Original Research Article

Temperature induction response (TIR): A novel physiological approach for thermotolerant genotypes in chickpea (*Cicer arietinum* L.)

Abstract

Pulses have been described as a "poor man's meat and affluent man's vegetable" in India. Chickpea (*Cicer arietinum L.*) is the third most legume crop grown during rabi season under receding soil moisture.Chickpeas is known to flourish in drought-prone conditions, but it seems to be sensitive to heat stress exceptionally during reproductive development, resulting in considerable yield loss. The performance of chickpeas under heat stress is more variable. It is crucial to develop screening tools to identify thermotolerant chickpea genotypes because of the increase in average global temperatures. In this view, a lab experiment was conducted to standardize the temperature induction response (TIR) protocol for chickpea seeds. The 70 % hydrated seeds were used for the experiment. This technique can be used as a potential tool to identify and select temperature tolerant lines at the seed stage itself from a large population. A set of six chickpea genotypes were screened for intrinsic tolerance using the standardized Thermo Induced Response (TIR) protocol. Among the genotypes JG-14, JG-11 and A-1 showed highest thermo tolerance in terms of higher survival of seeds (germination percentage) and seedlings with less per cent reduction in seedling survival, root and shoot growth. The genotypes with intrinsic heat tolerance can be explored for the development of varieties suitable for late sown conditions in Karnataka where chickpea is prone to terminal heat stress. The results of the study concluded and standardized the sub lethal temperature as 32 ºC to 50 ºC for 5 hours and 30 min,lethal temperature *i.e.*, challenging temperature as 58 ℃ for 3 hours and LD 50 as 52 ℃ for 3 hours at seed level itself.

Keywords: Chickpea, Lethal temperature, Sub lethal temperature, LD 50 thermotolerance, Seed level

Introduction

Pulses have been described as a "poor man's meat and wealthy man's vegetable" in India. Vegetable protein's significance has long been acknowledged across the world. With a mainly vegetarian population, India is the world's producer and consumer of grain legumes. Nonetheless, fast increasing demographic pressure and nearly stagnant output have created a significant challenge in resolving the nutritional imbalance. On the other hand, the country's growing population poses severe food security issues. To make up this shortfall in supply, besides further demand from burgeoning population, the only option at hand to cope up with this problem is to increase productivity.

Chickpea (*Cicer arietinum L.*) is a food legume and an important rabi season crop from the Fabaceae family that is produced all over the globe. It is the third most significant pulse crop. The desi chickpea variety has angular seeds with varying seed coat colours and is cultivated in the semi-arid tropics. It is a photosensitive and thermosensitive crop, with a crop length ranging from 90 to 120 days depending on genotype, soil moisture, temperature, and latitude. One of the causes for low chickpea production in India, in general, and particularly in Karnataka, may be ascribed to high temperatures. High temperature stress has an adverse effect on plant growth and physiological processes, resulting in permanent damage. As the temperature rises over a certain threshold, the size of the heat stress impact rapidly increases (Senthil Kumar et al., 2006). The effects can be estimated using quantitative and qualitative parameters such as seed emergence, seedling establishment, physiological parameters such as photosynthetic rate, transpiration rate (Rahbarian et al., 2011), biochemical parameters such as chlorophyll content (Mafakheri et al., 2010), osmotic regulation and reactive oxygen species.

The high temperature stress increases the temperature at cell level which damages the cell wall and increases the electrolyte leakage and has adverse effects on phenological phases of chickpea (Pouresmaeil*et al*., 2013). In general, heat stress has negative effects on gametogenesis, flowering and podding, thus causing drastic reduction in production and productivity (Senthil-Kumar, 2006).Acquired high temperature is a complex trait that is influenced by various of factors. One approach to improving thermotolerance is through 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 the field level (Selmani and Wasson, 1993). Another approach to improving thermotolerance at the laboratory level, from this perspective, a protocol called temperature induction response (TIR) technique has been developed. This approach is based on the fact that temperature stress develops gradually from sub lethal to lethal levels of stress. An array of response events was expressed during sub lethal temperatures and give cellular protection at lethal temperatures. Therefore, evaluating the relative performance of chickpea genotypes for high temperature tolerance using TIR technique and standardizing the lethal, LD-50 and sub lethal temperature at seed level is the main objective.

