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Validation of temperature induction response technique on combined effect of drought and heat stress in rice (*Oryza sativa* L.)

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ABSTRACT

Acquired tolerance traits can be evolved in plants by gradual induction of stress in a progressive manner. Here, we demonstrate a significant genetic variability in acquired tolerance traits among rice genotypes against combined effect of heat and drought stress after standardizing temperature induction response technique protocol.Expression of stress responsive genes during the sub-lethal induction stress and differences in the expression of these genes is the reason for observed genetic variability for stress tolerance. Five days germinated seedlings of rice varieties Jyothi (Ptb-39) and Vaishak (Ptb-60) were exposed to different temperatures to standardize both the lethal and induction temperature for TIR technique. Seedlings exposed to 52°C for 3 hr showed 100% mortality was selected as lethal temperature. TIR induced as well as non-induced plants of ten rice varieties (Nagina 22, Apo, CR Dhan 305, CR Dhan 307, Ptb-7, Ptb-15, Ptb-30, Ptb-39, Ptb-43 and Ptb-60) were exposed to both the abiotic drought and heat stress and were used to study the morpho-physiological and molecular responses. Based on the percentage of seedlings survival rate lethal temperature was standardized as 52°C for 3 h. Standardized induction temperature was 32-42ºC for 5 h and 42-52ºC for 30 min followed by 52ºC for 3 h based on the recovery growth of varieties. TIR induced plants exhibited better performance on morphological and physiological traits than non-induced plants in all the genotypes based on better stress tolerance and yield parameters. Nagina 22 and Apo were selected as the best genotypes for stress related traits. Tolerance of genotypes towards stress has been attributed to changing transcript levels of stress induced genes. The results revealed that temperature induction response technique can be used as a prospective tool for improving the performance of high yielding susceptible genotypes under stress conditions.

Keywords*:* Drought stress, heat stress, rice, temperature induction response technique

Rice (*Oryza sativa* L.) is a carbohydrate-enriched food that contributes more than 50% of caloric intake of world population, 90% of rice production and consumption concerted in Asia (Fitzgerald *et al*., 2009). Globally, rice is cultivated in an area of 163.2 million hectares, of which 45% is under rainfed cultivation with less productivity due to lack of resources and exposure to abiotic stresses (FAO, 2017). In recent years, rice cultivation has been threatened by major production constraints especially because of the adverse effects of climate change in India (Beena, 2015).To meet the targeted food production during 2025, there should be an increase of 40% in yield from drought-prone areas having difficult ecosystem (Kumar and Gautham, 2014). Farmers are adopting better cultural and management practices for rice production, but the productivity of rice is not increasing as expected (Beena *et al*., 2013). This fluctuation in rice yield in various agricultural regions is mainly due to changes in climatic factors (Jagadish *et al*., 2007), which include both biotic and abiotic factors. Among abiotic factors, drought and high temperature are considered as key stress factors with high potential impact on crop yield. According to IPCC (2014) the expected rise in global air temperature by 2100 is 0.2°C–0.4°C per decade which is expected to increase by 1.8°C– 4.3°C compared to the current point. It was reported that 0.51°C per 100 year is the warming trend for India during the period of 1901-2007. In tropical and subtropical areas, rice productivity is affecting mainly by high temperature (Shah *et al*., 2011), and the elevated temperature results in grain sterility injurious to rice yield (Prasad *et al*., 2006; Beena *et al*., 2018a). Worldwide food production is highly affected by drought. Occurrence of multiple stresses increase the severity of damage by additive effects of individual stresses. TIR (Temperature Induction Response) is a technique which is an efficient methodology to recognize thermo-tolerant genotypes or lines from a natural collection of genotypes or from a segregating mapping population. Induction of thermo- tolerance can be done by gradual increase in temperature to lethal temperature as would be experienced in natural environment (Larkindale *et al*., 2005). Plants develop the ability to withstand under lethal temperatures by acclimation through the mechanisms of avoiding heat damage along with the repair of heat-sensitive components (Kheir *et al*., 2012). As per the predicted climatic scenarios in the coming future, rice may expose

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to various kinds of stresses frequently in field condition. Therefore, it is imperative that the adaptability of rice to various abiotic stresses should be improved (Prince *et al*., 2015; Rejeth *et al*., 2020; Manikanta *et al*., 2020; Nithya *et al*., 2020; Pravallika *et al*., 2020). In this present study, an attempt was made to standardize the temperature induction response technique for rice genotypes and to validate the effect of TIR technique on combined effect of drought and heat stress in rice.

MATERIALS AND METHODS

Standardization of TIR technique in rice (Figure 1)

Rice varieties Jyothi (Ptb-39) and Vaishak (Ptb-60) were selected for standardization of TIR technique. Jyothi (Ptb-39) is a low land high yielding drought susceptible variety and Vaishak (Ptb-60) is an upland drought tolerant variety.

