like BAM181, IRGC126084, Moshi, Maha-Chai, Vandana and MTU1010 can be termed as heat sensitive genotypes on the basis the performance with respect to MSI.

B. Number of Productive Tillers

Panicle formation was determined during tiller bud initiation and subsequent phases. On a hill, every tiller had the capacity to develop panicles. Due to environmental factors, it failed to form panicles, and the tiller was retained as unproductive (without seed set) (Fageria, 2007). In the present investigation, the differential response noted among genotypes, treatments but non-significant for genotype and treatment interaction (G×T) at $p<0.05$. Normally, tiller and panicle development is affected when the daytime temperature reaches above 35°C compared to normal temperatures of 30–33°C (Chaturvedi *et al.*, 2017). The mean number of productive tillers decreased significantly from 12 in control to 10 under heat. The mean number of productive tillers was decreased negatively by 16.67% under heat compared to control. In control, number of productive tillers of genotypes ranged from 8 (Gaja-Baru) to 20 (RNR31479) and in heat treatment, it ranged from 6 (BAM181) to 16 (RNR31479) When compared to control, Moshi (36.36%) had exhibited highest decrease in number of productive tillers with response to heat while the least decrease was noticed in N22 (7.69) (Fig. 2). Finally, Sebastian and Selvaraj (2017) reported that heat caused negative effects on productive tillers.

C. Number of Filled and Unfilled grains

The number of spikelets and panicles forms the basis for enhancing the grain number. The process of grain formation starts with pollination. Higher temperatures interfere with the normal anther dehiscence (Prasad *et al*., 2006).

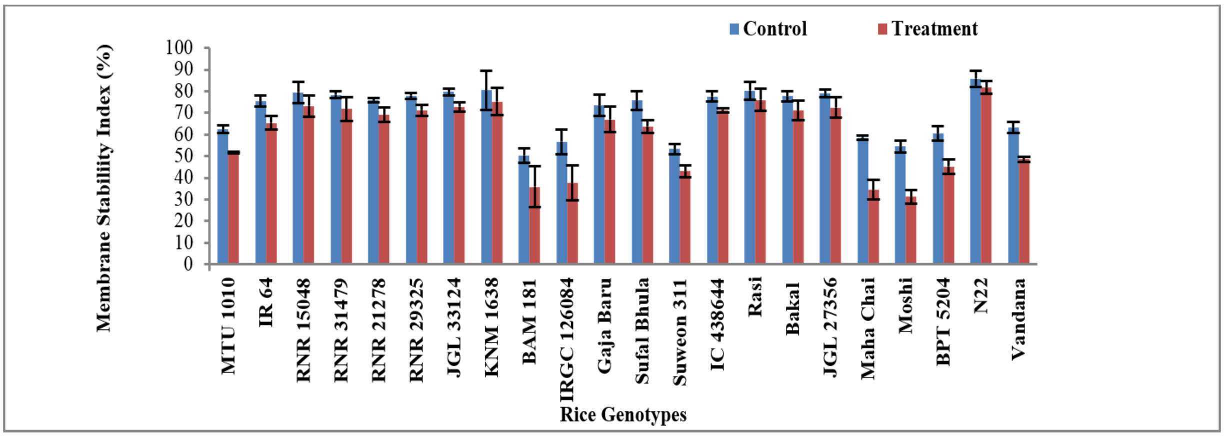

Fig. 1. Membrane stability index of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