Material and methods

Plant materials

Present investigation was conducted at Department of Crop physiology, College of Agriculture, University of Agricultural Sciences, Raichur with 6 genotypes. The experimental materials consisted of popular chickpea varieties *i.e.*, JG-11, JG-14, A-1, ICCV-4958, JAKI-9218 and GBM-2 wereobtained from Zonal Agricultural Research Station, Kalaburagi, Karnataka.

Identification of lethal temperature and LD 50: The temperature at which 0 per cent (100 per cent mortality of seeds) and 50 per cent survival of seeds in terms of germination is considered as lethal temperature and LD-50. To standardize lethal temperature and LD-50, 70% hydrated seeds of JG-11 were exposed to graded temperatures for different durations i.e., 1, 2 and 3 hrs. Later, the seeds were subjected for recovery at room temperature (30 ℃) and 60 % relative humidity for 48 hours and after the recovery period seeds were tested for germination (Anon., 2013). The per cent survival of seeds was recorded.

Identification of Sub lethal temperature: In order to standardize the sub lethal temperature which is required for induction of high temperature tolerance, the 70% hydrated seeds of JG-11 were exposed to a gradual graded induction temperature from32ºC- 46 °C (32 to 44 ºC for one hour each for every increment of 4 ℃ temperature and 46 ºC for two and half an hour), 32ºC -48°C (32 to 44 ºC for one hour each for every increment of 4 ℃ temperature and 46 to 48 ºC for two and half an hour), 32 ºC- 50°C(32 to 48 ºC for one hour each for every increment of 4 ℃ temperature and 50 ºC for one and half an hour), 32ºC - 52ºC (32 to 48 ºC for one hour each for every increment of 4 ℃ temperature and 48 to 52 ºC for one and half an hour), 32ºC - 54ºC (32 to 52 ºC for one hour each for every increment of 4 ℃ temperature and 54 ºC for half an hour)and 32ºC - 56 ºC (32 to 52 ºC for one hour each for every increment of 4 ℃ temperature and 52 to 56 ºC for half an hour), respectively. Later, suchseeds were exposed to the standardized lethal temperature of 58ºC for3 hrs, later kept for recovery at room temperature (30 ℃) for 48hrs. The per cent survival of seeds and per cent reduction in root, shoot growth and seedling vigour index was recorded and presented in the Table 2.

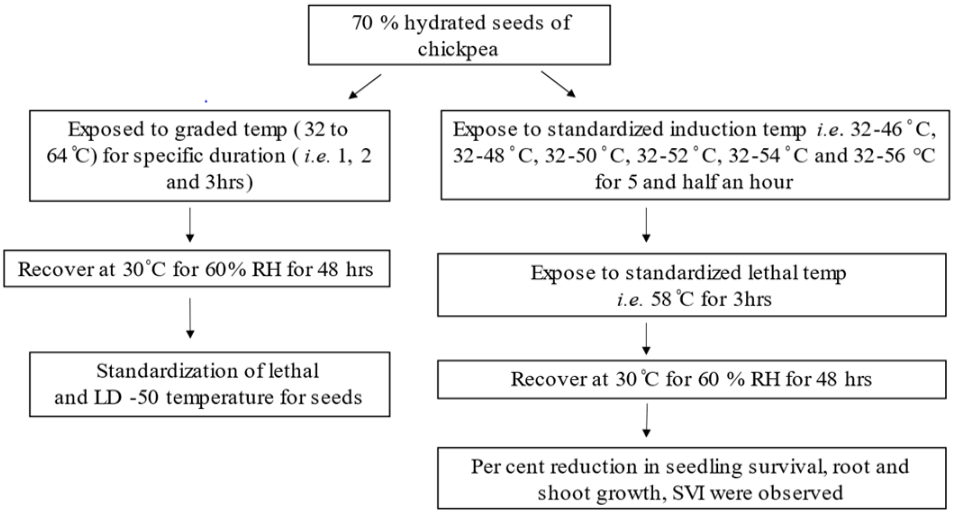

Fig 1: Protocol of Temperature Induction Response technique

Assessment of temperature induction response (TIR) and genotypes:

Chickpea seeds were surface sterilized by treating with 2 per cent carbendazim solution for 30 minutes and washed with the distilled water for 4-5 times and 70 per cent hydrated seeds were obtained by saturation method. Those hydrated seeds were subjected to different graded and induction temperatures for varying duration of hours in the temperature induction chamber. Later these seeds were exposed to lethal temperature @ 58°C for 3 hours. The following parameters were recorded from the seedlings.

