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RESEARCH PAPER

Temperature induction response technique - A physiological approach to identify thermotolerant genotypes in rice

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Abstract : Thermotolerance is one of the various acquired stress tolerance phenomenon observed in many living organisms, when the stress is imposed gradually. It is extremely important to develop screening tools for identification of thermotolerant genotypes in the context of increase in average global temperature. Temperature induction response (TIR) technique has been standardised to identify thermotolerant genotypes in rice. This technique involves exposing rice seedlings to gradual induction temperature immediately followed by lethal temperature and measuring growth of the surviving seedlings at the end of the recovery period of 72 h. The accuracy of the TIR technique depends on optimum induction cycle and lethal temperature. The standardization of induction temperature and lethal temperature is based on per cent growth reduction and survival percentage at the end of recovery period. The induction temperature was standardized as 36-44°C for 5 h and the lethal temperature as 52°C for 3 h. It is proposed that this technique can be used as a potential tool to identify and select temperature tolerant lines at the seedling level from a large population.

Key Words : Temperature induction response, Thermotolerance, Induction temperature lethal temperature, Cellular level tolerance

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INTRODUCTION

Prevalence of high temperature is the major limitation for the cultivation of crops in tropical conditions. The day temperatures to which the plants are exposed in many tropical areas are often above their optimal growth temperature and a small increase above optimum has large effect on growth rate (Howarth, 1996). To increase the productivity and to stabilize production in the ever changing environment, development of genotypes that are capable to survive better under high temperature stress is paramount important and inevitable.

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 upland cropping systems, many of which are prone to drought and high temperature. Rice responses to high temperature differ according to the developmental stages with the highest sensitivity recorded at the reproductive stage. Temperatures greater than 35°C at anthesis and lasting for more than 1h can lead to high sterility in rice (Jagadish *et al.*, 2007).

Acquired stress tolerances to temperature extremes are complex traits dependent on many attributes. One of the approaches to improve thermotolerance is to transfer superior alleles from intrinsically thermotolerant wild relatives, which require precise screening methods to measure the variability in thermotolerance. Various screening techniques based on specific physiological parameters such as single leaf photosynthetic capacity, quantification of chlorophyll fluorescence under stress are being used to screen thermotolerance at field level (Selmani and Wasson, 1993), but these measurements are highly influenced by environmental factors which are the major limitation. The

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best alternative, therefore, would be to develop suitable laboratory protocol for screening acquired thermo tolerance of rice genotypes. From this perspective, a protocol called temperature induction response (TIR) technique has been developed and standardised for rice. This technique has been used to screen thermotolerant varieties of pea (Shrikanthbabu *et al.*, 2002), groundnut (Gangappa *et al.*, 2006), sunflower (Senthil *et al.*, 2001). In the present study, an attempt was made to standardize the temperature induction response technique which is prerequisite for screening the temperature tolerance in rice genotypes.

MATERIAL AND METHODS

Seedling growth:

Rice seedling (cv. White Ponni) was selected to standardize protocol for TIR technique. This technique required standardization of age of seedlings, lethal temperature and induction temperature. The rice seeds (about 30-40) were soaked in water for 18 h and then allowed to germinate in Petriplates. Three day old seedlings or 1.5 cm length were selected for the experiment. The uniform seedlings from each genotype were transferred to three different sets of Petriplates for further studies.

Identification of lethal temperature:

The minimum temperature which causes more than 100 per cent mortality when the seedlings exposed without induction cycle is the lethal temperature. To determine the lethal temperature, rice seedlings were subjected to different lethal temperatures *viz.*, 48, 50, 52°C followed by recovery at 30 °C for 72 h.

Identification of optimum induction temperature:

Induction cycle is the sequence of temperature treatments during which the rice seedlings are exposed to an optimum sub lethal to lethal temperature followed by recovery at 30°C for three days. Such seedlings are referred as induced seedlings. In the present study, induction temperature was optimized by exposing the rice seedlings to different induction cycles *viz.*, 32-40°C for 5h, 34-42°C for 5h and 36-44°C for 5h with increase of 2 °C per hr. After this treatment the seedlings were exposed to a standardised lethal temperature. Later the seedlings were kept at 30°C for recovery.

