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Assessment of variability in acquired thermotolerance in rice (*Oryzasativa* L.) through temperature induction response (TIR) technique

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Abstract
High temperature stress is a major concern for crop production worldwide because it greatly affects the growth, development and productivity of plants. Plant response and adaptation to elevated temperature and the mechanisms underlying the development of heat tolerance, to be better understood for important agricultural crops. The unfavourable effects of heat stress can be mitigated by developing crop plants with improved thermotolerance using an assortment of genetic approaches. Acquiring thermotolearnce is a lively progression by which considerable amount of plants resources are diverted to structural and functional maintenance to escape damaged caused by heat stress. The present study was conducted at Department of Plant Physiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)during summer 2015 and 2016to adapt a novel temperature induction response (TIR) technique for identifying highly thermotolerance rice genotypes which can be used as donor source for developing high temperature tolerant rice genotypes to withstand future temperature rise, The screening observations for heat tolerance were recorded on five randomly selected plants in each plot on 430 rice accessions. Therefore, screening revealed that 93 accessions performed well for heat tolerance. The most of genotypes significant in quantitative characters but spikelet fertility was a more powerful indicator for screening at the reproductive stage than other stages. The high genotypic and phenotypic coefficient of variation was recorded for unfilled grains and total number of grains per panicle under high temperature stress during both summer seasons. Thus, this approach forms one of the potential screening tool techniques to evaluate genetic variability for intrinsic stress tolerance.

Keywords: Rice (*Oryzasativa* L.), Temperature induction response (TIR), Thermotolerance

Introduction
Rice (*Oryzasativa* L.) has a renowned relationship with the human since ages. It is the world's most important staple food crop, which not only provided food but also influenced traditions, religion, culture and life style since "Vedic period". Rice is among the world's most important and second most produced crop worldwide (FAO, 2015) [4].

Rice is cultivated around the world mainly in tropical and subtropical zones. Climate change resulting from increased atmospheric concentration of greenhouse gases will increase earth surface temperature (IPCC, 2007) [9]. Thence, in the future, rice will be grown in much warmer environment (Battisi and Naylor, 2009) [1] with a greater livelihood of high temperatures coinciding with heat sensitive recesses during the reproductive stage. This would significantly affect rice productivity, raising serious concerns about food security.

Chhattisgarh is popularly known as "Rice bowl of India". The summer rice is an important crop of Chhattisgarh. However, very low humidity and high temperature in summer results in higher fissuring and chalky grain. This increases broken percentage during milling and reduce head rice recovery. Identification of genotypes tolerant to high temperature are one of the simplest and effective method to improve the milling quality of the paddy. The high losses during milling are not economical for the millers for processing and ultimately farmers are facing the problem during marketing of their produce. In summer rice during reproductive stage temperature remains very high, which drastically reduces pollen fertility, resulting spikelet fertility and impact fisher in developing drastically reducing the Heed rice recovery (HRR).

High temperature stress is a major concern for crop production worldwide because it greatly affects the growth, development and productivity of plants. Plant response and adaptation to elevated temperature and the mechanisms underlying the development of heat tolerance, to be better understood for important agricultural crops. Under high temperature conditions, plants accumulate different metabolites (Such as antioxidant, osmo protectants, heat shock proteins (HSPs), etc and different metabolic pathways and processes are activated. These changes emphasize the importance of physiological and molecular studies to reveal the mechanisms underlying stress response. Therefore, an important to understand the response of rice to high temperature, with special emphasis on genotypic variation and morphological and characteristics of rice in relation to high temperature and also to gain insignificant to the mechanism of high temperature stress tolerance. Replacement of the sensitive cultivars by tolerant cultivars in the field, will increase the global rice production.

Materials and Methods

Method I

The screening of rice accessions against heat tolerance through temperature induction response (TIR) technique done during *Summer* 2015 as per the protocol proposed by Senthil Kumar *et al.* (2004).

