IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **13** | Issue 1 | January, 2018 | 165-170

DOI: 10.15740/HAS/IJPS/13.1/165-170
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Screening of acquired thermotolerant ragi [*Eluesina coracana* (L.) Gaertn] genotypes using T.I.R. technique

B. Sujatha, P. Sirisha and Y.V. Bharathi

SUMMARY

For present population ragi is the major food as it is considered as the power house of health benefits. The production of ragi is coming down slowly due to the climatic factors like temperature and drought. Breeding of selected genotypes with increased thermotolerance is therefore, one of the most vital objective in crop improvement programme. Temperature induction response (TIR) technique has been developed to identify thermotolerant lines. 24 ragi genotypes has been tested using temperature induction response (TIR) technique. Ragi seedlings were exposed to gradual increase in temperature range of 32-48^{0}C for 5hrs and later subjected to the lethal temperature of 54^{0}C for 2 hrs. These treated seedlings were allowed to recover at 30^{0}C and 60% relative humidity for 2 days. After recovery per cent survival, per cent reduction of root growth and per cent reduction of shoot growth was calculated. Among 24 ragi genotypes VR900, Indaf 8 and Udurumalliga were found resistant with low per cent reduction of root and shoot growth and the genotypes VR 1138, CO-7 and OUAT-2 were found susceptible with high per cent reduction of root and shoot growth. By using this TIR technique it is easy to identify thermotolerant lines from a large range of population at the seedling level itself

Key Words : Acquired thermotolerance, Temperature induction response, Lethal temperature

How to cite this article : Sujatha, B., Sirisha, P. and Bharathi, Y.V. (2018). Screening of acquired thermotolerant ragi [*Eluesina coracana* (L.) Gaertn] genotypes using T.I.R. technique. *Internat. J. Plant Sci.*, **13** (1): 165-170, **DOI: 10.15740/HAS/IJPS/13.1/165-170**.

Article chronicle : Received : 16.11.2017; **Revised :** 05.12.2017; **Accepted :** 19.12.2017

With the increasing population of the world enhancement of food production is a major necessity. Temperature and drought are the climatic changes which likely to affect the agricultural productivity adversely and cause drastic yield reduction in major crops. This effect is more drastic in arid and semiarid tropics like India, where the agricultural production is mainly dependent on climatic factors.

Productivity of rice, maize, sorghum, and ragi crops are negatively influenced with increase in temperature. Ragi [*Eluesina coracana* (L.) Gaertn] is the most important small millet in the tropics and is cultivated in more than 25 countries in Africa and South Asia predominantly as a staple food grain. For present population ragi is the major food as it is considered as

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
B. Sujatha, Department of Botany, Andhra University, Visakhapatnam (A.P.) India
Email : sudheer.ponnada@yahoo.com

Address of the Co-authors:
P. Sirisha, Department of Botany, Govt. Junior College, Gurla, Vizianagaram (A.P.) India

Y.V. Bharathi, Department of Botany, Government Degree College, Saluru, Vizianagaram (A.P.) India

the power house of health benefits. The production of ragi is coming down slowly due to the climatic factors like temperature and drought. At present 50% of the total households growing ragi against 100% few years back (Sub sectoral analysis).

So, there is necessity to bring out the new varieties withstand the adverse climatic factors like high temperature drought, salinity etc. The species which can show tolerance to the abiotic stress factors and yield maximum production are to be found to overcome the food scarcity and nutritional crisis. Breeding of selected genotypes with increased heat tolerance is therefore, one of the most vital objective in crop improvement programme. To increase the productivity and to stabilize production in the ever-changing environment, development of genotypes that are capable to survive better under abiotic stress is essential (Gangappa *et al.*, 2006).

Acquired thermotolerances to temperature extremes are complex traits dependent on many attributes. One of the approach to improve thermotolerance is to transfer superior alleles from intrinsically thermotolerant wild relatives, which require precise screening methods to measure the variability in thermotolerance (Harihar *et al.*, 2014). Several studies have shown that plants have ability to withstand otherwise severe temperature stress upon exposure to mild stress temperature. Stress referred to as induction stress. The phenomenon of adapting to a designated severe stress following a mild stress is known as acquired thermotolerance (Vierling, 1991).

Based on preliminary studies, an efficient screening technique referred to as the temperature induction response (TIR) technique has been developed to identify thermotolerant lines (Senthil Kumar *et al.*, 2003). According to this technique, the seedlings are exposed to optimum induction temperature before being exposed to a severe challenging temperature and subsequently allowed to recovery at room temperature.

