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**SHORT COMMUNICATION**

# IDENTIFICATION OF THERMOTOLERANT RICE GENOTYPES AT SEEDLING STAGE USING TIR TECHNIQUE IN PURSUIT OF GLOBAL WARMING

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**Global warming predicted to affect food security, especially rice, which is stable food crop as it is vulnerable to high temperature. In the present study, a novel temperature induction response (TIR) technique was adapted for identifying highly thermotolerant rice genotypes. This approach of TIR involves, first the identification of challenging temperature and induction temperature and later standardizing them before being used for screening the material for intrinsic stress tolerance. A lethal temperature of 55°C for 2 hours and induction treatment from 36°C-52°C for 5 hours was standardized in the laboratory. Using this standardized TIR protocol, highly thermotolerant rice genotypes were screened from 72 rice germplasm. Sufficient genotypic variability was noticed from zero level to 100% level of tolerance. Among the genotypes, NLR-145 showed highest thermotolerance in terms of 100 per cent seedlings survival and no reduction in root and shoot growth. NLR-40066, NLR-40070 and NLR-40050 also showed higher thermotolerance in terms of 90% seedlings survival and no reduction in root and shoot growth. This study revealed that TIR technique can be used for identification of thermotolerant rice genotypes. The identified rice varieties can be used as donor source for developing high temperature tolerant rice genotypes to withstand future temperature rise.**

***Key words:*** **Lethal temperature, temperature induction response, thermo tolerance**

Rice (*Oryza sativa* L.) is one of most important food grain crops with wide adaptability to diverse agro climatic conditions around the world. In India, it is cultivated in 44.8 m ha in the year 2009 with the total production of 93 million tones. Rice is extensively grown in irrigated cropping systems, allowing production in the warmer, high radiation, post-monsoon and summer months. Rice production has also intensified in rainfed - lowland and dry land (upland) cropping systems, many of which are prone to drought and high temperature (Coffman 1977).

The rising global temperatures are predicted to impact agricultural and food security. Among the agricultural crops, studies were mostly done on rice crop regarding impact of rising temperatures on crop growth and yields. The Intergovernmental Panel on Climate Change (IPCC) has indicated an increase in temperature causing severe shortage of water that exerts pressure on crop yields. IPCC (2007) has predicted that, every 1°C increase in night temperature will reduce rice yields by 0.3 tons per hectare. Similarly, 90% decrease in yield was reported when rice plants were exposed to high night temperatures (32°C) (Mohammed and Tarpley 2009). India is the worst sufferer, as rice is the principle food especially, in South India. Hence, development and identification of technologies/varieties where crop yields were less affected due to global warming is the research

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priority. A well defined response to high temperature stress is the loss of cellular membrane integrity and imbalance between photosynthesis, respiration and enzyme degradation. Under high temperatures stress, normally plants have ability to rapidly acclimate to withstand lethal temperatures (Vierling 1991). The acquisition of thermotolerant is a cell autonomous phenomenon and results from prior exposure to a conditioning pre treatment (sub lethal high temperatures).

From this background rice genotypes were screened for high temperature tolerance using temperature induction response (TIR) technique. This approach is based on the fact that temperature stress develops gradually from sub lethal levels to lethal levels of stress. An array of response events are expressed during sub lethal temperatures and give cellular protection at lethal temperatures (Abdullah *et al.* 2001). Present experiment was conducted at Phenotyping laboratory, Institute of Frontier Technologies, Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh with 72 Indica rice genotypes obtained from Agricultural Research Station, Nellore, Andhra Pradesh.

*Identification of lethal temperature treatment*: To assess the challenging temperatures for 100 per cent mortality, 42 hour old rice seedlings were exposed to different lethal temperatures (48, 50, 52, 54 and 55$^o$C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30$^o$C and 60 per cent relative humidity for 48 hours at the end of recovery period. Per cent mortality of rice genotypes after recovery was recorded (table 1). The lethal temperature of 55$^o$C for 2 hours was considered in this test, as maximum mortality (95%) of seedlings.

*Identification of sub lethal (induction) temperatures:* During the induction treatment, the seedlings were exposed to a gradual increase in temperature for a specific period. This temperature regimes and duration are varied from crop to crop are to be standardized. Based on the earlier studies in paddy (Narayana Swamy 2010), the duration of induction temperatures was used as five hours and the specific temperature regime was standardized in this laboratory. The germinated rice seedlings (42 hour old rice seedlings) were subject to gradually increasing temperatures for a period of five hours. After this induction treatment, seedlings were exposed to lethal temperatures i.e., 55$^o$C for two hours and then transferred to the normal temperature for recovery. The temperature regimes and durations are varied to arrive at optimum induction protocol (Table 2). The optimum sub lethal temperatures were arrived based on the per cent survival of seedlings. The sub lethal treatment which recovered least per cent seedlings survival reduction was considered as optimum temperature i.e., 36-52$^o$C for five hours.

*Thermo induction response (TIR):* Rice seeds were surface sterilized by treating with 0.1 per cent carbendizim for 30 minutes and washed with distilled water for 4-5 times followed by one per cent sodium hypochlorite for 1-2 minutes. Then the seeds were washed with distilled water for 4-5 times and kept for germination at 30$^o$C and 60 % relative humidity in the incubator. After 42 hours, uniform seedlings were selected in each genotype and sown in aluminum trays (50 mm) filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperature increasing from 36$^o$C–52$^o$C for five hours in the environmental chamber (“WGC-450” Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (55$^o$C) (induced) for 2 hours. Another sub set of seedlings were directly exposed to lethal temperatures (non induced).