The experiment material comprised of 430 rice accessions. The experiment was conducted under lab conditions. Heat stress was imposed on 21 days old seedlings in a frame of three times points by transferring them to a growth chamber maintained at 35 ^{0}C for 2hrs then at 42 ^{0}C for 2hrs followed by 48 ^{0}C. The 21 days old seedlings are exposed to a standardized induction temperature of 35^{0}C for 2hrs than 42^{0}C for 2hrs and again exposed to the standard challenging temperature 48^{0}C for 2hrs. At the end of the treatment period, seedlings are kept for recovery at a room temperature for 72 hrs. For comparison, one set of seedlings will have to be exposed directly to the challenging temperature (48 ^{0}C) without induction and one set as absolute control without any temperature. At the end of recovery period, the seedling derived from different treatments (like absolute control, with induction and without induction) are measured for their survival and recovery growth (%) over absolute control, the seedlings are classified as either tolerant or susceptible (Wahid *et al.* 2007) [15]. The percent survival (%) and reduction in growth (%) is calculated using the formula given below:

$$\text{Percent survival of seedlings (\%)} = \frac{\text{No. of seedling survival at the end of recovery}}{\text{Total no. of seedling taken for the experiment}} \times 100$$

$$\text{Reduction in growth per cent (\%)} = \left[\frac{(RGc - RGt)}{AGc} \times 100\right]$$

Where,

RGc is the recovery growth of absolute control, RGt in the recovery growth of treated, AGc in the Absolute control growth.

Method II

The field experiment was conducted during *summer* 2015 for quantitative characters of heat tolerant accessions of rice.

Heat Tolerance (HTol)

Scale for spikelet fertility: The rice genotypes were screened for heat tolerance on the basis of spikelet fertility percentage. The spikelet fertility recorded at growth stage 7-9. The different classes of spikelet fertility and their score are given: 1: More than 80%; 3: 61-80%; 5: 41-60%; 7: 11-40%; 9: Less than 11%.

Estimation of coefficient of variation

The coefficient of variation for different characters was estimated by formula as suggested by Burton and De Vane (1953).

(1) Phenotypic coefficient of variation (PCV)

$\sigma2p = \sigma2\ g + \sigma2\ e$

P C V (%) = $(\sqrt{\sigma2\ p} / X) \times 100$

(2) Genotypic coefficient of variation (GCV)

G C V (%) = $(\sqrt{\sigma2\ g} / X) \times 100$

Where,

σ2p = Phenotypic variance

σ2g = Genotypic variance

σ2e = Environment variance

X = General Mean

The estimates of PCV and GCV were classified as low, moderate and high according to Sivasubramanian and Madhava Menon (1973).

< 10% = Low

10 to 20% = Moderate

>20% = High

Heritability (broad sense)

It is the ratio of genotypic variance to the phenotypic variance (total variance). Heritability for the present study was calculated in a broad sense by adopting the formula as suggested by Hanson *et al*., (1956).

$$(ba) = \frac{\sigma 2g\ h2}{\sigma 2p} \times 100$$

Where,

*h*2 (bs) = Heritability in broad sense,

σ2g = Genotypic variance,

σ2p = Phenotypic variance

The estimates of heritability broad sense were classified as low, moderate and high according to Robinson (1966).

< 50% = Low heritability

50 to 70% = Moderate heritability

> 70% = High heritability

Genetic advance

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. Expected genetic advance (GA) was calculated as per the method suggested by Johnson *et al.* (1955).

G A = K. *h2*. σp

Where,

GA = Genetic advance

K = Constant (Standardized selection differential) having the value of 2.06 at 5 per cent level of selection intensity.

h2 = Heritability of the character

σ p = Phenotypic standard deviation

Genetic advance as percentage of mean

It was calculated by the following formula:

The estimation of genetic advance categories as;

$$\text{GA as percentage of mean} = \frac{\text{Genetic advance}}{\text{General mean}} \times 100$$

< 10% = Low
10 to 20% = Moderate
> 20% = High

Results and Discussion
Method I
The total 430 rice accessions were screened by TIR technique only 61.16% (263) accession survival and significant variation for acquired thermo tolerance was observed. The 430 entries were evaluated only 34 were exhibited thermotolerance to induced high temperature and percent survival of seedlings varied from 21 to 94% reduction in growth varied between 9 to 86%. Whereas, among genotypes, showed highest thermo tolerance of 100 percent seedling survival and reduction in root & shoot growth, 153 and 154, 299, also showed high thermo tolerance in temperature of 90% seedlings survival and no reduction in root and shoot growth.

Method II
Genetic variability parameters of quantitative characters
The mean, range and other variability parameters of quantitative characters of 93 selected rice genotypes are presented in Table 1.