Root and shoot length of induced and non-induced were recorded after recovery period. The control seedlings were maintained at 30°C through out the experiment period. The survival per cent was recorded after the recovery period. The per cent reduction over absolute control and the survival per cent was calculated (Senthil, 2001).

$$\text{Per cent reduction over absolute control} = \frac{(C - R)}{C} \times 100$$

C – Recovery growth of absolute control

R – Recovery growth of induced seedling

$$\text{Survival of the seedlings} = \frac{\text{No. of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

RESULTS AND DISCUSSION

The temperature induction response (TIR) has been effectively employed to idenify the thermotolerance genotypes in various crops. For developing thermotolerant crops, existence and quantification of genetic variability is pre-requisite in any crop improvement programs. By adapting the temperature induction response technique, the existence of significant genetic variability has been demonstrated across the genotypes of pea (Srikanth, 1999). There are evidences to show that genetic variability for high temperature stress tolerance is seen only upon induction stress (Uma *et al.*, 1995; Jayaprakash *et al.*, 1998; Kumar *et al.*, 1999; Krishnan *et al.*, 1989). Therefore, identifying thermotolerance at seedlings through TIR is potential option which requires crop specific standardization. Among the three lethal temperatures tried, highest mortality rate was recorded in 52°C for 3 h (98 %). As the temperature increases from 48^0 C to 50^0 C with increment of 2°C, the mortality increased from 25 to 98 per cent (Table 2). Even when the temperature was increased above 52^0 C, the mortality was found to be 100 per cent (data not shown), since the lethal temperature is decided based on lowest temperature with highest mortality, the 52^0 C is optimized as lethal temperature in rice. The lethal temperature standardised is comparable with lethal temperature reported in other crops

Table 1 : Effect of different induction cycle on growth of rice seedlings (cv. WHITE PONNI)

Treatments	Root length (cm)	Shoot length (cm)	Root +Shoot (R+S) (cm)	Per cent reduction over control	Survival per cent
Control	3.7	5.7	9.4	NA	97.0
32-40°C for 5h	2.3	3.6	5.9	37.2	49.1
34-42°C	2.5	5.2	7.7	18.1	70.1
36-44°C	3.1	5.3	8.4	10.6	87.0
Mean	2.90	4.95	8.1	-	84.3
S.E.±	0.25	0.54	0.90	-	7.88
C.D. (P=0.05)	0.58*	1.25*	2.21*	-	18.18*

Table 2 : Mortality per cent of rice seedlings exposed to high temperature stress

Treatments	Mortality per cent
Control	0
48^0C	25
50^0C	38
52^0C	98
Mean	40.2
S.E.±	4.1
C.D. (P=0.05)	9.4*

(Kumar *et al.*, 1999; Reddy, 2000).

Following standardization of lethal temperature, the seedlings were standardized for optimum induction cycles. The root length and shoot length was observed to be the highest in the treatment 36 to 44°C (3.1 and 5.3, respectively) and the lowest value was observed in 32 to 40°C (2.3 and 3.6, respectively). Among the three induction cycles, 36 to 44°C was found to be lower growth reduction (10.6) with higher survival per cent (87.0) (Table 1). Therefore, 36 to 44°C is standardised as optimum induction cycle in rice. During induction, synthesis and localization of some of the HSPs trigger several important physiological and biochemical parameters (Chen *et al.*, 1990). These changes impart tolerance when the seedlings exposed to lethal temperature.

In summary, the induced seedlings exhibited higher recovery growth at the temperature 36-44°C. By adopting the temperature induction response technique, it is feasible to identify thermotolerant lines from a large population at the seedling level. Hence, this method is a reliable method to screen for thermotolerance. This has a specific advantage of high-throughput and non-destructive technique. By adapting this technique, several basic issues in terms of relevance of stress adaptive mechanisms in addition to genetic variability can be efficiently studied. Besides, the tolerant seedlings identified technique can be established in the field and their progenies can be subsequently screened through recurrent selection to obtain highly tolerant cultivars.

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