Oryza Vol. 55 No. 2, 2018 (285-291)
DOI 10.5958/2249-5266.2018.00035.8

Evaluation of rice genotypes for acquired thermo-tolerance using Temperature Induction Response (TIR) technique

Beena R*, Veena Vighneswaran and Narayankutty MC

Regional Agricultural Research Station, Pattambi, Kerala Agricultural University,Thrissur, Kerala, India

**Corresponding author e-mail: beenaajithkumar@gmail.com*

Received : 26 May 2018 Accepted : 21 June 2018 Published : 27 June 2018

ABSTRACT

Global climate change is leading to asymmetric atmospheric warming with reduced temperature differences between day and night. Increase in temperature alters broad range of physiological processes, such as growth and development, pollination and fertilization and ultimately affecting the yield. Hot summers in many agricultural regions can negatively affect the vegetative and reproductive growth phases of such crops and can result in up to 80% losses in rice yield. However, heat stress has numerous specific effects depending on the genotype. Physiological observations both under field and greenhouse conditions show a variable degree of tolerance between different genotypes. In this study, a screening protocol was developed based on the principle of "acquired tolerance" in which exposure of seedlings to a sub-lethal level of specific stress is used to induce tolerance to a subsequent lethal level of stress. Seedlings were subjected to a gradual temperature increase from 38 to 48 °C for 3 h (induction treatment), immediately followed by challenging at 54°C for 3 h. Among the landraces, Njavara and Chenellu showed a mortality of 18 and 10% respectively, coupled with a less reduction in percent root and shoot growth when subjected to induction treatments. The physiological basis of thermo-tolerance in these lines was further confirmed, as these lines recorded a higher chlorophyll stability index and a strong antioxidant enzyme system with lesser lipid peroxidation in terms of malondialdehyde content values.

Key words: *Rice, Temperature induction response technique, thermo-tolerance, high temperature stress*

INTRODUCTION

Rice is an important crop and consumed widely across the globe as staple food. India is one of the world's largest producer of rice and occupies second position in production. It accounts about 20% of the world rice production. India has the largest area under rice cultivation with an area of 44 Mha. It is the dominant crop of the country and contributes 43% of the total food.

To meet the demand of the growing population and to achieve food security in country, the production levels need to be increased by 2mt every year (Thankapandian et al., 2010). Current IPCC projections indicate that the mean global temperature will rise 0.2 °C per decade in coming years. For every 1°C increase in temperature there will be 10% decrease in grain yield. High temperature especially high night temperature stress causes chalkiness and spikelet sterility in rice and contributes for the reduced grain yield.

Rice grows optimally between 20ºC to 35ºC and increase in ambient temperature of more than 10-15ºC relative to the optimum growing temperature can constitute heat stress (Wahid et al., 2007). According to Ziska and Bunce (1998) the rate of respiration to photosynthesis increases with increasing temperature. Climate change will likely result in more extreme climatic conditions in irrigated lowland and rain-fed upland regions in tropical countries. Heat injury can result in high spikelet sterility. Even just an hour of exposure to heat stress at anthesis could induce sterility and result in grain yield reduction (Jagadish et al., 2007). The sterility mainly results from reduced anther dehiscence, low pollen production and low numbers of germinating pollen grains on the stigma (Prasad et al.,

2006).

In Kerala, rice is grown under diverse ecologies from irrigated lowland, rain-fed upland, rain-fed wetland and deep water condition. High temperature induced sterility has not been an important problem in irrigated systems earlier. But at present the temperature may go up to 39ºC during second/third crop at Palakkad, Thrissur and Kuttanad tract of Alappuzha. These areas are the main rice growing areas of Kerala. Ilangovan et al. (2011) found that the decreased trends of rainfall and temperature were changed abnormally and influenced the soil fertility and rice yield in Pattambi. The last five years (2003-2008) the annual mean minimum temperature have increased by 0.5°C and 0.42oC, respectively during the month of July and June months when compared to 58 years of data. Kole wet lands; the rice bowl of central part of Kerala had experienced a drastic reduction in yield during the second crop season of 2009-2010. The study conducted revealed that the yield decline was due to high temperature accompanied with pest and diseases and weed infestation (Nandini et al., 2010).

MATERIALS AND METHODS

Plant materials

The present research work was conducted at the Department of Plant Physiology, R.A.R.S., Pattambi. The experimental materials consisted of NS-1, NS-2, NS-3, NS-4, IET 22218, IET 22216, IET 20924, Njavara, Jyothi and Chenellu. The seed materials were obtained from Department of Plant Breeding and Genetics, R.A.R.S., Pattambi.