Table 1: Genetic variability parameters for different quantitative characters in heat tolerant accessins**.**

S.N.	Characters	Mean	Min.	Max.	GCV%	PCV%	h^2 (bs)	GA	SEm	CV%	GA as % 0f mean
1	Days to 50% flowering	83.96	75.00	96.00	5.20	6.51	64%	7.18	2.3273	3.9199	8.55
2	SPAD value (%)	31.40	26.25	42.05	11.24	12.00	88%	6.81	0.9344	4.2076	21.68
3	Panicle harvest index (%)	62.82	48.89	99.97	5.20	7.21	54%	7.16	2.3270	11.68	7.55
4	Flag leaf area(cm)	29.26	14.03	42.62	7.72	8.88	55%	4.59	0.3651	25.50	14.64
5	Membrane injury percentage (%)	24.37	24.25	46.05	10.14	11.00	66%	5.81	0.8344	3.2016	19.58
6	Panicle length (cm)	24.52	19.79	29.49	7.72	8.38	85%	3.59	0.5651	3.2597	14.64
7	Plant height (cm)	122.96	83.75	160.70	15.02	15.22	87%	37.53	2.1593	2.4834	30.52
8	Canopy Temp.	32.10	23.26	42.51	8.11	10.12	56%	6.18	0.7342	3.2112	20.19
9	Photosynthetic rate	17.02	14.00	19.90	6.82	7.82	54	4.32	0.3125	24.30	12.54
10	Transpiration rate	1.94	0.74	3.63	13.52	14.26	62	0.62	0.0321	2.1512	28.86
11	Number of Filled grain/panicle	117.91	55.05	303.55	36.23	36.73	87%	86.81	5.0243	6.0259	73.62
12	Number of unfilled grains/panicle	39.35	10.90	109.30	53.73	54.53	92%	42.49	0.5020	15.64	107.47
13	Total number of grains/panicle	145.00	73.00	319.40	34.31	34.85	87%	100.93	6.2177	6.0642	69.61
14	Spikelet fertility (%)	81.24	56.61	96.04	9.03	9.90	90%	13.79	2.3262	4.0494	16.98
15	Spikelet sterlity (%)	81.24	9.53	40.50	30.75	32.31	90%	14.57	0.195	9.9244	60.27
16	100 grain weight(g)	2.57	1.61	3.57	15.69	15.87	83%	0.82	0.0430	2.3637	31.96
17	Yield per plant (g)	18.72	8.35	29.45	25.99	26.12	89%	9.97	0.3478	2.6276	53.26

Days to 50% flowering
The days to 50% flowering were recorded between 75 days (R 1762-780-1-242-1) to 96 days (R 1966-49-1-20-1) with a mean of 83.96 days.

Fooland (2005) [5] reported that growth chamber and greenhouse studies suggest that high temperature is most deleterious when flowers are first visible and sensitivity continues for 10-15 days. Reproductive phases most sensitive to high temperature are gametogenesis (8-9 days before anthesis) and fertilization (1-3 days after anthesis) in various plants.

Chlorophyll content (%)
The SPAD value ranged from 26.25% (R 2127-918-1-369-1) to 42.05% (R2112-88-6-1-340-1) with an average of 32.04%.

Hilaliand Thimd (2015) reported that twenty genotypes of rice were evaluated for their physiological tolerance of cellular membranes, stomatal and photosynthetic pigments under high temperature stress.

Panicle Harvest Index
The Panicle harvest index was ranged from 48.89% (R1629-234-5-1882-2) to 99.97% (R 2127-918-1-369-1) with an average of 62.82%.

Kim *et al* (1996) reported that the rate of increase in dry matter in the panicle after the heading decreased under high temperature. This could be partly due to the increase in the number of sterile spikelets.

Flag leaf area (cm)
The Flag leaf area were ranged from 14.03 cm (R 2095-864-3-319-1) to 42.62cm (R 1902-3887-2-339-1) with an average of 29.26 cm.

Hsiao *et al.* (1976) reported that reduction in flag leaf area due to high temperature appeared to be a consequence of slowed cell enlargement.

Membrane injury percentage
The Membrane injury percentage were ranged from 24.25% (PSBRC-68) to 46.05% (R 1973-87-1-31-1) with an average of 24.37%.

High temperature affect membrane stability through lipid peroxidation, leading to the production of peroxide ions and malondialdehyde (MDA). (Wahid *et al*. 2007) [15]

Panicle length (cm)
The panicle length were ranged from 19.79 cm (R2112-88-6-1-340-1) to 29.49 cm (R 1661-605-84-1) with an average panicle length of 24.5 cm.