Many earlier studies have demonstrated that genetic variability for high temperature tolerance is noticed only upon induction treatment prior to severe stress (Burke, 2001; Krishnan *et al.*, 1989; Kumar *et al.*, 1999; Srikanth Babu *et al.*, 2002; Jayaprakash *et al.*, 1998 and Uma *et al.*, 1995). This technique has been used to screen thermotolerant varieties of rice (Vijayalakshmi *et al.*, 2015; Harihar *et al.*, 2014; Renuka Devi *et al.*, 2013 and Sudhakar *et al.*, 2012), ragi (Venkatesh Babu, 2013), cotton (Kheir *et al.*, 2012), groundnut (Gangappa *et al.*, 2006), sunflower (Senthil Kumar *et al.*, 2003), pea (Srikanth Babu *et al.*, 2002), sunflower (Kumar *et al.*, 1999).

MATERIAL AND METHODS

Present investigation was conducted at Physiology lab of Botany Department, Andhra University, Wltair, Andhra Pradesh, using BOD incubator with 24 ragi genotypes obtained from Agricultural research station, Vizianagaram, Andhra Pradesh, using the standardized TIR protocol.

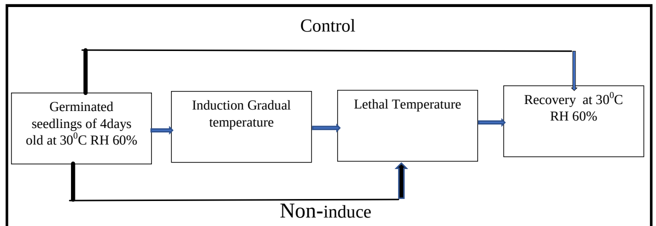

Fig. A : Standerdized temperature induction response (TIR) protocol for ragi

Identification of lethal temperature:

To assess the challenging temperatures, 5-day old ragi seedlings were exposed to different lethal temperatures (46, 48, 50, 52 and 54°C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 72 hours. At the end of recovery period the per cent mortality of the seedling was calculated and recorded in Table 1.

$$\text{Per cent mortality of seedlings} = \frac{\text{No. of seedlings died at the end of recovery}}{\text{Total no. of seedlings sown in the tray}} \times 100$$

Identifications of sub lethal (induction) temperature:

To identify the sub lethal (induction) temperature 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. The germinated ragi seedlings (5 day - old) were subject to gradually increasing temperature ranges (28-44^{0}C, 30-46^{0}C, 32-48^{0}C, 34-50^{0}C, and 36-52^{0}C) for a period of five hours with 4^{0}C increase for every 1hr. After this induction treatment, seedlings were exposed to lethal temperature for two hours and then transferred to recovery. The per cent survival of seedlings for different induction ranges were calculated and was recorded in Table 2.

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

Thermal induction response (TIR):

24 varities of ragi seeds were surface sterilized and kept for germination at 30ºC and 60% relative humidity in the incubator. After 5 days uniform seedlings were selected in each genotype and sown in aluminium trays (50mm) filled with soil mixed with vermi compost and vermiculite in 2:1:1proportions. These trays with seedlings were subjected to sub lethal temperatures for five hours in the BOD incubator. Later these seedlings were exposed to lethal temperatures for 2 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non-induced). Induced and non-induced ragi seedlings were allowed to recover at 30^0C and 60% relative humidity for 72 hours. Along with induced and non-induced treatments a control treatment was maintained at 30^0C without exposing to sub lethal and lethal temperatures.

Per cent reduction in root growth :

$$\text{Actual root growth of control seedlings} = \frac{\text{Actual root growth of treated seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

Per cent reduction in shoot growth :

$$\text{Actual root growth of control seedling} = \frac{\text{Actual root growth of treated seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Identification of lethal temperature:

