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Short Communication

Identification of thermo tolerance urdbean [*Vigna mungo* (L) Hepper] genotypes using temperature induction response technique

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ABSTRACT

High temperature stress in plants reduces crop yield because it negatively affects several plant physiological processes. Screening and identification of cultivars for high-temperature stress conditions is essential under changing environments. In the present study, the temperature induction response (TIR) technique was used for screening the high-temperature tolerant genotypes in urdbean (*Vigna mungo* (I) Hepper). In the present investigation, induction temperature and lethal temperature have been standardized based on percent growth reduction and survival percentage at the end of the recovery period. The induction temperature was observed at 38 to 48 °C as sub-lethal i.e., challenging temperatures for 4 h and 30 min, while the lethal temperature was observed at 50 °C for 3 h. This technique can be used as a potential tool to identify and select temperature-tolerant genotypes at the seedling stage from a large population. A total of nineteen urdbean genotypes were screened and evaluated for thermo tolerance. By using standardized optimum induction and challenging temperature, cellular level tolerance was assessed in the studied genotypes. Based on root length and shoot length of induced seedlings over control seedlings, the cellular level tolerance in terms of least reduction in growth and highest survival percentage was calculated and PGRU 95014 IPU 94-1, IPU 2K -22 and PGRU 95016 identified as heat tolerant genotypes. These can be used as donors in breeding programs.

***Key words*:** Lethal temperature, Sub-lethal temperatures, Thermotolerance, Urdbean.

Urdbean (*Vigna mungo* (I) Hepper) is an important food legume and widely used in daily diet. Globally, many crop species are sensitive to high temperature (HT) stress, which ultimately affects countries' economies by reducing crop production (Abbas, 2022). Pulses including urdbean are very sensitive to drought, water logging, and high temperatures. High-temperature stress is frequently defined when temperature rises beyond the level of a threshold for a certain period and abundantly causes irreversible impairment to the growth and development of plants. When the temperature rises frequently to 10–15°C beyond the ambient temperature for a short period, it is described as heat shock or heat stress. HT is a major abiotic stress factor, which limits crop production (Cao *et al*., 2022) and causes harmful effects on plants. HT reduced the crop yield by direct damage to enzymes and tissues (Moore *et al*., 2021), impaired flowering (Tian *et al*., 2012), and triggered oxidative stress at the reproductive stage (Khan *et al*., 2022). In addition to this, it was found that the reproductive stage of crops such as tomato, rice, maize, cotton, and soybean is more susceptible to HT stress (Bawa *et al*., 2022; Jagtap *et al*., 2020; Jansma *et al*., 2022; Park *et al*., 2021; Wu *et al*., 2022) because, when the temperature rises at the inflorescence/panicle stage or during the flowering stage, the fertility of the crop is reduced, or sometimes the abortion of the flower may occur (Zhang *et al*., 2022). The most important effects on the reproductive phase that affect pod set, seed set and yield are flowering time, asynchrony of male and female floral organ development, and impairment of male and female floral organs. The importance of high temperature >30 °C, the temperature range of 32 - 35 °C during flowering also produces distinct effects on grain yield (Devasirvatham *et al*., 2012). Various screening techniques based on specific physiological parameters such as single-leaf photosynthetic capacity, and quantification of chlorophyll fluorescence under stress are being used to screen

thermotolerance at the field level (Selmani and Wasson, 1993), but these measurements are highly influenced by environmental factors which are the major limitation. The best alternative, therefore, would be to develop a suitable laboratory protocol for screening acquired thermo tolerance of urdbean genotypes. Earlier the temperature induction response (TIR) technique of exposing young seedlings to sub-lethal and lethal temperatures has been used and validated for screening high tolerant genotypes in other crops viz. rice (Sudhakar *et al.*, 2012; Sapna Harihar *et al*., 2014), sunflower (Senthil Kumar *et al*., 2003), cotton (Ehab abou Kheir *et al.*, 2012), groundnut (Gangappa *et al.*, 2006) and pea (Venkatachalayya *et al.*, 2001). Therefore, the present study aimed to standardize the temperature induction response technique and its use for screening the high-temperature tolerance genotypes in urdbean.