1. Standardization of TIR technique in rice

A. Standardization of challenging temperature for rice using TIR technique

Germinated rice seedlings were subjected to different temperature for various durations eg. 49, 50, 51, 52, 55°C for 1, 2, 3 h durations. Later the seedlings were allowed to recover at 30°C for 72h and at the end of recovery period the percent survival was recorded. The temperature treatment which resulted in 90% mortality of the seedlings was defined as the severe temperature stress to challenge the seedling and assess their genetic variability.

B. Determination of optimum induction temperature treatment of seedlings

The germinated seedlings were subjected to different induction temperature by gradual temperature induction. Temperature treatment at which maximum recovery growth was observed to be defined as optimum induction temperature.

Treatments used for this study were:

1. Temperature was increased from 28°C to 42°C in 2.5h and maintained at 42°C for 2h

2. Temperature was increased from 28°C to 44°C in 4.5h

3. Temperature was increased from 28°C to 45°C in 5h

4. Seedlings were maintained at 35°C for 1h

5. Seedlings were maintained at 40°C for 1h

6. Seedlings were maintained at 45°C for 2h and immediately transferred to the challenging temperature.

Seedling vigor index = Germination percentage x Total length of plant

Percent survival of seedlings = (Number of seedlings survived at the end of recovery/ Total number of seeds sown) X 100

Percent reduction in root growth =[(root growth of control seedlings-root growth of treated seedlings)/ root growth of control seedlings)] x 100

Percent reduction in shoot growth= [(shoot

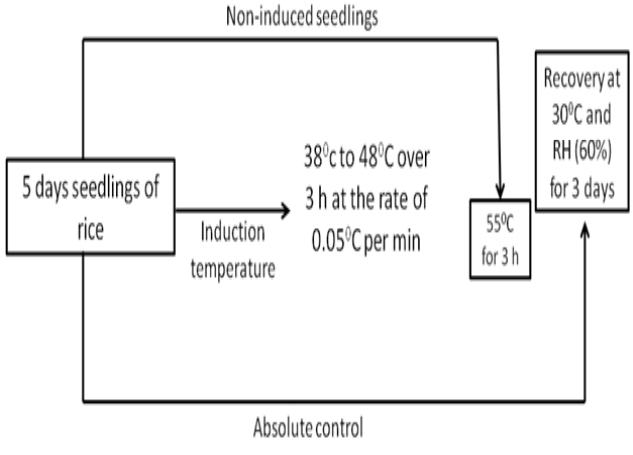

Fig. 1. Protocol to induce high temperature toelrance through the temperature induction response technique.

growth of control seedlings- shoot growth of treated seedlings)/ shoot growth of control seedlings)] x 100

Lipid peroxidation

Lipid peroxidation was estimated using TBA reaction described by Bishayee and Balasubramaniam (1971). 100 micro litres of tissue homogenate in tris buffer of pH 7 was incubated in a reaction mixture containing KCl (100µL), ascorbic acid, ferrous ammonium sulphate and tris buffer for 1hr at 37°C. After incubation 1ml of TCA was added and mixed thoroughly. Heated with 2ml of TBA in boiling water bath for 15min. Mixture was allowed to cool and then centrifuged at 2000rpm. The supernatant was read for absorbance at 530 nm. The amount of malondialdehyde formed was calculated from a standard curve of malondialedehyde.

Estimation of Antioxidant Enzymes

1. Estimation of peroxidase (EC.1.11.1.7)

The peroxidase activity in plants was estimated following the method described by Reddy et al., (1995). Leaf sample of 200mg was homogenized in one ml of 0.1M phosphate buffer (pH 6.5) and centrifuged at 5000 rpm for 15 minute at 4°C. To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added and adjusted to read zero at 430nm. The enzyme reaction was started by adding 0.5ml of one percent hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured every 30 second up to 3 minute. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

2. Estimation of catalase (EC.1.11.1.6)

The catalase (CAT) activity in plants was quantified following the method described by Luck (1974). 200mg of leaf sample was prepared in phosphate buffer. The homogenate was centrifuged at 5000rpm for 15 minutes at 4°C and the supernatant was used for the enzyme assay. The H_2O_2- phosphate buffer (3.0ml) was taken in an experimental cuvette. This was followed by the rapid addition of 40µl enzyme extract was mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm. The enzyme solution containing H_2O_2 free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

3. Superoxide dismutase (EC.1.5.1.1)

Superoxide dismutase activity was quantified following the method described by Kakkar et al.(1984). Leaf samples of (0.5g) from third fully opened leaves were ground with 3.0 ml of potassium phosphate buffer, centrifuged at 2000ppm for 10 minutes and the supernatant was used for the assay. The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30°C for 90 second and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of-butanol, allowed to stand for 10 minute and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

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).