In the Table 1 per cent mortality of ragi seedlings at different temperatures is recorded. 46, 48, 50, 52 and 54^0C are the different challenging temperatures for lethal treatments studied for 1 hr, 2 h and 3 hr durations. As the temperature increased per cent mortality of ragi seedlings also increased with increased durations. For 46, 48 and 50^0 C of lethal temperatures the per cent mortality is low (below 55%). For 52^0C it is 56% for 2 hrs and 80% for 3hrs. When observe the 54^0C the per cent mortality is 96% for 2 hrs and 99% for 3hrs. Among the 5 different temperatures studied 54^0C for 2 hours with 96% of mortality is standardised as lethal temperature considered for further induction treatment of 24 ragi seedlings. Similar critical lethal temperature has been reported in other crops like rice (Vijayalakshmi *et al.*, 2015; Harihar *et al.*, 2014 and Renuka Devi *et al.*, 2013), ragi (Venkatesh Babu, 2013), cotton (Kheir *et al.*, 2012), groundnut (Gangappa *et al.*, 2006), sunflower (Senthil Kumar *et al.*, 2003 and Kumar *et al.*, 1999), pea (Selvaraj *et al.*, 2011), sugarcane (Gomathi *et al.*, 2014); soyobean (Uwimana *et al.*, 2016); tomato (Chandola *et al.*, 2016 and Senthil Kumar *et al.*, 2001).

Identification of sub lethal (induction) temperature:

In the Table 2 the per cent survival of ragi seedlings at different sub lethal (induction) temperature ranges (28-44^0C, 30-46^0C, 32-48^0C, 34-50^0C and 36-52^0C) for 5 hrs are recorded. With increase in temperature range from 28-44^0C, 30-46^0C, 32-48^0C per cent survival of seedlings increased as 80%, 84% and 90%, respectively. And ranges for 34-50^0C and 36-52^0C it decreased as 82% and 78%, respectively. Among the 5 different temperature ranges studied 32-48^0C for 5 hrs is identified as sub lethal (induction) temperature range as it shown maximum per cent survival of seedlings (90%). Similar critical sub lethal temperature ranges have been reported in other crops like rice (Harihar *et al.*, 2014 and Renuka Devi *et al.*, 2013), ragi (Venkatesh Babu *et al.*, 2013), cotton (Kheir

Table 1 : Percentage mortality of ragi seedlings at different lethal temperatures

Sr. No.	Temperature (^{0}C)	Percentage mortality of ragi seedlings after recovery/duration of temperature (%)		
		1 hr	2 hr	3 hr
1.	46	0	0	20
2.	48	0	10	36
3.	50	0	28	54
4.	52	0	56	80
5.	54	30	96	99

et al., 2012), groundnut (Gangappa *et al.*, 2006), sunflower (Senthil Kumar *et al.*, 2003; sugarcane (Gomathi *et al.*, 2014); soyobean (Uwimana *et al.*, 2016); tomato (Chandola *et al.*, 2016).

Thermal induction response (TIR):

Following standardized lethal and sub lethal (induction) temperatures the thermotolerance of 24 ragi genotypes has been tested using temperature induction response (TIR) technique.

The 5-day old ragi seedlings were exposed to gradual increase in temperature range of 32-48^{0}C for 5hrs and later subjected to the lethal temperature of 54^{0}C for 2 hrs. these treated seedlings were allowed to recover at 30^{0}C and 60% relative humidity for 2 days. After recovery a) per cent survival, b) Per cent reduction of root growth and c) per cent reduction of shoot growth was calculated.

Statistical analysis :

To calculate parameters standard deviation (S.D.) is also calculated to measure the spreading of the values from mean and also to determine the deviation of the varieties observed among themselves.

At the end of recovery the ragi genotypes exposed to gradual induction temperature prior to lethal temperature showed higher seedling survival per cent compared to the direct lethal temperature (non-induced). The genotypes differed significantly for Per cent reduction of root and shoot growth after induction

Table 2 : Per cent survival of ragi seedlings at different induction (sub lethal) temperature ranges

Sr. No.	Temperature ranges (Induction treatment for 5 hrs)	Per cent survival of ragi seedlings (%)
1.	28-44	80
2.	30-46	84
3.	32-48	90
4.	34-50	82
5.	36-52	78