Identification of lethal temperature

The germinated rice seedlings (120 h) of two rice varieties Jyothi (Ptb-39) and Vaishak (Ptb-60) were exposed to various temperatures for a period of time for diverse durations *viz*., 49, 50, 51 and 52°C for 2, 2 ½ and 3 h followed by recovery for 72 h at room temperature. The percentage of survival was recorded at the completion of the recuperation cycle (Senthil, 2001). The temperature at which 100 per cent mortality of seedlings was observed was determined to be the lethal or challenging temperature. This temperature was used to challenge the seedlings to assess the genetic variability.

Identification of optimum induction temperature

The optimum induction temperature was defined as temperature at which there was maximum recovery in development and the lowest reduction in percent in growth (Senthil, 2001). To find out what temperature is best for induction, rice seedlings were subjected to different treatments *viz*, 28-40 ºC for 5 hr and 40-52 ºC for 30 min,32-40 ºC for 5 hr and 40-52 ºC for 30 min and 32-42 ºC for 5 hr and 42-52 ºC for 30 min followed by standardized lethal temperature followed by recovery for 72 hr at room temperature.This experiment was done in a temperature-controlled incubator. In first treatment (28-40 ºC for 5 hr and 40-52 ºC for 30 min) where the temperature was increased gradually from lowest temperature (28 ºC) to highest temperature (40 ºC) within that particular period of time (5hr) and then temperature was increased gradually from 40 to 52 ºC within 30 min.

Root and shoot length of induced and control seedlings were recorded after recovery period. The seedlings in the control group were kept at room temperature throughout the experimental period. Over absolute power, the recovery growth and per cent reduction in recovery growth were estimated (Kumar *et al*., 1999).

Per cent survival of seedlings = (No. of seedlings survived at the end of the recovery/ total no. of seeds sown) X 100.

Recovery growth (cm)

Before exposing the seedlings to various temperature treatments initial measurement (root and shoot length) of seedlings were made. Similarly, after treatments the final seedling growth was also measured and based on the initial and final growth of the seedlings, recovery growth of seedlings was determined.

Recovery Growth = Final growth – Initial growth

Per cent reduction in recovery growth (% RRG)

The recovery growth measured in the absolute control was used to calculate the % reduction in recovery growth of treatments.

$$\% \text{ RRG} = \frac{\text{Recovery growth of control seedlings} - \text{Recovery growth of}}{\text{Recovery growth of control seedlings}} \times 100$$

To study the effect of TIR technique for rice with a combination of drought and heat stress treatment

Ten rice varieties were used to study the effect of TIR technique for a combination of drought and heat stress, specifically, Nagina 22 (N-22), Apo, CR Dhan 305, CR Dhan 307, Ptb-7, Ptb-15, Ptb-30, Ptb-39, Ptb-43 and Ptb-60 (Table 1). Among the genotypes selected, N-22 is a drought and heat tolerant variety. Apo, Ptb-7, Ptb-30, Ptb-43 and Ptb 60 are upland drought tolerant varieties. CR Dhan 305, CR Dhan 307 and Ptb-39 are high yielding varieties suitable for irrigated lowland system. Ptb-15 is a traditional lowland rice land race with deep root system suitable for irrigated lowland system. Five days old germinated seeds of rice varieties were exposed to standardized TIR technique. TIR induced plants along with non-induced plants were exposed to combined drought and heat stress. Drought stress was imposed by withholding irrigation for five days from panicle initiation and heat stress was imposed by maintaining plants at 40-42°C from panicle initiation to maturity in a controlled polyhouse facility. Non-induced plants without stress were kept as absolute control. Various physiological and molecular observations were made on ten days after stress induction.

Statistical analysis

ANOVA was performed for each variable; subsequently ANOVA was used to determine whether there were differences among the rice genotypes. Treatments in the experiments were arranged in a completely randomized design (CRD), with five replications. The data on various parameters were analyzed statistically as per the procedure of Gomez and Gomez (1984).

Morpho-physiolgical observations

Plant height, root length, root volume were measured as per standard protocol.

Photosynthetic rate and stomatal conductance:

The Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) was used to measure stomatal conductance and photosynthetic rate at morning time between 9 am and 11 am and were expressed in m H_2O moles m^{-2} S^{-1} and µ CO_2 moles m^{-2} S^{-1} respectively.

Cell membrane stability index

The Blum and Ebercon (1984) protocol was used to measure the cell membrane stability index.

Chlorophyll stability index

Chlorophyll content of leaf samples were estimated as per the procedure by Arnon (1949).

RT- PCR analysis

Expression level of *DRO 1* (Deeper Rooting 1) was studied in induced and non-induced plants of one tolerant genotype (Nagina 22) and one susceptible genotype (Ptb 39- Jyothi) under combined drought and heat stress by using RT (Reverse Transcriptase) PCR. RNA was isolated using TRIZOL™ reagent.

RNA isolation was done by Triazol method. The Complementary DNA (cDNA) synthesis was done using Thermo scientific verso cDNA Synthesis kit Product code AB-1453/A. About 4µl of 5X cDNA synthesis buffer, 2µl of dNTP mix, 1µl of anchored oligo dT, 1µl of RT Enhancer, 1µl of Verso Enzyme Mix and 5 ìl of RNA template (1ng of total RNA) were added to an RNA*ase* free tube. The total volume of the reaction was 20 ìl using sterile distilled water. The Eppendorf Master Cycler (thermal cycler) was programmed to perform cDNA synthesis. The following cycling conditions were employed, 30 min at 42ºC and 2 min at 95ºC.