In the present investigation, 19 urdbean genotypes were used to screen the thermo-tolerant genotypes. These genotypes were collected from the Division of Crop Improvement, ICAR-Indian Institute of Pulses Research, Kanpur. Temperature induction response (TIR) technique was standardized for seedlings age, lethal temperature and induction temperature. For this, seeds (about 15-20) of urdbean genotypes were germinated by soaking them in sterile distilled water and then allowed to germinate in moist Petri plates at room temperature. Two-day-old seedlings or 1.5 cm length of seedlings were selected for the experiment. The uniform seedlings from each genotype were transferred to three different sets of Petri plates for further studies.

The term Challenging or "lethal temperature" refers to a temperature treatment that, when applied to seedlings without an induction cycle, results in a mortality rate of over 90%. This temperature treatment must be applied for a specified amount of time and measured in hours. The germinated urdbean seedlings were subjected to various challenging temperatures (44, 46, 48, and 50 ^{0}C) for varying durations (1, 2 and 3 hours) without any prior induction for determining the challenging temperature. As a result, exposed seedlings were allowed to recuperate at 30 ^{0}C and 60% relative humidity for 48 hours. At the end of the recovery period, the temperature at which 90% mortality of the seedlings occurred was considered the challenging temperature. To assess the genetic variability for seedling survival, the percent mortality of urdbean genotypes after recovery was noted (Table 1). The lethal temperature of 50 ^{0}C for 3 hours was considered in this context, at which maximum mortality (100%) of seedlings was observed.

During the induction treatment, the seedlings were exposed to a progressive rise in temperature for a specific period because temperature regime and duration vary in each crop. For this, germinated seedlings of urdbean were kept in gradually increasing temperatures i.e. 38 to 48 ^{0}C for a period of four and half hours. After this induction treatment, seedlings were exposed to lethal temperature i.e., 50 ^{0}C for three hours, and then allowed to recuperate at 30 ^{0}C and 60% relative humidity for 48h. The temperature regimes and durations are different to arrive at the optimum induction protocol (Table 1). The optimum sub-lethal temperatures were based on the percent survival of seedlings i.e., 38 ^{0}C - 48 ^{0}C.

To determine the lethal temperature, the percentage of seedling mortality and the reduction in growth were measured at the end of the recovery period. Lethal or challenging temperature was defined at which seedling growth was reduced 90% and 100% or seedlings died. The following formula was used to determine the lethal temperature.

$$Recovery\ \%\ mortality\ of\ seedlings = \frac{No.\ of\ seedlings\ died\ after\ treatment}{Total\ no.\ of\ seedlings\ sown\ in\ the\ tray} x100$$

Urdbean seeds were surface sterilized by treating them with 2% bavistin solution for 30 minutes. They were then cleaned four times with

Table 1. Percent mortality of urdbean seedlings at different lethal temperatures and sub-lethal temperature ranges.

Temperature range [Induction Temperature °C (4 hours 30 min)]	Percent survival of the seedling	Temperatures	Percent mortality of urdbean seedlings after recovery at different Temperatures duration		
			1 hour	2 hour	3 hour
32-50	60	44 ^{0}C	00	20	30
32-48	70	46 ^{0}C	00	30	50
34-48	80	48 ^{0}C	50	64	87
38-48	90	50 ^{0}C	76	90	100
35-50	50	-	-	-	

distilled water and kept for germination in an incubator at 30 ^{0}C temperature and 60% relative humidity. Uniform seedlings of each genotype were chosen and planted in 50 mm aluminum trays that were filled with soil. The plant growth chamber (Thermotech L-7003) was used to expose these trays of seedlings to sub-lethal temperatures (gradual temperature increases from 38 ^{0}C to 48 ^{0}C) for four and a half hours. Afterward, these seedlings were subjected to three hours of induced exposure to lethal temperatures (50 ^{0}C). Another set of seedlings was directly exposed to lethal temperatures (non-induced). Induced and non-induced urdbean seedlings were allowed to recover at 30 ^{0}C and 60% relative humidity for 48 hours. The following parameters were recorded from the seedlings.

$$Percent\ survival\ of\ seedlings = \frac{No.of\ seedlings\ survived\ at\ the\ end\ of\ recovery}{Total\ no.of\ seedlings\ sown\ in\ the\ tray} x100$$

$$Percent\ reduction\ in\ root\ growth = \frac{Root\ length\ of\ control\ seedling - root\ length\ of\ treated\ seedling}{Root\ growth\ of\ control\ seedlings} x100$$

$$Percent\ reduction\ in\ shoot\ growth = \frac{Shoot\ length\ of\ control\ seedling - Shoot\ length\ of\ treated\ seedling}{Shoot\ growth\ of\ control\ seedlings} x100$$