Table 3 : Screening of the rmotolerant ragi genotypes

Sr. No.	Variety name	Per cent survival of seedlings (%)	Per cent reduction in root growth (%)	Per cent reduction in shoot growth (%)
1.	VR 900	100	-3.57	-19.09
2.	VR 1132	100	-21.47	6.85
3.	VR 1133	88	-20.55	3.19
4.	VR 1134	84	-6.08	1.92
5.	VR 1135	76	-15.65	-15.49
6.	VR 988	84	11	13.82
7.	VR 1131	88	9.18	0.18
8.	VR 1136	92	42.85	18.92
9.	VR 1137	100	-12.62	36.10
10.	VR 1076	72	25.92	13.20
11.	VR 1138	100	27.66	37.22
12.	VR 1139	96	24.83	5.48
13.	Indaf 8	100	-47.05	-23.54
14.	GPV 45	88	-2	1.85
15.	HR 911	100	16.08	-6.19
16.	OUAT-2	100	29.26	19.50
17.	CO-7	92	42.63	30.88
18.	K-7	100	43	38.70
19.	GN-4	100	3.04	2.07
20.	Kalyani	72	-1.12	-2.16
21.	Udurumalliga	100	-18.88	-27.89
22.	Bharathi	92	41.66	27.08
23.	Sri Chaitanya	92	25.31	3.74
24.	Hima	92	35.22	32.72
	Mean	92	9.527	8.3025
	S.D.	±8.869	±24.354	±18.79

Table 4 : Categorization of varieties based on per cent reduction in root growth

Name of the variety showing - %values	Name of the variety showing reduction in root growth value near 10 %	Name of the variety showing reduction in root growth value >10 %
VR 900	VR 1131	VR 988
VR 1132	GN -4	VR 1136
VR 1133		VR 1076
VR 1134		VR 1138
VR 1135		VR 1139
VR 1137		HR 911
Indaf 8		OUAT-2
GVP 45		CO-7
Kalyani		K-7
Uduru malliga		Bharathi
		Sri Chaitanya
		Hima

Table 5 : Categorization of varieties based on per cent reduction in shoot growth

Name of the variety showing -% value	Name of the variety showing reduction in shoot growth value < 10 %	Name of the variety showing reduction in shoot growth value >10%
VR 900	VR 1131	VR 988
VR 1135	VR 1132	VR 1136
Indaf 8	VR 1133	VR 1137
HR 911	VR 1134	VR 1076
Kalyani	VR 1139	VR 1138
Uduru malliga	GPV 45	OUAT -2
	GN-4	CO-7
	Sri Chaitanya	K -7
		Bharathi
		Hima

treatment only. The per cent survival of ragi seedlings varied from 72 to 100 %, the per cent reduction in root growth varied from – 47.05 to 42.63 and per cent reduction of shoot growth varied from – 27.89 to 38.70 (Table 3).

The genotypes were categorized based on per cent reduction of root growth (Table 4), and per cent reduction of shoot growth (Table 5) into resistance, moderately resistance and susceptible varieties. The genotypes which has shown 100% seedling survival, low per cent reduction of root growth and shoot growth are identified as resistance varieties and the genotypes which has shown low seedling survival, high per cent reduction of root growth and shoot growth are identified as susceptible varieties.

After careful observation it was confirmed that among 24 ragi genotypes VR900, Indaf8 and Udurumalliga were found resistant with low per cent reduction of root and shoot growth and. The genotypes VR 1138, CO-7 and OUAT-2 were found susceptible with high per cent reduction of root and shoot growth.

Conclusion :

By using this TIR technique it is easy to identify thermotolerant lines from a large range of population at the seedling level itself. Hence, this method is unique for screening of thermotolerance. It has a specific advantage of high through put and non-destructive technique. It is a robust and constructive technique to identify genetic variability in cellular thermotolerance within a short period of time and it is also suitable for screening of large number of genotypes.

Acknowledgement :

We are very much thankful to Dr.T.S.S.K. Patro, Sr. Scientist and Head of ARS, Vizianagaram, A.P.for providing research material.

REFERENCES

Burke, J.J.(2001).Identification of genetic diversity and mutations in higher plant acquired thermotolerance. *Physiologia Plantarum*, **112**(2) : 167-170.

Chandola, Ankita, Pandurangam, Vijai and Srivastava, Jai Prakash (2016). Genotypic variations in tomato

(*Lycopersicon esculentum* Mill.) for acquired thermotolerance to temperature induction response. *Internat. J. Agric., Environ. & Biotechnol.,* **9**(4):649-657.

Gangappa, E., Ravi, K. and Kumar, N. Veera (2006). Evaluation of groundnut (*Arachis hypogeae* L.) genotypes for temperature tolerance based on temperature induction response (TIR) technique. *Indian J. Genetics,* **66**(2):127-130.