The amplification was done using Thermo scientific amplification kit. For each 50 µL reaction: 25 µL of PCR Master Mix (2X), 2 µL of Forward primer (ATATGGGCGTACGGTAGCTG) (0.1-1.0 µM), 2 µL of Reverse primer (AGAGATTGGGGAGGGACAAA) (0.1-1.0 µM), 5 µL of Template DNA (10 pg - 1 µg) were used. The components were made upto 50 µL with sterile distilled water (nuclease-free). Initial denaturation at 95°C for 3 min, followed by denaturation at 95°C for 30 sec, annealing at 60 °C for 30 sec and extension at 72°C for 1 min was performed for 35 cycles, with the final extension taking place at 72°C for 5 minutes. The PCR product was segregated by agarose gel electrophoresis after amplification.

RESULTS AND DISCUSSION

Lethal temperature was standardized from four different temperatures at three different durations and the percentage seedling survival after the treatment was presented in Table 2. Results showed that as the temperature increased survival rate was decreased. Seedlings exposed to 52°C for 3 hr showed 100% mortality and hence this treatment was selected as lethal temperature. Harihar *et al*. (2014) reported lethal temperature of rice as 52°C for 3 h with 98 % mortality. Vijayalakshmi *et al*. (2015) determined that a temperature of 54°C for 3 h was the best challenging temperature for screening of rice seedlings for inducing thermotolerance at cellular level. In the case of bananas, a similar analysis was carried out, where 55°C for 2h was selected as lethal temperature with 11% survivability (Vidya *et al*., 2017). Beena *et al*. (2018b) also studied the principle of "acquired tolerance" states that exposure to sub-lethal temperature was required to induce thermo-tolerance in rice. In that experiment, the seedlings were exposed to a gradual temperature rise over 3 h from 38 to 48 °C, which was considered as the induction treatment. Following that, seedlings were immediately exposed to a high temperature setting for 3 h at 54°C. The results showed that among the landraces, Njavara and Chenellu had the least reduction in per cent root and shoot growth with a mortality of 18 and 10% respectively as a result of the induction treatments. The physiological basis of thermo-tolerance was explained by strong antioxidant system with lower lipid peroxidation as recorded by the lesser malondialdehyde content with a higher chlorophyll stability index. Seedling recovery growth was presented in Table 3. Seedlings were subjected to induction temperatures before exposing to standardized lethal temperature. Mean recovery growth was maximum (7.8 cm) for the third treatment (32-42ºC for 5 h and 42-52ºC for 30 min followed by 52ºC for 3 h). The least

Table 1: List of rice accessions and their parentage used in the study

Sl.No.	Genotypes	Parentage or Pedigree
V_1	N22 (Nagina)	A selection from Rajbhog
V_2	Apo	IR55423-01
V_3	CR Dhan 305	R 77080-B34-3/IRRI 123 2
V_4	CR Dhan 307 (Maudamani)	Dandi / Naveen // Dandi
V_5	Ptb 7 (Parambuvattan)	Landrace
V_6	Ptb 15 (Kavunginpoothala)	Landrace
V_7	Ptb 30 (ChuvannaModan)	Landrace
V_8	Ptb 39 (Jyothi)	Ptb-10 x IR-8
V_9	Ptb 43 (Swarnaprabha)	Bhavani x Triveni
V_{10}	Ptb 60 (Vaishak)	Selection from Ptb 43 (Swarnaprabha)

Table 2: Percentage of seedling survival under of rice genotypes under different treatments

Sl.No.	Treatments	Jyothi (Ptb39)	Vaishak (Ptb60)	Mean A
1	49°C for 2 hrs	96.7	98.4	97.5
2	49°C for 2 ½ hrs	95.0	95.0	95.0
3	49°C for 3 hrs	91.7	91.7	91.7
4	50°C for 2 hrs	73.3	88.3	80.8
5	50°C for 2 ½ hrs	66.7	78.3	72.5
6	50°C for 3 hrs	50.0	70.0	60.0
7	51°C for 2 hrs	48.3	46.7	47.5
8	51°C for 2 ½ hrs	41.7	41.7	41.7
9	51°C for 3 hrs	23.3	22.9	23.1
10	52°C for 2 hrs	11.7	13.3	12.5
11	52°C for 2 ½ hrs	9.8	10.0	9.9
12	52°C for 3 hrs	0	0	0
13	Control (Ambient temperature)	100.0	100.0	100.0
	Mean B	**54.5**	**58.2**	
		LSD (0.05)	**SEm±**	
	G	**1.6**	**0.6**	
	T	**4.1**	**1.4**	
	GxT	**5.9**	**2.1**	

Table 3: Recovery growth and per cent reduction in recovery growth of seedlings (in parentheses) after different induction temperature treatments