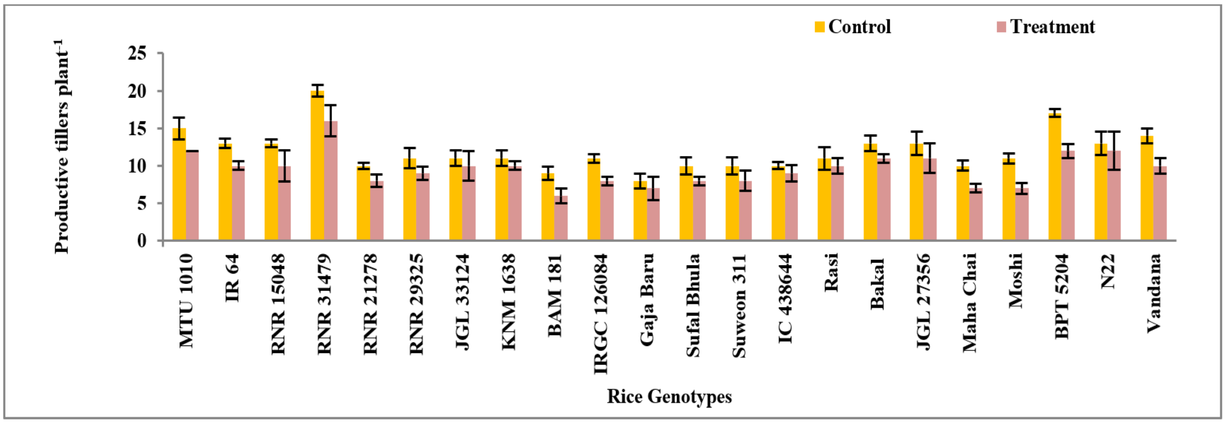

Fig. 2. Productive tillers of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

Due to heat stress, the pollen grain in the anther dries up leading to the mortality of pollen grains (Wang *et al*., 2019). Thus, heat stress interferes with the process of pollination leading to more number of unfertilized eggs. Therefore, the number of filled and unfilled grain in panicle is an important parameter (Kumar *et al*., 2020) to study heat stress tolerance.

In the present investigation, the number of filled and unfilled grains per panicle showed significant difference among genotypes, treatments and interaction (G×T) at $p<0.05$. The mean number of filled grains per panicle declined significantly from 100 in control to 69 under heat respectively, while the mean number of unfilled grains increased significantly from 14 (control) to 41 respectively.

Under control, the genotypes RNR15048 (181) exhibited higher number of filled grains per panicle whereas in terms of number of unfilled grains per panicle, the genotypes RNR21278 (30) recorded higher number of unfilled grains. Under treatment, the genotypes RNR15048 (153) exhibited higher number of filled grains per panicle whereas in terms of number of unfilled grains per panicle the genotypes Moshi (87) recorded higher number of unfilled grains (Fig. 3). In the present investigation is in accordance with the studies of Chang-lan *et al*. (2005) and Thuy *et al*. (2021), where they reported that number of filled grains declined under heat; simultaneously the number of sterile grains were increased under heat condition (Aghamolki *et al*., 2014).

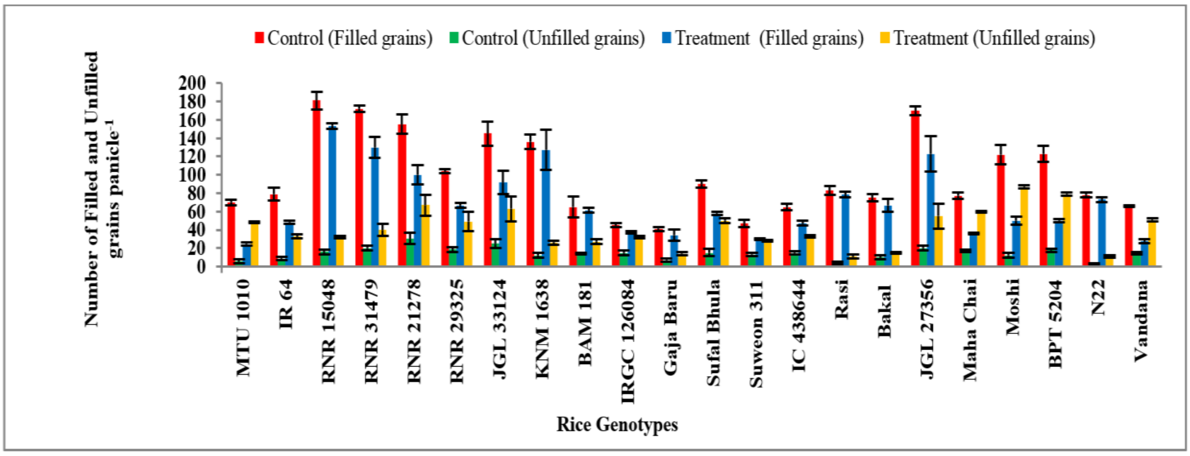

Fig. 3. Number of Filled and Unfiled grains per panicle of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

Heat stress hinders the rupturing of cell layers in anther (anther dehiscence) (Tazib *et al*., 2015) due to disturbance in the moisture content of anther (Das *et al*. 2014,). Number of pollen grains (Fu *et al*., 2016) and their viability (Jyothi *et al*., 2022) adhesion and germination on the stigma were reduced (Endo *et al*., 2009), curtailed the pollen tube length (Zhang *et al*., 2018) and modified the anther morphology (Jagadish *et al*., 2010) all of which severely interspersed the process of pollination and fertilization process. From the above findings, indicating the probable reasons for reduction of fertility in spikelet (filled grains) during reproductive stage.