Gomathi, R., Siyamala, S., Vasantha, S. and Suganya, A. (2014). Optimization of temperature conditions for screening thermotolerance in sugarcane through temperature induction response (TIR) technique. *Internat. J. Sci.,* **3** : 5-18.

Harihar, Sapna, Srividya, S., Vijayalakshmi, C. and Boominathan, P. (2014) .Temperature induction response – A physiological approach to identify thermotolerant genoypes in rice. *Internat. J. Agric. Sci.,* **10** : 230-232.

Jayaprakash, T.L., Ramamohan, G., Krishna Prasad, B.T., Ganesh Kumar, Prasad, T.G., Mathew, M.K. and Udaya Kumar M. (1998). Genotypic variability in differential expression of Lea2 and Lea3 genes and proteins in response to salinity stress in finger millet (*Eleusine coracona* Gaertn) and rice (*Oryza sativa* L.) seedlings. *Ann. Bot.,* **82** : 513–522.

Kheir, Ehab Abou, Sheshshayee, M.S., Prasad, T.G. and Udayakumar, M. (2012). Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *J. Cotton Sci.,* **16** : 190-199.

Krishnan, M., Nguyen, H.T. and Burke, J.J. (1989). Heat shock protein synthesis and thermotolerance in wheat. *Plant Physiol.,* **90** : 140–145.

Kumar, G.T., Krishnaprasad, B.T., Savitha, M., Gopalakrishna, R., Mukhopadyay, K., Ramamohan, G. and Udayakumar, M. (1999). Enhanced expression of heat-shock protiens in thermos-tolerant lines of sunflower and their progenies selected on the basis of temperature induction response. *Theor. Appl. Genet.,* **99** : 359-367.

Renuka Devi, K., Siva Shankar, A. and Sudhakar, P. (2013). Sceening of rice genotypes for thermotolerance by TIR technique. *Internat. J. Appl. Biol. & Pharmaceu. Technol.,* **4**(4):205-210.

Selvaraj, M.G., Burow, G., Burke, J.J., Belamkar, V., Puppala, N. and Burrow, M.D. (2011). Heat stress screening of peanut (*Arachis hypogaea* L.) seedlings for acquired thermotolerance. *Plant Growth Regulation,* **65**: 83-91.

Senthil-Kumar, M., Srikanthbabu, V., Mohan Raju, B., Ganeshkumar, Shivaprakash, N. and Udayakumar, M. (2003). Screening of inbred lines to develop thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. *J. Experimental Bot.,* **54**(392) : 2569-2578.

Senthil-Kumar, Muttappa, Kumar, Ganesh, Venkatachalayya Srikanthbabu and Udayakumar, Makarla (2007). Assessment of variability in acquired the rmotolerance: Potential option to study genotypic response and the relevance of stress genes. *J. Plant Physiol.,* **164**:111-125.

Srikanth Babu, V., Ganesh Kumar, Krishnaprasad, B.T., Gopalaswamy, R., Savitha, M. and Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *J. Plant Physiol.,* **159** : 535-545.

___Sub – sectoral analysis of Ragi.

Sudhakar, P., Latha, P., Rameshbabu, Sujatha, K and Raja Reddy. (2012). Screening of paddy genotypes for thermotolerance to using TIR technique in pursuit of global warming. Indian journal of Plant Physiology. (In Press).

Uma, S., Prasad, T. G. and Kumar, M.U.(1995).Genetic variability in recovery growth and synthesis of stress protiens in response to polyethylene glycol and salt stress in finger millet. *Ann. Bot.,* **76**(1) : 43-40.

Uwimana Marie Ange, Srividhya, S., Vijayalakshmi, C. and Boominathan, P. (2016). Temperature induction response reveals intrinsic thermotolerant genotypes in soyabean. *Legume Res.,* **39** (6):926-930.

Venkatesh Babu, D., Sudhakar, P. and Reddy, Y. Sharath Kumar (2013). Screening of thermotolerant Ragi genotypes at seedling stage using TIR technique. *Bioscan,* **8**(4): 1493-1495.

Vierling, E. (1991). The roles of heat shock protiens in plants. *Annu. Rev. Plant Physiol. & Plant Molecular Biol.,* **42** : 579-620.

Vijayalakshmi, D., Srividya, S., Vivitha, P. and Raveendran, M. (2015). Temperature induction response (TIR) as a rapid sscreening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify novel donors for high temperature stress tolerance. *Indian J. Plant Physiol.,* **20** (4):368-374.