Treatments	Jyothi	Vaishak	Mean
T1-28-40 °C for 5 hrs& 40-52 °Cfor 30 min	5.2 (47.7)	5.1 (42.4)	5.3 (53)
T2-32-40 °Cfor 5 hrs& 40-52 °Cfor 30 min	6.6 (41.8)	5.7 (35)	6.1 (38.4)
T3-32-42 °Cfor 5 hrs& 42-52 °Cfor 30 min	8.8 (21.8)	6.8 (22.5)	7.8 (22.2)
T4-Control (Ambient temperature)	11.3	8.8	10.0
Mean	**8.0 (38.9)**	**6.6 (33.3)**	
		LSD (0.05)	**SEm±**
	G	**0.4 (5.3)**	**0.1 (1.7)**
	T	**0.5(4.3)**	**0.2(1.4)**
	G x T	**0.7(NS)**	**0.2(2.4)**

Table 4: Root volume (cm^3) of rice genotypes under combined drought and heat stress

Genotypes	Induced plants under combined stress	Non-induced plants under combined stress	Control plants	Mean
V_1	30.9	29.3	28.2	29.5
V_2	30.7	30.3	29.5	30.2
V_3	19.8	18.5	32.8	23.7
V_4	21.6	20.4	32.7	24.9
V_5	26.1	22.1	32.6	26.9
V_6	38.3	32.0	44.6	38.3
V_7	33.2	31.5	37.0	33.9
V_8	19.3	16.6	23.7	19.9
V_9	19.8	17.7	27.8	21.8
V_{10}	29.4	25.9	33.8	29.7
Mean	**26.9**	**24.4**	**32.3**	
		LSD (0.05)	**SEm±**	
	G	**1.8**	**0.6**	
	T	**0.9**	**0.3**	
	GxT	**2.9**	**1.0**	

Table 5: Photosynthetic rate (ì mol CO_2 m^{-2} s^{-1}) and Stomatal conductance (m moles m^{-2} s^{-1}) of rice genotypes under combined drought and heat stress

Genotypes	Photosynthetic rate (ì mol CO_2 m^{-2} s^{-1})				Stomatal conductance (m moles m^{-2} s^{-1})			
	Induced plants under combined stress	Non-induced plants under combined stress	Control plants	Mean	Induced plants under combined stress	Non-induced plants under combined stress	Control plants	Mean
V_1	19.3	18.5	20.2	19.3	888.3	929.7	816.0	878.0
V_2	21.7	21.2	24.9	22.6	834.3	818.3	753.3	802.0
V_3	14.6	14.7	24.3	17.9	632.0	608.0	766.0	668.6
V_4	19.3	22.2	22.1	21.2	631.0	616.3	807.7	685.0
V_5	19.6	17.6	25.2	20.8	740.0	695.7	684.3	706.6
V_6	18.0	19.4	25.4	20.9	827.3	796.7	766.7	796.8
V_7	20.6	18.7	23.7	20.9	852.7	836.0	736.7	808.4
V_8	10.7	8.6	21.5	13.6	434.0	386.0	850.7	556.8
V_9	12.2	11.6	21.2	15.0	583.3	574.0	853.3	670.2
V_{10}	20.8	19.2	24.4	21.5	662.7	586.7	876.0	708.4
Mean	**17.7**	**17.2**	**23.3**		**708.6**	**684.7**	**791.1**	
	LSD (0.05)	**SEm±**			**LSD (0.05)**	**SEm±**		
G	**1.0**	**0.4**			**26.0**	**9.179**		
T	**0.6**	**0.2**			**14.3**	**5.028**		
GxT	**1.7**	**0.6**			**45.1**	**15.899**		

reduction in recovery growth (22.2%) (Table 2) was also recorded in the third treatment (32-42ºC for 5 h & 42-52ºC for 30 min followed by 52ºC for 3 h) and hence this treatment was selected as optimum induction temperature. Harihar *et al*. (2014) reported 36-44ºC for 5h as the induction temperature in rice. Vijayalakshmi *et al*. (2015) reported a gradual increase in induction temperature in rice for 3 h from 38 to 48°C.

Plant height was measured from the base of the shoot to tip of the topmost panicle at maturity. Differences in the plant height was significant with respect to genotype and treatment. Except the variety N-22, all other varieties

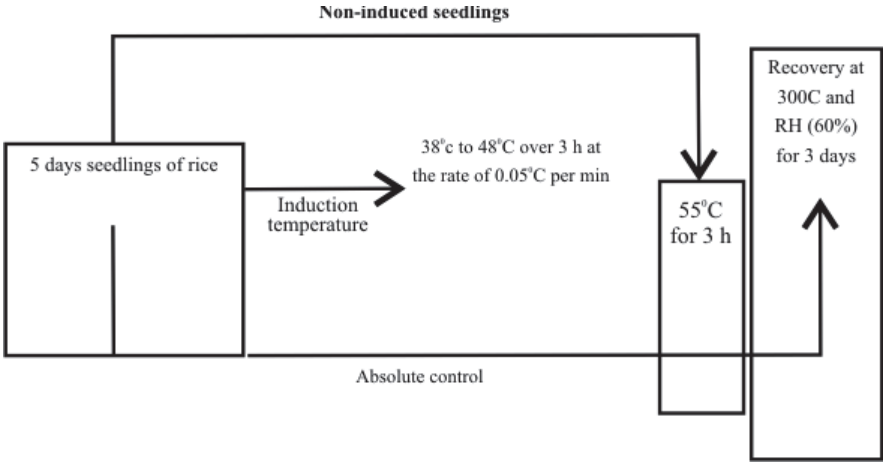


Fig.1: Protocol to induce high temperature tolerance through the temperature induction response (TIR) technique (Kumar *et al*., 1999)