Kumar *et al.* (1999) [10] noted significantly variation for plant height, number of tiller per plant, flag leaf area, number of panicle per plant, number of spikelets per plant, yield per plant and test weight.

Plant Height (cm)
The plant height were ranged from 83.75 cm (RSR2011-12-1) to 160.70 cm (R 1919-573-1-160-1) with an average plant height of 122.96 cm.

The variation in plant height existed in different environment and was reduced due to high temperature and moisture availability. Reduction in plant height under stress has been reported by Nandaranjan and Kumaravelu (1994).

Canopy temperature

The canopy temperature were ranged from 23.26^{0}C (R 2196-1150-1-472-1) to 46.05^{0}C (Narendra 97) with an average canopy temperature of 32.10^{0}C.

The leaves may be 13^{0}C above air temperature indirect sunlight. If this temperature was added to a reasonable midsummer temperature of 35^{0}c, the leaf must then be at a temperature near 48^{0}c.

Photosynthetic rate

The photosynthetic rate were ranged from 14.00 (R 1966-49-20-1) to 19.90 (r1919-537-2-151-1) with an average of 17.02.

Heat stress tends to reduce the phoyosynthetic rate and induces early senescence and shortens the grain filling period.

Transpiration rate

Transpiration rate were ranged from 0.74 (R RF 75) to 3.63 (Karmamasuri) with an average of 1.94.

The transpiration rate significantly decreased with progress of heat stress and such reduction seems to be associated with decreasing plant water stress under water deficit condition. (Subramaninian and Maheshwari, 1990).

Number of filled grains per panicle

The number of filled grains per panicle is an important yield contributing trait, as it directly affects grain yield. It ranged from 55.05 (R 1801-132-2-13-1) to 303.55 (R1973-111--1-41-1) grains per panicle with an average of 117.91 filled grains per panicle.

Chakrabarti *et al*. (2010) [3] studied the effect of high temperature on pollen as well as spikelet sterility in Basmati and non-Basmati rice. Rise in temperature increased pollen sterility and reduced germination of pollen grains on the stigma. At 35.5 ^{0}C, variety Pusa Sugandh 2 (Basmati) recorded a pollen sterility of 17% and 26% reduction in pollen germination. Increased temperature during the grain filling period also increased spikelet sterility in rice variety. Non-Basmati rice varieties were less affected by increased temperature than Basmati types.

Number of unfilled grains per panicle

The number of unfilled grains per panicle were ranged from 10.90 (R 2095-864-3-319-1) to 109.30 (R1781-672-1-373-1) with an average of 39.35.

Baker *et al*. (1995) reported the trend of grain filling with increasing temperature due to decrease in number of filled grain per panicle.

Number of grains per panicle

Number of grains per panicle were ranged from 73 (R 1871-210-1-98-1) to 319.40 (Narendra 97) with an average of 145.00 grains per panicle.

Spikelet fertility percent

The spikelet fertility percent were ranged from 56.61 (R 1973-87-1-31-1) to 96.04% (R RF 75) with an average of 81.24%.

Prasad *et a*l. (2006) [6] reported that the high temperature significantly decreased spikelet fertility and can be used as a screening tool for heat tolerance during the reproductive phase.

Spikelet sterility percent

The spikelet sterility percent were ranged from 9.53% (R2164-1069-440-1) to 40.50% (R 1973-87-1-31-1) with an average of 24.17%.

Jagdish *et al*. (2011) studied that rice crops will be frequently exposed to water deficit and heat stress at the most sensitive flowering stage, causing spikelet sterility and yield losses.

100 grain weight (g)

100 grain weight were ranged from 1.6 g (R 2032-486-1-129-1) to 3.5 g (R 2095-864-3-319-1) with an average of 2.57g.

Zhang *et al.* (2013) [16] studied thirty six varieties of rice, grown in temperature controlled field chambers. Significant difference of grain yield between low night temperature and high night temperature was decreased by 16.7%, 9.6% and 8.0% than low night temperature in the four consecutive seasons, respectively.

Yield per plant (g)

The yield per plant was ranged from 8.35g (Chinnor) to 29.45g with an average of 18.72 g per plant.