As compared with tolerant check N22, the genotype Rasi, RNR15048, KNM1638, Bakal, BAM181 and Gaja-Baru had shown less reduction in number of filled grains per panicle. Some genotypes like Moshi, Vandana, MTU1010, BPT5204, Maha-Chai showed significant reduction in number of filled grains per panicle. The genotypes Moshi, Vandana, MTU1010, BPT5204, Maha-Chai had reported more number of unfilled grains per panicle under heat. The genotypes N22, Rasi, RNR15048, KNM1638, Bakal, BAM181 and Gaja-Baru maintained their number of filled grains and did not show much variation in the number of unfilled grains even under heat. Our results are in agreement with the Zakaria *et al*. (2002) wherein heats reduced the grain yield by increasing the number of unfilled grains.

D. Panicle length

Under heat conditions, the leaf had insufficient water content at panicle initiation and anthesis stages, which ultimately limits the carbohydrate metabolism and cell proliferation of the floral organ and resulted in a reduction in panicle length (Radhakrishna *et al*., 2018). The present study reported the mean panicle length decreased significantly from 20.4 cm in control to 19.1 cm with heat. The mean panicle length was decreased negatively by 6.37% under heat compared to control. Significant variation noticed among the tested genotypes for panicle length which had decreased in all the genotypes with response to heat. In control, panicle length of genotypes ranged from 16.4 cm (IRGC126084 and Gaja-Baru) to 27.0 cm (Moshi) and in heat treatment, it ranged from 14.9 cm (IRGC126084) to 23.5cm (Moshi). In comparison with control, Vandana (18.28%) had exhibited highest decrease in panicle length with response to heat while the least decrease was noticed in RNR15048 (3.57%) (Fig. 4). The panicle length of rice genotypes was significant among genotypes, treatments but non-significant for interaction (GxT) at $p<0.05$.