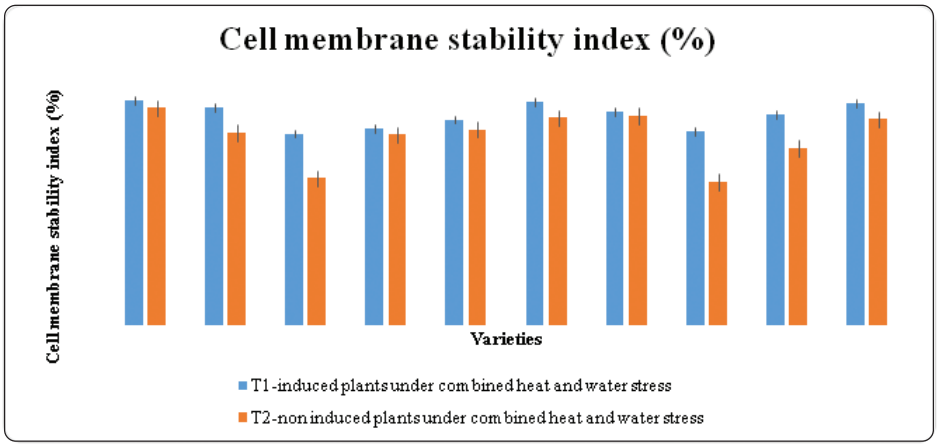


Fig. 2: Variation in cell membrane stability index (%) of rice varieties under combined drought and heat stress

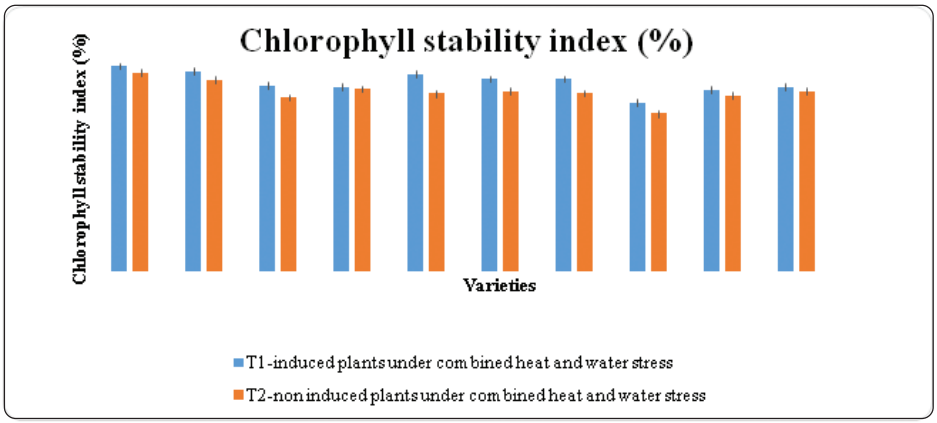
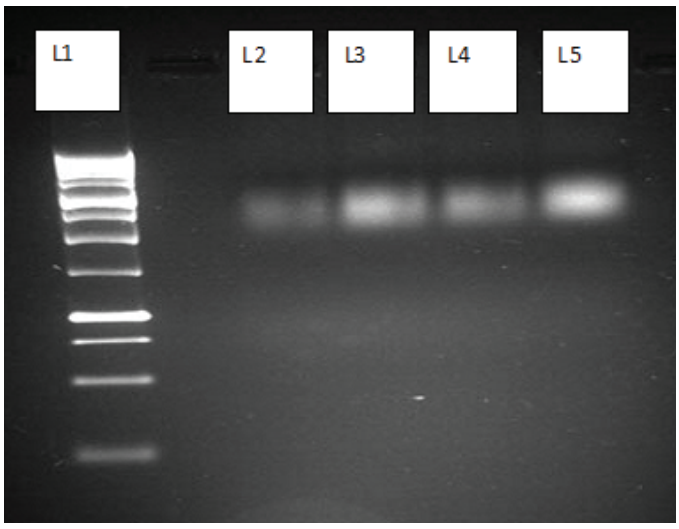


Fig. 3: Variation in chlorophyll stability index (%) of rice varieties under combined drought and heat stress



L 1-100bp Ladder;L2- Non induced- Ptb 39;L3- Induced– Ptb 39;L4- Non induced–N-22; L 5- Induced –N-22

Plate 1: Expression analysis of gene *DRO1* using Reverse Transcriptase PCR in induced and non-induced plants of Ptb-39 and N-22 under combined drought and heat stress

showed reduction in plant height under stress condition. Among the varieties, Ptb-15 (144.8 cm) recorded the highest mean value, followed by variety Ptb-43 (141.1 cm) and the variety Ptb-39 (96.1 cm) recorded the minimum plant height followed by Apo (107.7 cm). Induced plants showed least reduction in plant height as compared to non-induced plants. Similar results were reported by Chandola (2015) in tomato, where plant height was found to increase in induced plants as compared to non-induced plants. Prasad *et al*. (2006) reported that there was reduction in plant height and stem growth as a result of prolonged exposure to high temperatures.

