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Temperature induction response reveals intrinsic thermotolerant genotypes in soybean

Uwimana Marie Ange, S. Srividhya, C. Vijayalakshmi and P. Boominathan*

Department of Crop Physiology,
Tamil Nadu Agricultural University, Coimbatore-341 003, India.
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ABSTRACT

The responses of soybean genotypes to high temperature for intrinsic tolerance was studied using Temperature Induction Response (TIR) technique in order identify the genotypes tolerant to high temperature stress. Seven soybean genotypes subjected to lethal and sub lethal temperatures showed significant variation for acquired thermotolerance. Thermotolerant genotypes ADT 1 and CoSoy1 identified by the TIR technique, demonstrated higher survival percentage, and lower growth reduction. Further the tolerant genotypes identified based on TIR also showed higher antioxidant enzymes activity implying the critical role of antioxidant in cellular thermotolerance. This clearly demonstrated that TIR can be effectively used for screening high temperature tolerance genotypes in soybean. This study also established the fact that the alternations in antioxidants during induction are vital for imparting tolerance to high temperature stress.

Key words: Acquired thermotolerance, Antioxidant enzymes, Sub lethal temperature.

INTRODUCTION

Soybean (*Glycine max* L. Merr.) accounts for approximately 50 per cent of total production of oilseed crops in the world. About 90 per cent of the world's soybean production occurs in the tropical and semi-arid tropical region, which are characterized by high temperature and low or erratic rainfall. In the tropics, most of the crops are near their maximum temperature, consequent of which crop yield may decrease even with minimal increase in temperature (Thuzar *et al.*, 2010). There is an increase in the soybean seed yield as temperature increased from 18/12°C (day/night) to 26/20°C, however, decrease in yield is observed when the plants were grown at temperature greater than 26/20°C (Sionit *et al.,* 1987). Raising temperature from 29/20°C to 34/20°C during seed filling decreased soybean seed yield by 22 per cent (Dornobos and Mullen, 1991). Besides, Lobell and Asner (2003) reported that each 1°C increase in the average growing season temperature leads to 17 per cent decrease in soybean seed yield.

Plants overcome high temperature stress by adapting several physiological and biochemical mechanisms (Senthil Kumar *et al.*, 2006). Thermotolerance can also be induced by gradual increase in temperature from sub-lethal to lethal temperature as would be experienced in natural environment (Larkindale *et al.,* 2005). Plants growing in natural habitat at regular ambient temperature may experience high temperature that would be lethal in the absence of acclimation and hence, ability to acquire thermotolerance is of significant importance to plants (Srikanthbabu *et al.*, 2001).

Based on this concept, the temperature induction response (TIR) technique has been developed (Kumar *et al.*, 1999) and its relevance was recently validated in rice (Sapna harihar *et al.*, 2014). In this technique, the seedlings are initially exposed to a non-lethal induction stress to facilitate expression of stress responsive genes subsequently followed by lethal temperature. The seedlings that survive after lethal temperature with high recovery growth can be developed into thermotolerant lines (Senthil Kumar, 2001).

MATERIALS AND METHODS

Seven popular soybean genotypes were used in this study and screened for thermotolerance using TIR approach. This involves initial identification of optimum challenging and induction temperature for screening genotypes for intrinsic thermotolerance (Senthil kumar, 2003).

Preparation of seedlings : Three sets of three days old soyabean seedlings were taken in petriplates and initial seedling length was measured. One set of seedlings was exposed to standardized induction temperature treatment in humidity controlled growth chamber for three hours. Once the induction treatment is completed, the seedlings were exposed to standardized challenging (lethal) temperature using growth chamber. Along with the induced seedlings, another set of seedlings exposed directly to lethal temperature for 3 hours. At the end of treatment, the petriplates from growth chamber was removed and kept at room temperature for 72 hrs for recovery of seedlings. One more set of measured seedlings was kept at room temperature throughout the experiment served as absolute control.

*Corresponding author's e-mail: boominathan.p@tnau.ac.in

Optimization of induction and lethal temperature : The genotype Cosoy3 was used to optimize the induction and lethal temperature of soyabean. The minimum temperature at which 75 per cent mortality of seedlings and 50 percent reductions in seedlings growth was taken as lethal or challenging temperature to assess the difference in cellular thermotolerance. To standardize the optimum induction temperature three days old soybean seedlings (Cosoy3) were subjected to different induction temperature cycles following which they transferred to a standardised challenging temperature. The seedlings were exposed to following temperatures gradually for 5 h at the rate 2°C per h. After subjecting the seedling to challenging temperature, seedlings were allowed to recover at 30°C for 72 h.

30-38 ˚C
32-40 ˚C
34-42 ˚C
36-44 ˚C

At the end of recovery period, the seedlings survival percentage and recovery growth was measured. Based on per cent seedlings survived and per cent reduction in recovery growth, the tolerant lines were selected. The per cent reduction over absolute control and the survival per cent was calculated using the formula suggested by Senthil Kumar (2001).