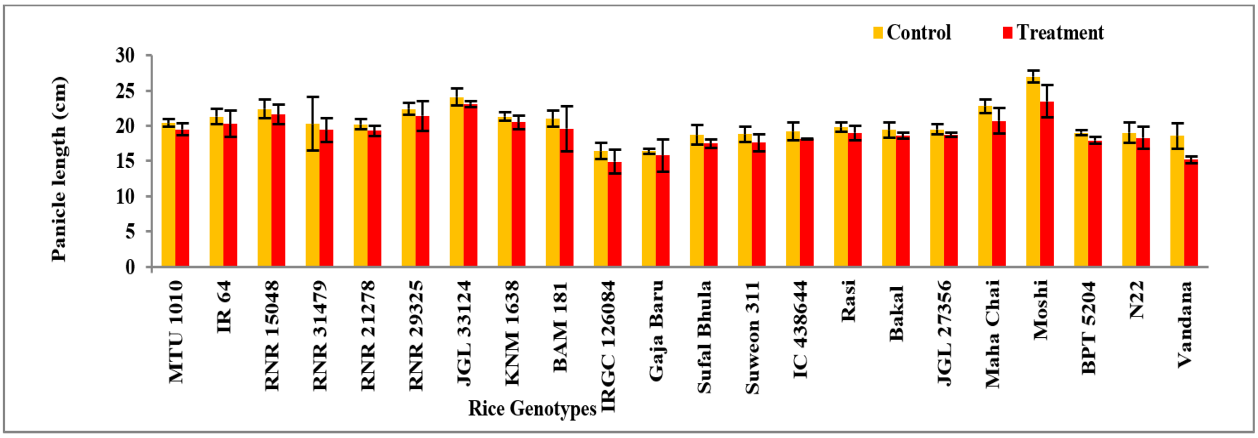

Fig. 4. Panicle length of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

E. Total dry matter

The total dry weight can be used as a measure of photosynthetic outcomes of a plant (Yoshida *et al*., 1981). Due to heat, the functional leaf photosynthesis (source) and the development of vascular bundles (flow) were hampered (Wu *et al.*, 2022) and hence the partition between leaf, culm, leaf sheath and panicle dry matter varied significantly. The panicle weight and grain yield decreased, whereas the straw weight increased due to dry matter accumulation in culm and leaf sheath. It resulted that more organic matter retained in the field and provoke the emission of greenhouse gas and also intervenes the carbon cycle (Guo-hua *et al*., 2013).

Temperature rises between 1.6°C to 3.1°C causes wide variation in the grain filling process (Liu *et al*., 2021). Rising temperature affects the endosperm capacity for starch storage by retarding the cell growth of the endosperm (Morita *et al*., 2005). Therefore, the rate of synthesis and storage of the starch was reduced (Xie *et al*., 2011). Alteration in source-sink ratio disturbs the allocation of dry matter to grain (Tu *et al*., 2022). In a manner, the present experiment shown that the mean of total dry matter decreased significantly from 1014 gm^{-2} in control to 815 gm^{-2} with heat. The mean total dry matter was severely reduced by 19.63% under heat when compared to control.

Significant difference was noticed among the tested genotypes for total dry matter which had sharply declined in all the genotypes with response to heat. In control, total dry matter of genotypes ranged from 564 gm^{-2} (Gaja-Baru) to 1689 gm^{-2} (Moshi) and in heat treatment, it ranged from 425 gm^{-2} (IRGC126084) to 1248 g m^{-2} (RNR15048). Vandana (31.72%) had exhibited highest decrease in total dry matter with response to heat over control while least decrease reported in N22 (6.64%) (Fig. 5). The effects of genotypes, treatments and interaction (GxT) were found significant at $p<0.05$.

F. Harvest Index (HI)

Harvest index is one of the main components for determining the grain yield. It was measured by the ratio between economic yield and biological yield. Adverse effects of heat causes negative impact on grain yield (Kumar *et al*., 2021) by reducing the spikelet fertility. The spikelet fertility was declined at reproductive stage and it was mainly related with impairment of pollen production and pollen shedding. Therefore, harvest index also declined (Prasad *et al*., 2006). The present investigation has shown significant variation among genotypes, treatments but non-significant for interaction (G×T) at $p<0.05$.