In all the varieties root length was increased under stress as compared to control. Induced plants showed 9.8% increase whereas non-induced plants showed only 1.5% increase as compared to control. Mean value for root length was maximum in variety Apo (47.9 cm) and variety Ptb-15 (47.8 cm). According to Porter and Gawith (1999), root growth decreases under heat stress because root growth is favoured under very narrow range of temperature. Greater root length will minimize the occurrence of stress through development of a healthy root system, which in case of drought, permits to absorb water from deeper soil layer (Lopes and Reynolds, 2010; Manikanta *et al*., 2020).

Among the treatments, there was significant reduction in root volume under stress as compared to control plants (Table 4). Both induced as well as non-induced plants under stress showed reduction in root volume. The reduction was recorded more in non-induced plants (24.5%) than induced plants (16.7%). Variety Ptb-15 showed the maximum value for root volume (38.3 cm^3). Even though most of the genotypes showed reduction in root volume under stress, genotypes such as N-22 and Apo showed increase in root volume under stress. This increase was attributed to their ability to withstand under drought by increasing root biomass and absorb moisture from deeper layers of soil. This result was supported by the findings of Ekanayake *et al*. (1985); Beena *et al*. (2017), who reported that deep and thick root system allows access to water in deeper layer and considered it as an important trait determining drought tolerance in rice.

Mean value for stomatal conductance was reduced significantly under combined stress condition (Table 5). But the varieties N22, Apo, Ptb-7, Ptb-15 and Ptb-30 showed higher values as compared to absolute control. In induced plants, conductance through stomata was higher as compared to non-induced plants in order to maintain optimum CO_2 intake. Morales *et al*. (2003) reported that in tomato pre-conditioning plants with high temperature showed high osmotic adjustment by maintaining the osmotic potential and stomatal conductance, and better growth than non-conditioned plants. Significant genotypic differences for starch, soluble sugars, titrable acidity (TA), total soluble solids (TSS), lycopene content, yield attributes *viz*., number of fruits/plants, fruit set %, average fruit weight (g) and yield per plant (g/plant) were observed among tomato

genotypes under high temperature stress condition (Vijayakumar *et al*., 2021). Tolerance of plants to simultaneous occurrence of both drought and heat stress mainly depends on the maintenance of leaf temperature by better stomatal conductance (Mittler and Blumwald, 2010).

Data recorded for photosynthetic rate of rice varieties is presented in Table 5. Observed mean value was maximum for Apo (22.6 ì mol CO_2 m^{-2} s^{-1}) and minimum for Ptb-39 (13.6ì mol CO_2 m^{-2} s^{-1}). In control set Ptb-15 recorded maximum photosynthetic rate (25.4 ì mol CO_2 m^{-2} s^{-1}) followed by Ptb-7 (25.2 ì mol CO_2 m^{-2} s^{-1}). The rate of photosynthetic activity was observed, a reduction under high stress condition and the reduction was more distinct in the case of non-induced plants except in CR Dhan 307(Maudamani) and Ptb15 (Kavuginpoothala). These two varieties were identified for abiotic stress tolerance Nithya *et al*. (2020) Oh-e *et al*. (2007) reported that photosynthetic rate was reduced by 40-60% at the mid-ripening stage under high temperature, thereby leading to enlarged rate of senescence of the flag leaf. Under water limiting conditions, there are two ways through which photosynthesis is influenced. Reduction in photosynthetic rate can be due to the impairment of metabolic activities or by closure of stomata thereby decreasing the flow of CO_2 into mesophyll cells which is a pathway regulation (Flexas *et al*., 2004).

Cell membrane stability index of rice varieties under stress is presented in Fig. 2. In all the varieties stability was reduced under stress and the reduction was recorded more in non-induced plants. TIR induced plants showed higher membrane stability under stress as compared to non-induced plants. These results are in line with findings of Chandola (2015) he reported that thermally induced plants showed increase in membrane stability as compared to the plants which were not induced at seedling stage. Fig. 3 showed the variation in chlorophyll stability index of ten different rice varieties when there was a drought and a heat stress at the same time. Chlorophyll stability was reduced under stress and it was more in the case of non-induced plants than induced plants. Similar results were obtained by Chandola (2015), where reduction in chlorophyll stability was recorded under all the treatment without induction. Maximum reduction of 25.7% was reported in plants subjected to combination of heat and water stress without thermal induction. Decreased chlorophyll content and chlorophyll stability index under combined drought and heat stress was found in wheat by Sairam *et al.*(1997) and Beena (2005). Burke (1998) reported that acclimated wheat plants showed higher chlorophyll stability.