Per cent reduction over absolute control = [(C-R)/C] x 100

Where,

C – Recovery growth of absolute control

R – Recovery growth of induced seedling

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

Measurement of enzymes associated with cellular level tolerance: The antioxidant enzymes activity of the induced seedlings was assessed in the seven contrasting soybean genotypes. Peroxidase activity (change in OD value at 430nm g^{-1} min^{-1}) was determined according to Peru (1962) and Angelini *et al.* (1990). Catalase activity was assayed from the rate of H_2O_2 decomposition extinction coefficient of 39.4 mmol as measured by the decrease in the absorbance at 240 nm, following the procedure of Aebi (1974). The data on various parameters were analyzed statistically as per the procedure of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Standardization of optimum lethal temperature and induction temperature for TIR : Results depicted in Table 1 showed reduction in shoot and root growth of the induced seedlings over control with increase in the lethal temperature while survival per cent decreased. At the challenging temperature of 48°C for 3h, lowest survival per cent (30%) and highest growth reduction (71.1%) was observed. The trend observed was similar in all the experiments conducted. Both total growth and per cent survival show a significant variation among different challenging temperatures. Based on above parameters, lethal temperature was standardized as 48°C. This lethal temperature is the same as that of peas (Srikanthbabu *et al*., 2001) while in rice it was higher (52°C) as recorded by Sapna (2011). Highest lethal temperature was recorded in groundnut (55°C) by Gangappa *et al.* (2006). The standardization of lethal temperature is inevitable to employ TIR for screening genotypes for cellular level tolerance as the temperature should be really lethal for seedling without induction.

Following standardization of lethal temperature, the induction cycles were optimized as 34-42°C for 3 h @ 2°C per h. Optimum induction protocol was arrived based on the least reduction and higher survival compared to control seedlings. Higher survival percent (55.0%) and least growth reduction (34.8%) of seedlings were observed when seedlings were induced with a gradual temperature induction of 34°C to 42°C for 3 h (Table 1). Both total growth and per cent survival showed a significant variation among different induction temperatures. This induction cycle varies with crop species which depends on intrinsic ability of plant system to acquire thermotolerance.

Genetic variability for intrinsic thermotolerance among soybean genotypes : Based on standardised the lethal and

Table 1: Standardization of lethal temperature and optimum induction temperature for TIR technique in soybean genotype (Cosoy3)

Standardization of lethal temperature				Standardization of optimum induction temperature			
Treatments	Shoot and root length (cm)	Per cent survival	Per cent reduction of shoot and root length over control	Treatments	Shoot and root length (cm)	Per cent survival	Per cent reduction of shoot and root length over control
Control	4.17	100	-	Control	6.19	100	NA
44°C	3.92	100	6	30-38°C	2.44	16.6	60.4
46°C	2.86	70	31.4	32-40°C	3.19	13.3	48.3
48°C	1.21	30	71.1	34-42°C	4.03	55	34.8
50°C	0	0	100	36-44°C	3.97	33.3	35.8
52°C	0	0	100	Mean	3.96	43.64	44.8
Mean	2.52	60	15.43	SEd	0.36	1.75	
SEd	0.21	0.54		CD (0.05)	0.81*	3.91*	
CD(0.05)	0.45*	1.21*					

induction temperature, all the seven soyabean genotypes were screened for intrinsic cellular level tolerance. The results in Table 2 showed that the variety Cosoy1 was found to have the least reduction in growth (55.7%) followed by ADT1 (62.0%). Highest survival percentage was recorded in ADT1 (75.0) followed by Cosoy1 (71.4). Highest reduction in growth and lowest survival per cent was observed in MACS450 (96.3 and 12.5 respectively). Thus ADT1 and Cosoy1 were identified as thermotolerant genotypes while MACS450 was found to be highly susceptible. These three genotypes were selected for further evaluation of thermotolerance under pot culture experiment (data not shown).

By adapting the temperature induction response technique, the existence of significant genetic variability has been demonstrated across the genotypes of pea (Srikanthbabu, 1999). The thermotolerant lines from the sunflower were identified in open pollinated population, cv. Morden (Kumar *et al.*, 1999). This is first study, TIR was demonstrated in soybean.

Assessment of antioxidant enzymes of TIR exposed soybean genotypes : Under abiotic stress, most of plants produce reactive oxygen species (ROS) which leads to oxidative stress (Gulen and Eris, 2004). The major scavenging enzymes necessary for reducing the ROS are peroxidase, catalase and superoxide dismutase. These enzymes remove free radicals and prevent the membranes and DNA from oxidative damage. Decrease in antioxidant activity in stressed tissues results in higher levels of active oxygen species (AOS) that may contribute to plant cell injury (Fadzillah *et al.*, 1996).

Significant difference among genotypes was observed for peroxidase activity in both induced and control treatments. Results indicated that peroxidase activity is found to be higher in induced seedlings than control in all genotypes (Table 3) . Peroxidase activity was found high in Bragg (9.57 and 10.45) and low in ADT1 (2.09 and 4.00) in control and induced seedlings respectively. The per cent increase in peroxidase activity was higher in ADT1 (91.4) followed by Cosoy1 (73.3) and least per cent increase in peroxidase activity was found in MACS450 (7.9).