A lethal temperature of 50 ^{0}C for 3 hours and induction treatment from 38-48 ^{0}C for four and half hours was standardized using TIR (Thermo Induction Response) and considered as the best lethal and induction temperatures for phenotyping of urdbean seedlings for intrinsic heat tolerance at the cellular level (Table 2). The experimental data were recorded and the genotypes that showed contrasting values for survival of seedlings, and reduction in root and shoot growth were presented in Table 2. The effect of TIR on genotypes revealed variable results, such as acquired tolerance was variably recorded in urdbean genotypes, where either survival of seedlings was affected in 19 genotypes (IPU 94-2, PKGU-1, HPU 120, PGRU 95016, IPU 2K 99-224, IPU 99-200, IPU 99-18, UH 32-3, IPU 94-1, IPU 94-31, LBG 26, LBG 17, IPU 2K-22, IPU 2K-21, PGRU 95014, Shekhar, LBG20, LBG 99-79 and IPU 2K-221) or root growth alone was affected in 5 genotypes (PGRU 95016, IPU 2K 99-224, IPU 99-200, IPU 99-18, and UH 32-3 or only shoot growth alone was affected in 3 genotypes (PKGU 1, LBG 26 and LBG 17).

In the genotypes LBG 20 and LBG 99-79 the seedling survival, shoot, and root growth were completely affected despite the recovery conditions maintained after exposure to sub-lethal to lethal temperatures. Despite exposing to 50 ^{0}C, germination, and seedling growth were not affected in PGRU 95016, PGRU 95014, IPU 94-1, and IPU 2K -22 probably due to acquired of thermo-tolerance. The technique of exposing young seedlings to sub-lethal and lethal temperatures has been validated in many crop species such as pea (Venkatachalayya *et al.,* 2001), sunflower (Senthil-kumar *et al.,* 2003), groundnut (Gangappa *et al.* 2006), rice (Sudhakar et *al.,* 2012; Renukha *et al*., 2013; Vijayalakshmi *et*

Table 2. Screening of thermo tolerant urdbean genotypes through Thermo Induced Response (TIR) technique

S.No.	Genotypes	Germination percent	Percent reduction in root growth	Percent reduction in shoot growth
1	IPU 94-2	50(47.00)	42.03(40.20)	18.50(25.22)
2	PKGU-1	90(70.59)	28.75(32.11)	0.00(0.00)
3	HPU 120	60(48.01)	32.53(34.44)	20.55(22.77)
4	PGRU 95016	100(93.2)	0.00(0.00)	0.00(0.00)
5	IPU 2K 99-224	40(35.35)	0.00(0.00)	30.15(28.71)
6	IPU 99-200	70(55.86)	0.00(0.00)	9.37(15.70)
7	IPU 99-18	80(63.47)	0.00(0.00)	10.35(18.78)
8	UH 32-3	100(89.02)	0.00(0.00)	8.35(15.73)
9	IPU 94-1	70(52.75)	0.00(0.00)	0.00(0.00)
10	LBG 26	50(44.03)	31.50(34.50)	0.00(0.00)
11	LBG 17	100(90.04)	16.57(23.10)	0.00(0.00)
12	IPU 2K-22	90(70.61)	0.00(0.00)	0.00(0.00)
13	IPU 2K-21	90(71.60)	20.22(26.40)	27.37(31.99)
14	PGRU 95014	100(89.02)	0.00(0.00)	0.00(0.00)
16	IPU 2K-221	40(38.10)	23.99(28.76)	33.28(34.94)
17	Shekher	100(91.00)	0.00(0.00)	0.00(0.00)
Mean		72.35	11.51	9.29
S.E. ±		5.92	0.691	0.892
CD (P=0.05)		19.1	2.45	2.97

al., 2015) and cotton (Ehab abou Kheir *et al.,* 2012). This temperature induction response technique has been demonstrated to reveal genetic variability in intrinsic stress tolerance at the cellular level (Narayanaswamy, 2010). In conclusion, our results suggest that the TIR technique is a powerful, rapid, and constructive technique to identify genetic variability for high-temperature tolerance in urdbean and it can be used to screen a large number of genotypes. The PGRU 95016 [(100(93.2)], PGRU 95014 [100(89.02)], IPU 94-1 [70(52.75)], and IPU 2K -22 [90(70.61)] genotypes identified in the present study can be used as a donor for developing high-temperature tolerant urdbean for late-sown conditions where urdbean is prone to terminal heat stress.

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