1. **Root length (cm)**

   At the end of germination test, ten normal seedlings were randomly selected from each replication at the final count and root length of 10 seedlings were measured from collar region to the tip of longest or primary root with the help of a cm scale and average root length was computed and expressed in cms.

2. **Shoot length (cm)**

   At the end of germination test, ten normal seedlings were randomly selected from each replication at the final count and shoot length of 10 seedlings were measured from base to the tip of the main stem with the help of a cm scale and average shoot length was computed and expressed in cms.

3. Seedling survival (%)

After the recovery period of 48 hrs, the number of survived seedlings were counted in all the three treatments and used in calculation of per cent seedling survival (Raghavendra *et al.,* 2017).

$$\text{Seedling Survival (\%)} = \frac{\text{Number of seedlings Survived at the end of recovery period}}{\text{Total Number of seedlings placed in the tray}} \times 100$$

4. Per cent reduction in root growth

After the recovery period of 48 hrs, the per cent reduction in root growth was calculated by using the formula

$$\text{Per cent reduction in root growth} = \frac{\text{Actual root growth of treated seedlings (cm)}}{\text{Actual root growth of controlled seedlings (cm)}} \times 100$$

5. Per cent reduction in shoot growth

After the recovery period of 48 hrs, the per cent reduction in root growth was calculated by using the formula

$$\text{Per cent reduction in shoot growth} = \frac{\text{Actual shoot growth of treated seedlings (cm)}}{\text{Actual shoot growth of controlled seedlings (cm)}} \times 100$$

6. Germination (%)

The germination per centage was calculated by using the formula

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds kept for germination}} \times 100$$

Results and Discussion

The temperature induction response (TIR) has been effectively employed to identify 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 and Kumar et al., 1999). Therefore, identifying thermotolerance at seed level through TIR is potential option which requires crop specific standardization.

Standardization of lethal (challenging) and LD-50 temperature at seed level

The chickpea genotype JG-11 was screened for intrinsic tolerance using the standardized Thermo Induction Response (TIR) protocol. The experimental data were recorded and presented in Table 1 and Fig 1. The temperature at which 0 per cent (100 per cent mortality of seeds) and 50 per cent survival of seeds in terms of germination is considered as lethal temperature and LD-50. To standardize lethal temperature and LD-50, 70% hydrated seeds of JG-11 were exposed to graded temperatures for different durations *i.e.,* 1, 2 and 3 hours. Later, the seeds were subjected for recovery at room temperature *i.e.,* 30 ℃ for 48 hours.

The results indicated that, 0 per cent and 50 per cent survival of seeds was recorded at temperature 58 ºC for 3 hrs (T_{14}) and 52 ºC for 3 hrs (T_{11}), respectively. Therefore, 58 ºC for 3 hrs is considered as a lethal temperature and 52 ºC for 3 hrs is considered as a LD-50 for chickpea seeds and can be used for screening chickpea germplasm lines for thermotolerance studies. Further, the present study was standardized at seed level as the seeds have less succulency when compared to the seedlings. It is well established fact that genotype JG-11 is considered as a thermotolerant that may be the reason for little higher temperature in this study as compared to the reports of (Raghavendra *et al.,* 2017) in chickpea who reported that 50 ℃ as lethal temperature.