Huang *et al*. (2009) [6] studied the effects of high temperature on the yields of three rice cultivars and 20 new strains. The results showed that the yields of the most cultivars and new strains were reduced for the high temperature at reproductive stage.

Coefficient of variation

The estimation of phenotypic and genotypic coefficient for respective quantitative characters in 93 selected rice genotypes. Among rice genotypes the highest coefficient of variation was observed for number of unfilled grainsper panicle (GCV=53.73, PCV=58.38) followed by number of grains per panicle (GCV=34.31, PCV=34.85).In addition to this other characters which shows high magnitude of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) (> 20%) were number of unfilled grains/panicle (53.73 and 54.53), followed by number of filled grains (36.23 and 36.73), total number of grains/panicle (34.31 and 34.85), spikelet sterility% (30.75 and 32.31) and yield per plant (25.99 and 26.12).The moderate estimation of GCV and PCV (10-20%) was observed for hundred grain weight (15.69 and 15.87), plant height (15.02 and 15.22),transpiration rate (13.52 and 14.26), chlorophyll (11.24 and12.00), and membrane injury percentage (11.24 and11.00).The low estimation of GCV and PCV (< 10%) were observed for spikelet fertility percentage (5.20 and 6.51), canopy temperature (8.11 and 10.12), flag leaf area (7.72 and 8.88), panicle length (7.72 and 8.38),photosynthetic rate (6.82 and 7.82), panicle harvest index (5.20 and7.21) and days to 50% flowering (5.20 and 6.51) respectively for GCV and PCV.

In the present study, for the different quantitative characters, the high magnitude of genotype and phenotypic coefficient of variation were observed for total number of grains per panicle, number of filled grains per panicle, number of unfilled grains per panicle and spikelet sterility percentage. Similar findings for days to 50 5 flowering and yield per plant have been reported by Markam (2013) [11].

Heritability

The heritability of the phenotypic value dependents on the estimates of heritability. Therefore high heritability helps in the effective selection based on a particular character. The magnitude of heritability was categorized as high (>70%), moderate (30-70%) and low (<30%). Among the all heat tolerant rice accessions, highest heritability in broad sense was observed for no. of unfilled grains (92.0) followed by spikelet fertility % (90.0). In addition to the above following characters exhibited high estimation of heritability were no. of unfilled grains per panicle (92.0), spikelet fertility percentage (90.0), spikelet sterility percentage (90.0), yield per plant(89.0), plant height (87.0), no. of filled grains (87.0), total no. of grains per panicle (87.0), panicle length (85.0) and panicle length (85.0),100 grains weight (83.0). The moderate estimation of heritability was found for membrane injury percentage (66.0), days to 50% flowering (64.0), transpiration rate (62.0), canopy temperature (56.0), flag leaf area, panicle harvest index (54.0), photosynthetic rate (54). None of the characters showed low heritability. High heritability values indicate that the characters under study are less influenced by environment in their expression.

The high estimation of heritability broad sense was observed for days to 50% flowering, which is accession to finding of Satyanarayan *et al*. (2005) [13]. The high estimation of heritability was also observed for plant height, yield per plant, panicle length, filled grains per panicle, spikelet sterility percentage is accordance to finding of Bisne (2009) [2].

Genetic advance as percentage of mean

The estimates of genetic advance as percent of mean provide more reliable information regarding the effectiveness of selection in improving the traits. Genetic advance denotes the improvement in the genotypic value of the new population over the original population. The genetic advance as per cent of mean was categorized as high (>20%), moderate (10-20%) and low (<10%).The highest genetic advance as percentage of mean was observed for number of unfilled grains per panicle (107.97) followed by number of grains per plant (69.61). In addition to other characters which exhibited high estimation of genetic advance mean was found for filled grains per panicle (73.62) followed by spikelet sterility percentage (60.27), yield per plant (53.26), hundred grains weight (31.96) and plant height (30.52), transpiration rate (28.86), chlorophyll content (21.68) and canopy temperature (20.19). The moderate estimation of genetic advance as percentage of mean was found for flag leaf area (14.64), membrane injury percentage (19.58), panicle length (14.64), spikelet fertility percentage (16.98) and photosynthetic rate (12.54).The low estimation of genetic advance as percentage of mean was found for days to 50% flowering (8.55), panicle harvest index (7.55).

The highest genetic advance as percentage of mean was found for unfilled grains for panicle, which is accordance to the finding of Markam (2013) [11].

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