Short communication

Screening of rice (*Oryza Sativa* L.) genotypes for high temperature stress under Temperature Induction Response

ABSTRACT

Plants are exposed to multifarious external environmental conditions that affect plant growth, development and productivity. Heat stress is one of the abiotic stress that occurs due to high ambient temperature. Also, it is a serious threat to crop production worldwide. Rice is a major food crop cultivated all over the globe. In the present study, temperature induction response (TIR) was carried out for screening the rice genotypes. Initially, three rice genotypes namely Swarnamukhi, Kasalth and N22 (tolerant check) were used to standardize the optimum induction temperature and lethal temperature. Optimum induction temperature was 46-54^{o} C and the lethal temperature 56^{o} C were standardized for rice. At this temperature, a total of 58 diverse genotypes were screened for high-temperature stress tolerance. Among the genotypes tried, sivapu chithiraikar(94.33 %), kavya(93.67%), kasalath(94.23), samleshwari (93.56), poongar (87.34), vedhividangan (75.10), Naveen (89.56), surakuruvai (84.90), swarnamukhi (92.10), vellaikatrai (80.50) and kavuni (92.60) expressed higher seedling survival percentage of tolerance (93.5 %) to high temperature and remaining were identified as temperature susceptible as compared to control (100% survival). There was a considerable variation among the genotypes screened for acquired thermotolerance. Results suggested that TIR is a simple and powerful technique and can be used to screen a large population at the seedling stage to identify thermotolerant lines.

Keywords: Induction response, Lethal temperature, Rice, Thermotolerance

1. INTRODUCTION

"Global warming has become a serious worldwide threat. Ambient temperature is rising at a considerable rate a part of the current global climate change. Climate models predict that the global mean temperature will continue this trend, increasing by 1–4°C in every forthcoming century and heat waves will occur frequently" (Hoegh-Guldberg *et al*., 2018). Rice (*Oryza sativa* L.) is an important cereal crop that "feeds more than half of the world's population. The unfavorable temperature may significantly affect plant growth, development and productivity. Under climatic scenarios, rice will be exposed to unfavorable conditions by increasing mean temperatures. To alleviate this threat, it is necessary to improve the adaptability of rice genetically to cope with the warming climates. Heat acclimation is a physiological response manifested in the acquired thermo-tolerance of organisms or cells to lethal high temperatures following exposure to a moderate heat stress condition. One of the approaches to improve thermo-tolerance is to transfer superior alleles from intrinsically thermotolerant wild relatives. A large number of genetic variations exist for intrinsic toleraznce among the varieties and landraces of rice. It is important to develop a rapid and reliable lab protocol that allows the simultaneous screening of a large number of genotypes".

From this perspective, a protocol called the temperature induction response (TIR) technique has been developed and standardized for rice. This technique has been used to screen thermotolerant varieties of pea and sunflower, Groundnut and Cotton. The present study, therefore, was planned to assess the genetic variability of the widely grown rice genotypes and landraces using the TIR protocol to identify sources for tolerance at the cellular level.

2. MATERIALS AND METHODS

2.1 Preparation of seedlings

Rice seedlings were selected to standardize the protocol for the TIR technique. This technique is required for standardization of the age of seedlings, lethal temperature and induction temperature. The rice seeds (30-40 seeds) were soaked in water for 18 hours and then allowed to germinate in petriplates. The three-day-old seedlings or 1.5 cm in length were selected for this experiment. The uniform seedlings from each genotype were transferred to three different sets of petriplates. One set of seedlings was exposed to standardize the induction temperature in the humidity-controlled growth chamber for three hours. Once the standardization of induction treatment was completed, the seedlings were exposed to a standardized challenging temperature which means a lethal temperature. Along with the induced seedlings, another set of seedlings was exposed directly to lethal temperature for 3 hours. At the end of treatment, each petriplates from the growth chamber were removed and keep it at room temperature for 72 hrs for recovery of seedlings. Apart from this, another set of seedlings was kept at room temperature throughout the experiment to serve as absolute control.

2.2 Standarization of temperature

2.2.1 Identification of lethal temperature

The minimum temperature which causes more than 100 percent mortality when the seedlings were exposed without an induction cycle is the lethal temperature. To determine the lethal temperature, rice seedlings were subjected to different lethal temperatures viz., 44, 46 48, 50, 52, 54ºC followed by recovery at 30ºC for 72 h (Table 1)

2.2.2 Identification of optimum induction temperature

The induction temperature is the sequence of temperature treatments during which the rice seedlings were exposed to an optimum sub-lethal to lethal temperature followed by recovery temperature at 30ºC for three days. Such seedlings were referred to as induced seedlings. In the present study, induction temperature was optimized by exposing the rice seedlings to different induction cycles viz., 44-48ºC for 3h, 48-52ºC for 3h and 50-56ºC for 3h with an increase of 2ºC per h. "After induction treatment, the seedlings were exposed to a standardized lethal temperature. Later the seedlings were kept at 30ºC room temperature for the recovery period of 48 hours.The root and shoot length of induced and non-induced temperature treatments were recorded after the recovery period. The separate set of control seedlings was maintained at 30ºC room temperature throughout the experimental period. The survival percentage was recorded after the recovery period. The percent reduction over absolute control and the survival percent was calculated" by Senthil (2001).