Results in Table 3 showed that all genotypes recorded higher catalase activity in induced seedlings compared to control. Highest catalase activity was recorded in Cosoy1 in both control (6.6) and induced (17.0) seedlings. The lowest catalase activity was recorded in Hardee (1.7) in

Table 2: Cellular level tolerance of soybean genotypes based on TIR technique

Genotypes	Shoot and Root length (cm)				Survival per cent		
	Control	**Induced**	**Mean**	**Per cent growth reduction**	**Control**	**Induced**	**Mean**
Cosoy3	16.57	4.12	10.34	75.2	100	57.1	78.6
ADT1	13.02	4.95	8.98	62	81	75	78.1
Hardee	10.93	1.34	6.13	87.8	81.8	25	53.4
Cosoy1	15.97	7.08	11.53	55.7	100	71.4	85.7
MAUS61	4.99	0.19	2.59	96.2	85	16.7	50.8
MACS450	15.71	0.64	8.18	96.3	100	12.5	56.2
Bragg	11.5	4.1	7.8	64.3	90	28.6	59.3
Mean	12.67	3.2	7.93		91.1	40.9	66
	G	**T**	**GXT**		**G**	**T**	**GXT**
SEd	0.35	0.23	0.6		1.32	0.86	2.29
CD(0.05)	0.70*	0.61*	1.21*		2.67*	1.75*	4.63*

Table 3: Peroxidase activity (Ä 430 nm g^{-1} min^{-1}) and catalase activity (Ä 430 nm g^{-1} min^{-1}) of TIR exposed soybean seedlings

Genotypes	Control	Induced	Mean	Per cent increase over control	Control	Induced	Mean	Per cent increase over control
Cosoy3	3.00	4.47	3.74	48.9	3.96	5.89	4.93	49.0
ADT1	2.09	4.00	3.04	91.4	4.81	10.34	7.58	114.8
Hardee	3.46	4.53	4.00	31.1	1.72	3.38	2.55	96.5
Cosoy1	3.62	6.27	4.94	73.3	6.64	16.95	11.79	155.4
MAUS61	9.46	10.26	9.86	8.4	2.60	2.89	2.74	11.4
MACS450	9.27	10.00	9.64	7.9	2.52	2.54	2.41	2.0
Bragg	9.57	10.45	10.01	9.2	3.26	3.46	3.36	6.3
Mean	5.78	7.14	6.46		3.64	6.46	5.05	
	G	**T**	**GXT**		**G**	**T**	**GXT**	
SEd	0.65	0.35	0.93		1.99	1.06	2.81	
CD(0.05)	1.34*	0.72*	NS		4.08*	2.18*	NS	

control and MACS450 (2.5) in induced seedlings. However, highest per cent increase of catalase activity in induced seedlings was recorded in Cosoy1 (155.4), followed by ADT1 (114.8) and lowest in MACS450 (2.0).

In this study, it was observed that the level of catalase and peroxidase had increased in induced seedlings over control in all the genotypes indicating higher production of reactive oxygen radicals under high temperature stress. Interestingly, genotypes that emerged as high temperature tolerant based on TIR also showed higher per cent increase over control in catalase (155.5 and 114.8 % in Cosoy1 and ADT1 respectively) and peroxidase (91.4 and 73.3% in ADT1 and Cosoy1 respectively) activity. Similar trend of increase in catalase activity was demonstrated in rice (Cao *et al.*, 2009). The susceptible genotype (MACS450) recorded lesser increase in the activity of the antioxidant enzyme (7.87 and 4.9 in peroxidase and catalase activity respectively). This data strongly suggest that of the many attributes for cellular level tolerance, the antioxidant enzymes might be one of them.

Babu *et al.* (2007) found a significant increase in the activity of guaiacol-specific peroxidase under high temperature stress (46-48°C) in french beans. Mohammadi *et al.* (2011) reported higher antioxidant enzyme activity under drought stress conditions in chickpea. Kumar *et al.* (2011) observed an increase in the activity of the enzyme peroxidase under drought in pigeon pea leaves. Although many literature correlates antioxidant with abiotic stress tolerance mainly drought and high temperature, this is first study to elucidate the role of antioxidant enzymes in acquired thermotolerance in soyabean. Further, it is inferred that gradual increase in induction temperature would also lead to synthesis of higher level of antioxidizing enzymes. In addition to antioxidants, we have also estimated the nitrate reductase activity of induced and control seedlings to understand how nitrogen metabolism is affected under TIR. The results suggest that there could be no relevance of cellular thermotolerance with nitrate reductase activity as the data is non-significant (data not shown). These findings are corroborated by studies of Joseph *et al.* (1976) who reported that, nitrate reductase activity was independent of temperature in soybean.

The results of the experiment showed that TIR technique is a robust and constructive technique to identify genetic variability in cellular thermotolerance in soybean within a short period of time and it is suitable for screening a large number of genotypes. Thus, the genotypes identified as thermotolerant *viz.*, Cosoy1 and ADT1 also showed higher percent increase in peroxidase and catalase activity in induced seedling indicating that antioxidants might positively correlate with acquired thermotolerance in soybean.

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