RESULTS AND DISCUSSION

Standardization of TIR technique in rice

The rice variety Jyothi (Ptb 39) was used to standardize the challenging and induction temperature for rice seedlings. Challenging temperature is the temperature treatment for specific time duration (in hours) required to cause more than a 90% reduction in seedling survival in non-induced seedlings of Jyothi, while induction temperature is the temperature pretreatment for a specific time duration (in hours) that is required to significantly improve the growth recovery of seedlings of Jyothi subsequently exposed to a challenging temperature.

Seeds of Jyothi were soaked for 2-3 h and put

Table 1. Percent mortality of Jyothi seedlings at different lethal temperature.

Temp.(°C)	Percent mortality of Jyothi seedlings at different lethal temperature Duration of temperature treatment (h)		
	1	2	3
49	9	13	24
50	12	25	45
51	36	48	66
52	42	62	98
55	56	100	100
Temp.(T)	0.089		
Duration (h)	0.046		
T X h	0.136		

for germination in petri-plates. Five days old uniform seedlings of Jyothi were subjected to different challenging temperature for specific time duration such as 49, 50, 51, 52, 53, 55°C for 1, 2, 3, and 4h durations without prior induction or gradual temperature induction. For temperature induction response technique (TIR), temperature was raised from 38 to 48°C over 3h at the rate of 0.05°C min^{-1} before exposing plants to high temperature. Gradual temperature induction was followed by exposure to lethal temperature. Then these seedlings were immediately allowed to recover in an incubator at 30°C and 60% relative humidity (RH) for three days. At the end of recovery period, the number of seedlings that survived was recorded and percent mortality was calculated. For all experiments, three replications were maintained for each treatment and each replicate constituted of 10 seedlings. The lethal temperature identified for Jyothi was 52°C for 2h (Table 1). Thus an induction temperature of induction treatment from 38 to 48°C for 3 h was standardized using TIR (thermo induction response). Similar method of standardizing the lethal and induction temperature has been adopted in screening cotton (Khier et al., 2012) and ragi lines (Babu et al., 2013). The threshold and induction temperatures for tolerance differ in sunflower (Kumar et al., 2006) and groundnut (Lokesh et al., 2004).

Genetic variability for thermo-tolerance in rice

Genotypic variation in seedling mortality during recovery from challenging temperature ranged significantly from 25 to 96% in non-induced seedlings and 10 to 74% in induced seedlings (Table 2). Among the genotypes, Chenellu recorded the lowest seedling mortality (10: 25 %), lowest percent reduction in root (14:15 %), lowest percent reduction in shoot growth (14: 22 %) both under induced and non-induced conditions, respectively. Among other varieties, NS-1, Njavara also showed a lower reduction in shoot and root growth rate when subjected to induction treatment. Thus, Chenellu followed by NS-1 and Njavara showed higher intrinsic tolerance mechanism. Similar work was also done in land races of Tamil Nadu (Vijayalakshmi et al., 2015).

Assessment of physiological basis of high temperature stress tolerance/susceptibility in rice genotypes

Chlorophyll Stability Index (CSI) was significantly reduced after the induction treatment (Table 3). Highest

Table 2. Variation in thermo-tolerance of different rice genotypes in terms of seedling mortality and percent reduction in root and shoot growth using temperature induction response technique.

Genotypes	Seedling mortality (%)		Percent reduction in roots		Percent reduction in shoots	
	Induced	Non-induced	Induced	Non-induced	Induced	Non-induced
NS-1	15	37	17	32	18	28
NS-2	45	74	45	75	54	82
NS-3	48	69	56	68	49	51
NS-4	26	58	32	47	29	34
IET 22218	63	79	29	46	35	51
IET 22216	74	96	36	62	38	62
IET 20924	20	42	38	50	32	47
Njavara	18	29	20	34	15	24
Jyothi	22	37	18	28	21	34
Chenellu	10	25	14	15	14	22
Varieties (V)	0.824	0.462	0.429			
Induction state (I)	0.276	0.247	0.263			
V x I	1.291	0.648	0.841			

Table 3. Effect of temperature induction response technique on Chlorophyll Stability Index and lipid peroxidation of rice genotypes.