2.2.3 Optimization of induction and lethal temperature

The genotype namely N22, Kasalath and Swarnamukhi were used to optimize the induction and lethal temperature of rice. The minimum temperature at which 75 per cent mortality of seedlings and 50 per cent reductions in seedlings growth was taken as lethal or challenging temperature to assess the difference in cellular thermotolerance. To standardize the optimum induction temperature, seven days old rice seedlings (N22, Kasalth and Swarnamukhi) were subjected to different induction temperature cycles following which they transferred to a standardized challenging temperature. The parameters measures were:

1. Survival of the seedlings = No. of seedlings survived/Total number of seedlings * 100

2. Percent reduction in root growth = (Root growth of control seedlings - root growth of treated seedling)/Root growth of control seedlings) * 100

3. Percent reduction in shoot growth = (Shoot growth of control seedlings - shoot growth of treated seedling)/Shoot growth of control seedlings) * 100

Following standardization of lethal temperature, the seedlings were standardized for optimum induction cycles. The temperature range 50 to 54^{o}C was standardised as optimum induction cycle in rice (Table 2-4). According to (Hasanuzaman *et al.*, 2013) "synthesis and localization of some of the HSPs trigger several important physiological and biochemical parameters during induction. These changes impart tolerance when the seedlings exposed to lethal temperature. Hence, we concluded that the induced seedlings exhibited higher recovery growth at the temperature 50-54^{o}C". "By adopting the TIR technique, it was feasible to identify thermotolerant lines from a large population at the seedling level. A total of 58 diverse genotypes were screened for high- temperature stress tolerance. The collected data were analyzed statistically using ANOVA as per the procedure of" Gomez and Gomez (2010).

Table 1: Mortality per cent of rice seedlings exposed to high temperature stress

Treatments	Mortality per cent
Control 30 ºC	0
48ºC	15
50 ºC	52
52 ºC	60
54 ºC	75
56 ºC	95

Source:

Table 2: Effect of different induction cycle on growth of rice genotype (Kasalath)

Treatment	Root length	Shoot length	Root + shoot	Survival per cent
Control	3.86	8.59	12.45	95.00
46-50	5.79	7.96	13.75	82.52
48-52	5.28	5.89	10.97	72.86
50-54	3.53	6.64	10.17	70.31

Source:

Table 3: Effect of different induction cycle on seedling growth of rice genotype (Swarnamukhi)

Treatment	Root length	Shoot length	Root + shoot	Surival per cent
Control	2.78	7.16	9.93	90.00

46-50	4.01	5.60	9.61	73.27
48-52	3.78	5.41	9.19	70.13
50-54	3.30	5.15	8.45	65.46

Source:

Table 4: Effect of different induction cycle on seedlings growth of rice genotype (N22)

Treatment	Root length	Shoot length	Root + shoot	Survival per cent
Control	3.92	8.53	12.45	100
46-50	5.37	8.06	13.43	87.59
48-52	5.13	6.87	12.00	89.12
50-54	4.15	7.59	11.73	97.5

Source:

3. RESULT AND DISCUSSION

"The experimental data recorded on genotypes for survival of seedlings, reduction in root and shoot growth are presented in Table 5. Based on the percent reduction in recovery growth and percent survival after a recovery period, the rice genotypes were classified into three different categories *viz*., tolerant, moderately tolerant and susceptible. A tale of 85 rice genotypes was exposed to optimum induction and lethal temperature based on the standardization to screen and identify the temperature tolerant genotypes. The percent survival of seedlings varied from 12 to 94.33 percent with a mean survival percentage of 91% as compared with control (100%). The percent reduction in root growth varied from 6.31 to 76% with a mean of 35.74 percent and the percent reduction in shoot growth varied from 9.62 to 72.5 percent with a mean of 35.57 percent over control (Table 5). These results are in conformity with previous studies, which showed that acclimated plants survive upon exposure to severe stress, which otherwise could be lethal and is considered to be as thermotolerant" (Vijayalakshmi *et al*., 2015).

The genotypes namely sivapu chithiraikar, kavya, kasalath, samleeshwari, poongar, vedhividhyan, naveen, surakuruvai, swarnamukhi, vellaikatrai and kavuni showed "the highest thermotolerance in terms of higher seedlings survival percentage and less reduction in root and shoot growth (Table.5) Thereby these genotypes were able to survive even when they exposed to lethal temperatures. The Temperature Induction Response (TIR) technique was an ideal protocol to identify and select the thermotolerant genotypes. It involves exposing seedlings or plants to induce stress and subsequently challenging with severe temperature and selecting the surviving seedlings at the end of a recovery period. Survival and recovery growth of plants exposed to stress could be a potential tool to screen for stress tolerance and TIR has been shown in recent studies as a potential tool for empirical assessment for cell survival and recovery growth at the seedling or whole plant level" (Sudhakar *et al*., 2012). "A number of earlier studies have shown that the TIR technique is the best alternative to evaluate rice genotypes for thermotolerance" (Sapna Harihar *et al*.,2014).