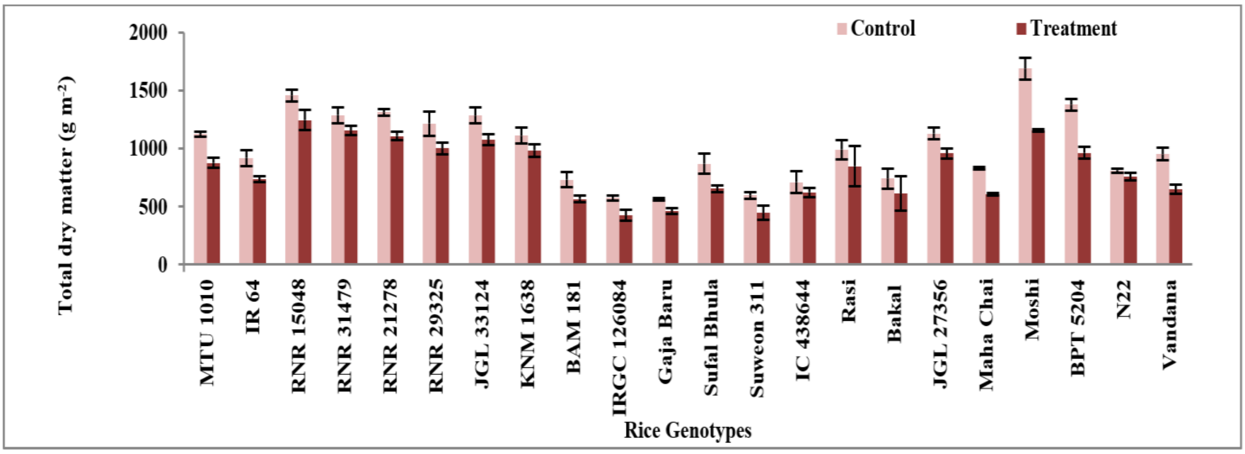

Fig. 5. Total dry matter of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

The mean HI decreased significantly from 49.4% in control to 42.6 % with heat. The mean HI was decreased negatively by 13.77% under heat compared to control. Significant variation was noticed among the tested genotypes for HI with response to heat which had decreased in all genotypes. In control, HI of genotypes ranged from 39.3% (Gaja-Baru) to 54.7% (RNR 31479) and in heat treatment, it ranged from 30.4% (Moshi) to 49.5% (Rasi) (Fig. 6).

In comparison with control, Vandana (34.10%) had exhibited highest decrease in HI with response to heat while the least decrease was noticed in Rasi (5.35%). Additionally, Singh *et al*. (2010) reported that harvest index declined under heat during reproductive phase. Poor grain growth and disturbance in the nutrient allocation from shoot to panicle under heat and this could be the reason for declining harvest index (Johnson *et al*., 2011).

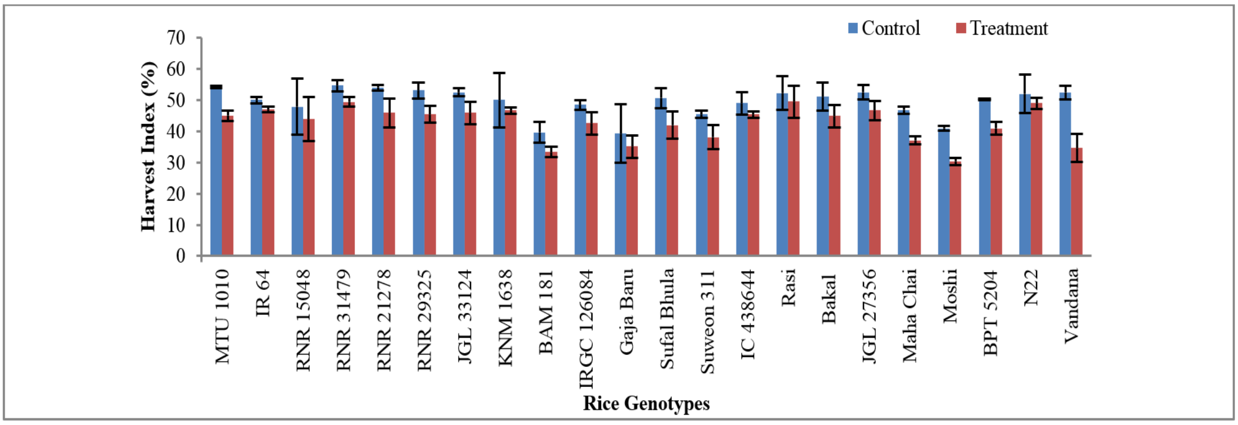

Fig. 6. Harvest index of 22 rice genotypes under control and heat treatment. The error bars represent standard error of means.

CONCLUSIONS

Heat stress in rice genotypes negatively impact the MSI, number of productive tillers, number of filled grains, panicle length, total dry matter and HI but positively increased the number of unfilled grains. Based on six traits as mentioned above, the genotypes Rasi, RNR15048, KNM1638, Gaja-Baru and Bakal performed at par with their control counterparts under heat stress. N22 a heat tolerant check also performed on par with its control. Therefore, these genotypes can be considered as heat stress tolerant genotypes. In response to heat, the genotypes Moshi, Maha-Chai and BPT5204 have shown reduction in the above mentioned parameters, which is similar to Vandana, a heat sensitive donor and therefore these genotypes can be considered susceptible to heat stress. Thus, it can be concluded from this study that heat stress causes a negative influence during the reproductive stage, morpho-physiological traits can be used to screen genotypes for heat stress tolerance studies. Further research is needed to confirm the findings of this experiment.

FUTURE SCOPE

Heat tolerant genotypes identified in this experiment can be used in breeding climate smart rice varieties and understanding the mechanism of heat stress tolerance in rice.

Author contributions. VN, CAK and AS designed the experiment, VN executed the experiment, DSR, AK analyzed data, VN and AS prepared the MS.

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Conflict of Interest. The authors declare no conflict of interest.

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