Induced and non induced rice seedlings were allowed to recover at 30$^o$C and 60% relative humidity for 48 hours. The following parameters were recorded from the seedlings

A lethal temperature of 55$^o$C for 2 hours and

$$\text{a) Per cent survival of seedlings} = \frac{\text{No. of seedlings survived at the end of recovery}}{\text{Total No. of seedlings sown in the tray}} \times 100$$

b) Per cent reduction in root growth = $\frac{\text{Actual root growth of control seedlings}}{\text{Actual root growth of treated seedlings / Actual root growth of control seedlings}} \times 100$

c) Per cent reduction in shoot growth = $\frac{\text{Actual shoot growth of control seedlings}}{\text{Actual shoot growth of treated seedlings / Actual shoot growth of control seedlings}} \times 100$

induction treatment from 36°C-52°C for five hours was standardized using TIR (Temperature Induction Response) and considered as best lethal and induction temperatures for phenotyping of rice seedlings for intrinsic heat tolerance at cellular level (Table 1 and Table 2).

**Table 1.** Per cent mortality of rice seedlings at different lethal temperatures.

| S.No. | Temperature (°C) | Per cent mortality of rice seedlings after recovery — Duration of temperature | | |
|---|---|---|---|---|
| | | 1 hour | 2 hour | 3 hour |
| 1. | 48 | 0 | 0 | 14 |
| 2. | 50 | 0 | 26 | 44 |
| 3. | 52 | 0 | 28 | 46 |
| 4. | 54 | 34 | 56 | 78 |
| 5. | 55 | 55 | 95 | 97 |

**Table 2.** Per cent mortality of rice seedlings at different induction temperature range.

| S.No. | Temperature range (°C) (Induction treatment for 5 hours) | Per cent survival of seedlings |
|---|---|---|
| 1. | 32-50 | 85 |
| 2. | 32-52 | 90 |
| 3. | 36-50 | 90 |
| 4. | 36-52 | 98 |
| 5. | 38-52 | 65 |

Induction treatment duration for 5 hours is fixed based on the earlier reports.

The experimental data were recorded and the genotypes which showed contrast values for survival of seedlings, reduction in root and shoot growth were only presented in Table 3. Among the 72 the genotype NLR-145, showed highest thermo tolerance in terms of 100% seedlings survival and no reduction in root and shoot growth. NLR-40066, NLR-40070 and NLR-40050 also showed higher thermo tolerance with no reduction in root and shoot growth, but seedling survival was reduced only by 10%. These varieties are able to survive even they expose to lethal temperatures. These results are in conformity with several studies, which showed that acclimated plants survive upon exposure to a severe stress, which otherwise could be lethal and is termed as thermo tolerance (Sun *et al.* 2001).

The effect of TIR on other genotypes revealed variable results. Such acquired tolerance was variably recorded in other rice genotypes, where either survival of seedlings was affected (NLR 34303) or root growth

**Table 3.** Screening of thermotolerant rice genotypes through TIR technique.

| S. No. | Entries | Percent survival of seedlings (%) | Percent reduction in root growth (%) | Percent reduction in shoot growth (%) |
|---|---|---|---|---|
| 1. | NLR 40054 | 90 | 53 | 03 |
| 2. | NLR 145 | 100 | 0.0 | 00 |
| 3. | NLR 3062 | 50 | 00 | 43 |
| 4. | NLR3160 | 60 | 00 | 31 |
| 5. | NLR3157 | 0 | 0 | 0 |
| 6. | NLR3061 | 80 | 5.13 | 43.8 |
| 7. | MLR34303 | 20 | 37.8 | 42.6 |
| 8. | NLR40066 | 90 | 0 | 0 |
| 9. | NLR40070 | 90 | 0 | 0 |
| 10. | NLR40055 | 100 | 0 | 17.64 |
| 11. | NLR40045 | 90 | 9.09 | 25.21 |
| 12. | NLR40050 | 90 | 0 | 0 |
| 13. | NLR3095 | 90 | 66.10 | 11.38 |
| 14. | NLR3097 | 100 | 66.3 | 32.67 |
| 15. | NLR3101 | 100 | 53.12 | 21.37 |
| 16. | NLR3109 | 80 | 0 | 60.95 |
| 17. | NLR3110 | 70 | 0 | 61.12 |

alone was affected (NLR 40054, NLR 40045, NLR 3095, NLR 3101, NLR 3097) or only shoot growth was affected (NLR 3062, NLR 3160, NLR 3061, NLR 40055, NLR 3109, NLR 3110). In the genotype NLR 3157 seedling survival, shoot and root growth were completely affected despite of recovery conditions maintained after exposing to sublethal and lethal temperature. The study revealed the existence of genotypic variability from 0 to 100 per cent thermotolerance in rice crop.

In spite of exposing seedlings to 55$^{o}$C, germination and seedlings growth were not affected in NLR 145, probably due to acquired thermo tolerance. Under natural conditions, development of thermo tolerance is a progressive phenomenon. Accordingly, plants experience sub lethal temperature initially before being exposed to severe levels. In response to this sub lethal temperature stress, several genes have been shown to either up regulated or down regulated which leads to development of considerable degree of tolerance in plants (Sun *et al.*, 2001).

This technique of exposing young seedlings to sub lethal and lethal temperature has been validated in many crop species (Kumar *et al.* 1999, Senthil Kumar *et al.* 2007). This novel temperature induction response technique has been demonstrated to reveal genetic variability in intrinsic stress tolerant at cellular level, (Narayanaswamy 2009). The present study also revealed that the TIR technique can very well be used in rice crop. The identified genotypes NLR 145, NLR 40066, NLR 40070 and NLR 40050 are showed to posses high level of thermo tolerance. These genotypes can be used as potent donor source for developing genotypes against high temperatures to withstand raising global temperature due to climate change.

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