Table 1:Effect of temperature on survival of chickpea seeds

Treatments	(%) Survival of seeds		
	Duration of Exposure		
	1 hr	2 hr	3 hr
T_1- 32 °C	96 (78.47)	92 (73.58)	90 (71.57)
T_2- 34 °C	96 (78.47)	91 (72.55)	88 (69.74)
T_3- 36 °C	91 (72.55)	88 (69.74)	84 (66.43)
T_4- 38 °C	89 (70.64)	83 (65.65)	81 (64.16)
T_5- 40 °C	84 (66.43)	81 (64.16)	78 (62.03)
T_6- 42 °C	76 (60.67)	70 (56.79)	68 (55.56)
T_7- 44 °C	73 (58.7)	68 (55.56)	65 (53.73)
T_8- 46 °C	70 (56.79)	65 (53.73)	61 (51.36)
T_9- 48 °C	64 (53.14)	61 (51.36)	59 (50.19)
T_{10}- 50 °C	62 (51.95)	58 (49.61)	56 (48.45)
T_{11}- 52 °C	57 (49.03)	55 (47.87)	50 (45.01)
T_{12}- 54 °C	54 (47.3)	51 (45.58)	45 (42.14)
T_{13}- 56 °C	33 (35.07)	27 (31.31)	28 (31.95)
T_{14}- 58°C	20 (26.57)	10 (18.44)	0 (0)
T_{15}- 60 °C	16 (23.58)	8 (16.43)	0 (0)

T_{16}- 62°C	13 (21.14)	5 (12.93)	0 (0)
T_{17}- 64°C	10 (18.44)	4 (11.54)	0 (0)
S. Em ±	0.47	0.44	0.42
CD @ 5%	1.34	1.26	1.21

Standardization of sub lethal temperature at seed level

To assess the sub lethal temperatures, 70 % hydrated chickpea seeds were exposed to gradual graded induction temperature from 32 ºC - 46 °C, 32 ºC - 48 °C, 32 ºC - 50 °C, 32 ºC - 52 ºC, 32 ºC - 54 ºC and 32 ºC - 56 ºC for 5 and half an hour. Later, such seeds were exposed to the standardized lethal temperature of 58 ºC for 3 hrs, later allowed for recovery at room temperature (30 °C) for 48 hours. The per cent survival of seeds and per cent reduction in root, shoot growth and seedling vigour index was recorded and presented in Table 2.

The results indicated that, an induction temperature of 32 ºC to 50 ºC [(32 to 48 ºC (1 hour) + 50 ºC (1 and half an hour)] for 5 and half an hour (T_4) is seems to be optimum or sub lethal temperature. Considerably, an induction temperature of 32 ºC to 50 ºC for 5 and half an hour has highest survival of seeds (87.25%), highest root growth (14.20 cm) and less per cent reduction in root growth (33.33 %), highest shoot growth (10.43 cm) and less per cent reduction in shoot growth (38.21 %), highest seedling vigour index (2148.97) and less per cent reduction in seedling vigour index (41.80 %) when compared with the other induction temperatures. Further, the present study was standardized at seed level as the seeds have less succulency when compared to the seedlings. As known that genotype JG-11 is considered as a thermotolerant and check variety for high temperature tolerance studies in many breeding programmes that can be the reason for little higher temperature in this study as compared to the reports of (Raghavendra *et al.*, 2017) in chickpea.

Table 2: Effect of sub lethal (thermo tolerance induction) temperature on survival, root, shoot growth and seedling vigour index in chickpea seeds

Treatments	**Survival of seeds (%)**	**% Reduction**	**Root length (cm)**	**% Reduction**	**Shoot length (cm)**	**% Reduction**	**Seedling vigor index**	**% Reduction**
T_1- control	96.72 (79.57)	-	21.3	-	16.88	-	3692.77	-
T_2- 32 - 46 °C	70.28 (56.97)	27.34	10.65	50.00	6.42	61.97	1199.68	67.51
T_3- 32 - 48 °C	80.12 (63.53)	17.16	12.10	43.19	7.93	53.02	1604.80	56.54
T_4- 32 - 50 °C	87.25 (69.08)	9.79	14.20	33.33	10.43	38.21	2148.97	41.81
T_5- 32 - 52 °C	45.19 (42.24)	53.28	9.50	55.40	6.21	63.21	709.93	80.78
T_6- 32 - 54 °C	22.46 (28.29)	76.78	5.37	74.79	3.24	80.81	193.38	94.76
T_7- 32 - 56 °C	15.74 (23.38)	83.73	3.18	85.07	1.99	88.21	81.38	97.80
S. Em ±	4.74		0.63		0.41		68.2	
CD @ 5%	NS		1.89		1.25		204.7	

To screen chickpea genotypes at seed level for thermotolerance under sub lethal temperature

A set of diverse six chickpea genotypes was screened for intrinsic tolerance using the standardized Thermo Induction Response (TIR) protocol. The experimental data were recorded and presented in Table 3.