Expression level of *DRO1* gene was analysed in root samples of induced and non-induced plants of two selected genotypes (tolerant- Nagina 22 and susceptible- Ptb-39), and the result is given in Plate 1. Relative expression of *DRO1* gene was recorded highest in induced plants of tolerant genotype, N22 followed by induced plants of susceptible genotype, Ptb-39. Relative intensity was comparatively less in non-induced plants of both the genotypes. Uga *et al*. (2011) reported that *DRO1* was involved in drought avoidance by controlling root angle under water stress. The most important utility of *DRO1* is that the rooting pattern can be modified from a shallow to a deep system, thereby improving the drought avoidance mechanism.

In this study, TIR protocol for rice was standardized as 32-42ºC for 5 hr and 42-52ºC for 30 min followed by 52ºC for 3 hr. Under combined drought and heat stress all the parameters analyzed were reduced and the reduction was more in non-induced plants. Tolerance of genotypes towards stress has been attributed to changing transcript levels of stress induced genes. Among the varieties, Nagina-22, Apo and Ptb-15 were performed better under stress condition in terms of morphological, physiological and biochemical parameters. TIR technique can also be used as a stress mitigation strategy for improving the performance of high yielding susceptible genotypes under stress conditions.

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REFERENCES

Arnon, D.I., 1949. Copper enzyme in isolated chloroplasts: polyphenoloxidase in Beta vulgaris. *Plant Physiol*., **24**: 1-15.

Beena, R. 2005. Studies on physio- morphological traits and genetic markers associated with drought responses in rice (*Oryza sativa* L.). Ph.D thesis submitted to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Beena, R., Praveenkumar, V.P., Vighneswaran, V., Sindhumol, P and Narayankutty, M.C. 2017. Phenotyping for root traits and carbon isotope in rice genotypes of Kerala. *Oryza, An Int. J. Rice*,**54**(3): 282-289.

Beena, R., V. Vighneswaran, Sudarsana Rao, S.R. ,Voleti. and Narayankutty, M.C. 2013. A rice variety suitable for alternate wetting and drying conditions of Kerala. *Annals Plant Physiol.*, **27**(1): 1-4.

Beena, R., Veena, V. and Narayankutty, M.C. 2018b. Evaluation of rice genotypes for acquired thermo-tolerance using Temperature Induction Response (TIR) technique. *Oryza,* **55**(2): 285-291.

Beena, R., Vighneswaran, V., Sindumole, P., Narayankutty, M.C. and Voleti, S.R. 2018a. Impact of high temperature stress during reproductive and grain filling stage in rice. *Oryza,* **55**(1):126-133.

Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.*, **21**(1), 43-47.

Burke, J.J. 1998. Identification of genetic diversity and mutations in higher plant acquired thermo-tolerance. *Physiol. Plant*, **11**: 167-170.

Chandola, A., 2015. Temperature Induction Response of Tomato Cultivars in Mitigation of Water and Heat Stress. Institute of Agricultural Sciences, Banaras Hindu University. 144p.

Ekanayake, I.J., O'Toole, J.C., Garrity, D.P. and Masajo, T.M. 1985. Inheritance of root characters and their relations to drought resistance in rice. *Crop Sci.*, **25**: 927-933.

Fitzgerald, M.A., McCouch, S.R. and Hall, R.D. 2009. Not just a grain of rice: the quest for quality. *Trends Plant Sci.*, **14**:133-139.

Flexas, J., Bota, J., Loreta, F., Cornic, G. and Sharkey, T.D., 2004. Diffusive and metabolic limitation to photosynthesis under drought and salinity in C3 plants. *Plant Biol.*, **6**: 269-279.

Food and Agriculture Organization of the United Nations. 2017. *Rice market monitor,* 20: 1–38.

Gomez, K.A., and Gomez, K.A. 1984. Statistical procedures for agricultural research in IRRI book. New York: Wiley.

Harihar, S., Srividhya S., Vijayalakshmi, C. and Boominathan, P. 2014. Temperature induction response technique - A physiological approach to identify thermo-tolerant genotypes in rice. *Int. J. Agri. Sci.*, **10**(1): 230-232.

IPCC. 2014. "Climate Change 2014: mitigation of climate change," in contribution of working 766 group III to the fifth assessment report of the intergovernmental panel on climate change. In: Edenhofer, R., Pichs-Madruga, Y.,Sokona, J. C., Minx, E., Farahani, S., Kadner, K., Seyboth, A., Adler, I., Baum, S., Brunner, P., Eickemeier, B.,Kriemann, J., Savolainen, S., Schlömer, C., Stechow, T. Zwickel (eds.), Cambridge University Press, New York, pp.1435.

Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R. 2007. High temperature stress and spikelet fertility in rice. *J. Exp. Bot.,***58**: 1627–1635.