Genotypes	Chlorophyll Stability Index (%)		Lipid peroxidation (nmol MDA g^{-1} fresh weight)	
	Before induction	After induction	Induced	Non-induced
NS-1	87.39	82.57	19.08	24.45
NS-2	84.52	78.24	24.45	28.28
NS-3	82.14	75.79	23.37	29.47
NS-4	86.16	72.08	20.78	27.62
IET 22218	75.24	62.46	35.94	42.04
IET 22216	76.63	59.74	38.47	61.18
IET 20924	85.34	80.27	27.06	36.27
Njavara	80.17	78.45	21.49	27.43
Jyothi	92.05	86.37	26.78	36.44
Chenellu	83.89	82.67	18.64	27.67
Varieties (V)	1.54		0.57	
Induction state (I)	0.45		0.32	
V x I	1.56		0.97	

Table 4. Effect of temperature induction response technique on peroxidase, catalase and SOD activity of rice genotypes.

Genotypes	Peroxidase activity (activity g^{-1} min^{-1})		Catalase (activity g^{-1} min^{-1})		Superoxide dismutase (activity g^{-1} min^{-1})	
	Induced	Non-induced	Induced	Non-induced	Induced	Non-induced
NS-1	3.12	1.32	0.32	0.12	1.02	0.85
NS-2	2.65	1.28	0.30	0.24	0.96	0.91
NS-3	2.87	1.47	0.23	0.23	0.54	0.52
NS-4	2.62	1.35	0.14	0.16	0.86	0.64
IET 22218	2.46	1.96	0.25	0.22	0.45	0.36
IET 22216	2.28	1.46	0.48	0.40	0.25	0.26
IET 20924	2.10	1.47	0.24	0.25	0.85	0.78
Njavara	2.49	1.45	0.16	0.25	0.46	0.38
Jyothi	2.12	1.05	0.31	0.30	0.75	0.84
Chenellu	3.45	2.14	0.27	0.28	0.95	0.87
Varieties (V)	0.224	0.06	0.09			
Induction state (I)	0.256	0.072	0.063			
V x I	0.591	0.148	0.241			

CSI was maintained by the variety Jyothi both before and after induction. After induction treatment, higher CSI was maintained by Jyothi (86.37%) followed by NS-1(82.57%) and Chenellu (82.6%). Lowest CSI was recorded by IET 22216 after induction. These results support the previous work in rice by Vijayalekshmi et al., (2015). The induction stress triggers several signaling pathways, which will alter the physiological and biochemical processes relevant to stress tolerance. Lipid peroxidation is generally considered as one of the conspicuous forms of damage resulting from oxidative stress. The extent of lipid peroxidation is a useful parameter for ascertaining the sensitivity of a plant to oxidative damage. Lipid peroxidation in terms of malondialdehyde (a secondary end product of the oxidation of polyunsaturated fatty acids) content was reduced after induction treatment. There was significant difference among the varieties and between treatment for malondialdehyde production. Chenellu and NS-1 recorded the lowest content of malondialdehyde after the induction treatment. CSI and lipid peroxidation are inversely related. The ability of a plant to respond to the induction treatment is a good reflection of its "intrinsic tolerance" ability at the cellular level.

Antioxidant Enzymes

Antioxidant enzymes were increased in seedlings subjected to induction temperature followed by exposure to challenging temperature (induced seedlings) to non-induced seedlings (Table 4). Peroxidase and SOD increased in all the genotypes under induced condition. Catalase activity was increased only in few

genotypes under induced condition. Highest peroxidase activity was recorded by Chenellu (3.45 activity g^{-1} min^{-1}) followed by NS-1 (3.12 activity g^{-1} min^{-1}) under induced condition. Highest catalase activity was recorded by IET 22216(0.48 activity g^{-1} min^{-1}) under induced condition. Highest SOD activity was recorded by NS-1(1.02 activity g^{-1} min^{-1}) followed by Chenellu (0.95 activity g^{-1} min^{-1}). Results revealed that, enzyme function is sensitive to changes in temperature. Heat-induced alteration in enzyme activity can lead to imbalance in metabolic pathways or heat can cause complete enzyme inactivation due to protein denaturation (Vierling, 1991). Genetic variability in stress tolerance is, therefore, a result of the extent of stress gene expression when plants experience such lethal stresses.