The results of the experiment indicated that, the temperature 32 ºC to 50 ºC for 5 and half an hour[(32 to 48 ºC (1 hour) + 50 ºC (1 and half an hour)] which is standardized through temperature induction response (TIR) technique obtaining highest per cent survival of seeds (less reduction in per cent germination), highest root growth (less reduction in per cent root growth) and shoot growth (less reduction in per cent shoot growth).

This temperature is screened for six chickpea genotypes, among the 6 genotypes JG-14 showed highest per cent survival of seeds (87 %) with less per cent reduction in germination (10.04 %), highest root growth (19.91 cm) with less per cent reduction in root growth (16.24 %) and highest shoot growth (14.35 cm) with less per cent reduction in shoot growth (30.09 %) which is in on par with JG-11 and followed by A-1 showed highest per cent survival of seeds (91.70 % and 91.11 %) with less per cent reduction in germination (10.51 % and 11.89 %), highest root growth (19.57 cm and 18.89 cm) with less per cent reduction in root growth (17.46 % and 23.85 %) and highest shoot growth (13.58 cm and 12.96 cm) with less per cent reduction in shoot growth (32.44 % and 35.38 %), respectively. These findings are found in continuity with (Raghavendra *et al.*, 2017) in chickpea. As the genotypes JG-14 and JG-11 was established as thermotolerant hence, the respective genotypes exhibited higher seedling survival, root and shoot growth when compared to the rest genotypes used in the study. Further, these genotypes can be used for high temperature tolerance studies in any of the breeding programmes.

CONCLUSION

Through the investigation it can be concluded that 58º C for 3 hrsis considered as lethal temperature, 52º C for 3 hrs is considered as LD 50, 32ºC to 50ºC for 5and half an hour is considered as sub lethal temperature at seed level. Among the 6 genotypes screened the genotype JG-14 was thermotolerant followed by genotype JG-11 with high seedling survival percentage, less per cent reduction in root andshoot growth.

Table 3. Screening of chickpea genotypes at seed level for thermotolerance under sub lethal temperature

Treatments	Seedling survival (%)				Root length (cm)				Shoot length (cm)			
	Control	Induced	Mean	% Reduc-tion	Control	Induced	Mean	% Reduc-tion	Control	Induced	Mean	% Reduc-tion
JG-11	96.16 (78.70)	86.05 (68.07)	91.70 (72.66)	10.51	20.27	16.73	19.57	17.46	16.21	10.95	13.58	32.44
JG-14	96.72 (79.57)	87 (68.87)	91.86 (73.43)	10.04	21.3	17.84	19.91	16.24	16.88	11.8	14.35	30.09
A-1	97.5 (80.91)	85.9 (67.95)	91.11 (73.26)	11.89	21.55	16.41	18.98	23.85	15.74	10.17	12.96	35.38
GBM-2	97.05 (80.12)	85.28 (67.44)	91.17 (72.72)	12.12	23.3	16.52	18.57	29.09	17.56	11.13	13.13	36.61
JAKI 9218	96.31 (78.93)	84.41 (66.75)	90.36 (71.92)	12.35	21.91	15.16	18.41	30.8	14.55	8.52	11.54	41.44
ICCV 4958	95.84 (78.24)	83.01 (65.66)	89.43 (71.03)	13.38	21.65	13.2	17.43	39.03	16.27	9.19	12.73	43.51
Mean	96.60	85.28			21.66	15.98			16.20	10.29		
	G	T	G×T		G	T	G×T		G	T	G×T	
S. Em ±	0.36	0.21	0.51		0.08	0.04	0.11		0.06	0.03	0.08	
CD @ 5%	1.05	0.61	NS		0.22	0.13	0.32		0.16	0.09	0.23	

Note: Lethal temperature: 58 ℃ for 3 hours; Induction temperature: [32 to 48 ºC (For every 4 ºC increment in temperature exposure to 1 hour) + 50 ºC (1 and half an hour)]

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