Kheir, E.A., Sheshshayee, M.S., Prasad, T.G. and Udayakumar, M. 2012. Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *J. Cotton Sci.*, **16**: 190-199.

Kumar, G., Krishnaprasad, B.T., Savitha, M., Gopalakrishna, R., Mukhopadhyay, K., Ramamohan, G. and Udayakumar, M. 1999. Enhanced expression of heat-shock proteins in thermo-tolerant lines of sunflower and their progenies selected on the basis of temperature-induction response. *Theor. Appl. Genet.*, **99**: 359-367.

Kumar, R. and Gautam, H., 2014. Climate change and its impact on agricultural productivity in India. *J. Climatol. Weather forecast.,* **2**(1): 1-3.

Larkindale, J., Hall, J.D., Knight, M.R. and Vierling, E. 2005. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermo-tolerance. *Plant Physiol.*, **138**: 882–897.

Lopes, M.S. and Reynolds, M. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.*, **37**: 147–156.

Manikanta, Ch.L.N., Beena R., Roy, S., Manju, R.V., Viji, M.M and Swapna, A. 2020. Physio-morphological plasticity of rice (*Oryza sativa* L.) genotypes exposed to water stress. *J. Tropical Agric.*, **58**(1): 139-145.

Mittler, R. and Blumwald, E. 2010. Genetic engineering for modern agriculture: challenges and perspectives. *Annu. Rev. Plant Biol,***61**: 443-462.

Morales, D., Rodriguez, P., Dellamico, J., Nicolas, E., Torrecillas, A. and Blanco, M.J. 2003. High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biol. Plant*, **47**: 203-208.

Nithya, N., Beena, R., Abida, P.S., Sreekumar, J., Roy, S., Jayalekshmi, V.G., Manju, R.V. and Viji, M.M. 2020. Genetic diversity and population structure analysis of bold type rice collection from Southern India. *Cereal Res. Commun.*, 1-18. https://doi.org/10.1007/s42976-020-00099-w.

Oh-e, M.M., Carey, E.E. and Rajashekar, C.B. 2009. Environmental stresses induce health-promoting phytochemicals in lettuce. *Plant Physiol. Biochem.*, **47**:578– 583.

Porter, J.R. and Gawith, M., 1999. Temperature and the growth and development of wheat: a review. *Eur. J. Agron.*, **10**: 23-36.

Prasad, P.V.V., Boote, K.J., Allen, L.H., Sheehy, J.E. and Thomas, M.G. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Res.*, **95**: 398–411.

Pravallika, K., Arunkumar, C., Vijayakumar, A., Beena,R. and Jayalekshmi, V.G. 2020. Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.). *J. Crop Weed*, **16**(2): 18-23.

Prince, S.J., Beena, R., Michael Gomez, S., Senthive, J.S. and Chandra Babu, R. 2015. Mapping consistent yield QTLs under drought stress in target rainfed environments. *Rice*, **8**:25.

Rejeth, R., Manikanta, Ch.L.N., Beena, R., Roy, S., Manju, R.V. and Viji, M.M. 2020. Water stress mediated root trait dynamics and identification of microsatellite markers associated with root traits in rice (*Oryza sativa* L.). *Physiol. Mol. Biol. Plants*, **26**(6):1225-1236. http:/ doi.org/10.1007/s12298-020-00809.

Sairam, R.K., Desmukh, P.S. and Shukla, D.S. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron Crop Sci.,***178**(3) :171- 177.

Senthil, K. 2001. Development and characterization of thermotolerant sunflower (*Helianthus annus* L) hybrid:An approach based on temperature induction response (TIR) and molecular analysis. PhD. Thesis, Department of crop Physiology, University of Agricultural sciences, Bangalore, Karnataka (India).

Shah, F., Huang, J., Cui, K., Nie, L., Shah, T. and Wang, C.C.K. 2011. Impact of high-temperature stress on rice plant and its traits related to tolerance. *J. Agr. Sci.,* **149**: 545-556.

Uga, Y., Okuno, K. and Yano, M. 2011. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *J. Exp. Bot.,***62**(8): 2485-2494

Vidya, S.M., Laxman, R.H., Bhatt, R.M., Rekha, A., Sunoj, V.S.J. and Ravishankar, K.V. 2017. Temperature induction response technique: a screening tool for evaluation of banana cultivars for thermo-tolerance. *Indian J. Plant Physiol.*, **22**(1):79- 84.

Vijayakumar, A., Shanija, S., Beena, R., Sarada, S., Sajitha, R.T., Roy, S., Manju, R.V. and Viji, M.M. 2021. High temperature induced changes in quality and yield parameters of tomato (*Solanum lycopersicum* L.) and similarity coefficients among genotypes using SSR markers. *Heliyon*, **7**(2):https:/ /doi.org/10.1016/j.heliyon.2021.e05988

Vijayalakshmi, D., Srividhya, S., Vivitha, P. and Raveendran, M. 2015. Temperature induction response (TIR) as a rapid screening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify novel donors for high temperature stress tolerance. *Indian J. Plant Physiol.*, **20**(4): 368-374.