CONCLUSION

In summary, it is found that the seedlings undergone Temperature Induction Response (TIR) Technique showed higher recovery growth at lethal temperature. Among the genotypes, Chenellu recorded the lowest seedling mortality, lowest percent reduction in root, lowest percent reduction in shoot growth both under induced and non-induced conditions, respectively. These seedlings showed also showed high chlorophyll stability index and antioxidant enzymes with less lipid peroxidation. It is an easy and non-destructive method to screen large population at the seedling stage. Hence, this method can be adopted as a reliable method to screen for thermo-tolerance. From this study, it is found that Chenellu showed higher recovery under TIR technique by higher rate of cellular level tolerance in terms of physiological traits. Field performance of induced seedlings has to be evaluated both under water stress and high temperature condition.

REFERENCES

Babu VD, Sudhakar P and Reddy YSK (2013). Screening of thermo-tolerant ragi genotypes at seedling stage using TIR technique. The Bioscan 8(4): 1493-1495

Chen GX and Asada K (1990). Hydrooxyurea and p-aminophenol are the suicide inhibitors of ascorbate peroxidase. Journal of Biological Chemistry 265: 2775-2781

Cushman JC and Bohnert HJ (2000). Genomic approaches to plant stress tolerance. Current Opinion in Plant Biology 3: 117-124

Deak M, Horvath GV, Davletova S, Torok K, Sass L, Vass I, Barna B, Kiraly Z and Dudits D (1999). Plants ecopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. Nature Biotechnology 17: 192-196

Fender SC and O'Connell MA (1990). Expression of heat shock response in a tomato interspecific hybrid is not intermediate between the two parental responses. Plant Physiology 93: 1140-1146

Gomez KA and Gomez KA (1984). Statistical procedures for agricultural research in IRRI book. New York: Wiley

Ilangovan R, Beevi P, Rao GSLHVP and Moosa PP (2011). Impact of climate change on soil degradation and rice productivity at Pattambi, Kerala in the humid tropics. In: Rao, G. S. L. H. V. (ed.), Climate Change Adaptation Strategies in Agriculture and Allied Sectors. Scientific publishers, India pp. 220-225

Jagadish SVK, Craufurd PQ and Wheeler TR (2007). Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. Crop Sci. 48: 1140-1146

Kheir EA, Sheshshayee MS, Prasad TG and Udayakumar M (2012). Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. The Journal of Cotton Science 16: 190-199

Krishnan M, Nguyen HT and Burke JJ (1989). Heat-shock protein synthesis and thermal tolerance in wheat. Plant Physiol. 90: 140-145

Kumar SM, Srikanthbabu V, Raju MB, Ganeshkumar Sivaprakash N and Udayakumar M (2003). Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. Journal of Experimental Botany 54(392): 2569-2578

Kumar SM, Kumar G, Srikanthbabu V and Udayakumar M (2006). Assessment of variability in acquired thermotolerance: Potential option to study genotypic response and the relevance of stress genes. Journal of Plant Physiology 164: 111-125

Lokesh B, Srikanthbabu V, Prasad TG, Badigannavar A and Udayakumar M (2004). Membrane thermo stability as a screening tool to identify high temperature stress tolerant groundnut (*Arachis hypogaea* L.) lines using temperature indication response approach. Journal of Plant Biology 31: 53-59

Nandini K, Jaikumaran U and Girija T (2010). Physiological

analysis on yield decline in rice (*Oryza sativa* L.) with high temperature in kole wet lands of Kerala [abstract]. In: Abstracts, National Conference of Plant Physiology on Physiological and Molecular Approaches for Crop Improvement under Changing Environment; 25th-27th November, 2010, Varanasi. Banaras Hindu University, Varanasi & Indian Society for Plant Physiology, New Delhi. Abstract No. 3: 97

Prasad PVV, Boote KJ, Allen LH, Sheehy JE and Thomas J MJ (2006). Species ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Res. 95: 348-411

Scharf KD, Hohfeld I and Nover L (1998). Heat shock response and heat stress transcription factors. Journal of Biosciences 23: 313-329

Shen B, Jensen RG and Bohnert H (1997). Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiology 113: 1177-1183

Thankapandian R, Jayaraj T, Redona, Edilberto D and Sheryl S (2010). Development of heat tolerant breeding lines and varieties in rice. Indian. J. Genet. Resour. 23(1): 1-3

Vierling E (1991). The roles of heat shock proteins in plants. Annual Review of Plant Physiology and Plant Molecular Biology 42: 579-620

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 thermo-tolerance in rice and to identify novel donors for high temperature stress tolerance. Indian Journal of Plant Physiology 20(4): 368-374

Wahid A, Gelani S, Ashraf M and Foolad MR (2007). Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199-223

Ziska LH and Bunce JA (1998). The influence of increasing growth temperature and CO_2 concentration on the ratio of respiration to photosynthesis in soyabean seedlings. Global Change Biol. 4(6): 637-643