Table 5. Screening of rice genotypes for thermotolerance through TIR technique

S. No	Genotypes	Survival percentage		Percentage of Reduction	
		Control	Induced	Shoot growth	Root growth
1.	Norungan	100.00	80.00	10.85	13.33
2.	Vandana	95.00	51.67	11.85	34.19
3.	Mallam punchai	80.00	42.60	38.00	42.74
4.	Sivapu Chithiraikar	100.00	94.33	8.00	4.70
5.	KarungKuruvai	90.00	41.33	44.00	51.56
6.	Mysurmalli	95.00	10.00	46.50	31.39
7.	Kavya	97.00	93.67	1.66	10.76
8.	Jeerga samba	97.00	32.10	61.36	60.53
9.	Kullakar	96.00	50.00	70.87	29.08

10.	Kasalath	100.00	94.23	6.30	14.78
11.	N22	97.00	59.20	12.44	16.67
12.	Mapillai Samba	95.00	74.00	15.28	21.93
13.	Swarnamukhi	91.00	92.10	4.76	15.42
14.	Kaivarasamba	93.00	38.45	48.00	12.55
15.	Varapukudhanchan	97.00	44.80	9.09	40.29
16.	Swarna	85.00	15.60	18.84	33.77
17.	Samleeswami	80.00	93.56	9.65	7.17
18.	Hasan serai	82.00	44.50	13.64	25.37
19.	Poongar	93.00	87.34	15.95	4.02
20.	Cherveruppu	90.00	52.30	21.43	13.51
21.	Vedhividankan	98.00	75.10	12.13	11.19
22.	Kallunidaikar	95.00	34.70	22.38	26.43
23.	Kalanamak	90.0	62.60	38.67	22.24

		0			
24.	Pokkali	97.0 0	32.56	38.06	12.70
25.	Iuppaipoosamba	100. 00	41.90	20.33	40.46
26.	Naveen	100. 00	89.56	8.14	16.67
27.	Whitepooni	90.0 0	56.78	15.73	33.08
28.	Kavuni	96.0 0	92.60	9.26	14.74
29.	Varisuriyan	77.0 0	37.00	52.19	17.14
30.	Surakuruvai	95.0 0	84.90	10.87	12.70
31.	Thondi	91.0 0	24.80	30.77	30.48
32.	Kayamma	95.0 0	15.60	45.45	18.33
33.	Mayur pankhi	93.0 0	34.00	57.55	20.83
34.	Maranellu	89.0 0	10.20	22.50	37.14
35.	Lalmeeta	96.0 0	32.40	44.00	39.85
36.	Kadaikannan	100. 00	63.33	32.19	17.34

37.	Karuthakar	100.00	75.10	80.40	2.80
38.	Lunishree	83.00	45.00	24.60	16.04
39.	Vellaikatrai	97.00	80.56	6.98	12.90
40.	Bhavani	94.00	62.60	26.41	15.07
41.	Athurkichaisamba	100.00	69.45	22.00	10.92
42.	Kattuyanum	100.00	41.90	13.86	25.22
43.	Panidubi	78.00	35.40	34.86	42.47
44.	APO	98.00	80.67	8.90	12.99
45.	Kattaikar	100.00	62.60	24.05	17.19
46.	Nootripathu	100.00	58.20	18.48	31.38
47.	Vellaichithiraikar	94.00	78.09	8.16	16.22
48.	Uppumilagai	95.00	56.00	28.53	14.55
49.	Thuyamalli	100.00	56.00	49.80	30.14
50.	Samba Machanam	100.	34.33	11.25	13.46

		00			
51.	Salem Samba	98.00	48.33	50.94	26.07
52.	Rasagadam	96.00	67.80	0.92	54.55
53.	Nammalvarkavuni	100.00	75.32	0.32	3.65
54.	Kuzhivedichan	100.00	67.09	56.70	50.12
55.	Kudavazhai	98.00	45.30	94.00	22.66
56.	Kichadi samba	100.00	43.20	24.55	10.45
57.	Karuthasamba	95.00	38.60	54.55	22.22
58.	Adukar	97.00	25.80	30.82	12.68
SE d		**1.343**	**0.752**	**0.456**	**0.379**
CD		**2.653**	**1.458**	**0.900**	**0.750**

4. CONCLUSION

The rice genotypes viz., Sivapuchithiraikar, Kavya, Kasalath, Samleeshwari, Poongar, Vedhividankan, Naveen, Surakuruvai, Swarnamukhi, Vellaikatri and Kavuni were screened for high temperature tolerance on the basis of survival percent and the percent reduction in shoot and root growth. Therefore, these rice genotypes will survive under high temperature stress at seedling stage.

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Image 1: Overview of experiment