Vegetable Science (2017) 44 (2) : 20-25

Evaluation of French bean genotypes for high temperature tolerance using Temperature Induction Response (TIR) technique

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Received: July 2017 / Accepted: November 2017

Abstract

French bean is a cool season legume crop requires a temperature of 17⁰C-28°C for ideal growth. It is grown mainly during *kharif* and *rabi* seasons. But its cultivation is severely constrained during summer months due to prevailing high temperatures. Further, under climate change conditions high temperature episodes are likely increase and considerably affect pod yield. The identification of suitable cultivars is a prerequisite for adaptation to high temperature situations. Thus, the temperature induction response (TIR) technique, which involves exposure of seedlings to sub lethal temperatures to induce inherent cellular tolerance and consequent exposure to challenging temperatures was standardized and employed for screening French bean genotypes for high temperature stress tolerance. Two day old French bean seedlings were subjected to various challenging temperatures from 43°C to 50°C for 3 hours and were allowed to recover at room temperature for 72 hours. Based on the least survival (11%) and highest growth reduction (92%) during recovery, 45°C for 3 hours was identified as challenging temperature. Consequently the induction temperature was identified by subjecting French bean seedlings to different induction temperature regimes. The gradual induction temperature from 30°C to 40°C for 3 hours at which 60% of the seedlings survival was observed during recovery and identified as the optimum induction temperature. Subsequently, ten French bean genotypes were screened by subjecting them to the standardized induction temperature of 30°C-40°C for 3 hours followed by challenging temperature of 45°C for 3 hours. The genotypes showed differences in percent seedling growth reduction during recovery period over control. The genotypes, (IC-525224 x IC-525239) IPS-1, (IC-525224 x IC-525239) 1-12 and Arka Anoop, with 60%, 65.27 %, 66.30% reduction in seedling growth showed tolerance to high temperature stress. In this study we standardized the TIR technique as a screening method to evaluate and identify temperature tolerant French bean genotypes.

Keywords: French bean, High temperature tolerance, TIR, Induction temperature, Challenging temperature

Introduction

French bean (*Phaseolus vulgaris* L.), due its high nutritional value, is an important vegetable crop. It grows well under optimum mean temperature range of 17⁰C - 28⁰C and due to which its cultivation is restricted to *kharif* and *rabi* seasons. However, the demand for French beans during summer months is encouraging farmers to cultivate the crop during March and April months. But the crop is exposed to great deal of high temperature stress during these months. The tropical regions, where prevailing temperatures are already high, would further face the adverse effects of high temperature stress under climate change conditions. High temperature adversely affects the crop growth, development and economic yield and causes reduction in shoot dry mass, growth and net assimilation rates in a number of plants (Wahid et al. 2007). It is observed that high temperatures prevailing during summer months lead to drastic reductions in bean yield. Lowest pod set was observed in plants exposed to high temperature for 1–6 days prior to anthesis (Graham and Ranalli 1997). Exposure to 35/20°C or 35°C caused reduced pollen viability (Halterlein 1980). The reproductive stage is sensitive to high temperatures and reduced pod and seed set is observed due to enhanced abscission of flower buds, flowers, and pods in beans (Konsens et al. 1991). The most critical time when high temperatures adversely affect French bean yield is from flowering through pod filling. Reduction in number of pods per plant is primarily affected by temperatures above the optimum (McWilliams 1980). Wang (1962) observed 25°C to 26°C as an upper optimum during blooming and 36°C as the

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upper threshold where growth ceased. High temperatures during flowering explained 68% of the variation in French bean seed yield (Ridge and Rye 1985). Due to the requirement of mild temperature conditions, the French bean crop is grown mainly during *rabi* season, where the crop gets optimum temperatures. But, growing French bean crop beyond *rabi* season puts the crop at risk due to adverse effects of high temperature stress.

Plants overcome high temperature stress by adopting several physiological and biochemical mechanisms. Thus, in order to adapt the French bean crop cultivation during summer months where, prevailing temperatures are around 35°C to 39 °C, it becomes necessary to identify suitable genotypes. Several techniques have been adopted for screening genotypes for high temperature stress tolerance. Several workers adopted the temperature induction response (TIR) technique, where seedlings are exposed to an induction temperature and subsequently to a challenging temperature which is otherwise lethal to non-induced seedlings (Senthil-Kumar et al. 2003). It is a potential and versatile technique for identifying thermo-tolerant genotypes (Gangappa et al. 2006). Hence, the present study was conducted with an objective to standardize the TIR technique for screening French bean genotypes and to identify tolerant genotypes.

Materials and Methods

Plant Material: The studies on standardization of challenging and induction temperatures were conducted using French bean cv. Arka Sharath. The seeds were surface sterilized by treating with 0.1% Carbendizum for two minutes and washed with distilled water for 4-5 times. The seeds were soaked for 24 hrs and 100 seeds were placed on the moist germination sheet and another moist sheet was placed over it. The rolled up sheets were kept in incubator for 48 hours maintained at 30°C for 8 hrs and 20°C for 16 hrs. The two day old seedlings (approximately 0.5 cm in length) were exposed to induction and challenging temperatures. The subsequent screening of French bean genotypes was done using the ten germplasm lines (Table 1) available in the Division of Vegetable Crops, ICAR-IIHR, Bengaluru, India.

Table 1: French bean genotypes used for screening

Sr.No.	Genotype
1	(IC-525224 x IC-525239)1-5
2	(IC-525224 x IC-525239)-5
3	(IC-525224 x IC-525239)-9
4	(IC-525224 x IC-525239)-12
5	(IC-525224 x IC-525239)-2
6	(IC-525224 x IC-525239)IPS-1
7	(IC-525224 x IC-525239)1-12
8	(IC-525224 x IC-525239)-1
9	Arka Sharath
10	Arka Anoop

Determination of challenging temperature: Twenty five (two day old) seedlings of French bean cv. Arka Sharath were placed on the moist germination paper in an aluminium tray and three such trays were used as replicates. The seedlings were exposed to challenging temperatures of 43°C, 45°C, 48°C, and 50°C for 3 hours at 60% RH and were allowed to recover at room temperature for 72 hours. At the end of the recovery period the percent seedling survival was recorded.

Determination of optimum induction temperature: In order to determine the optimum induction temperature twenty five two day old seedlings of cv. Arka Sharath placed on the moist germination paper in aluminium tray replicated thrice were subjected to different induction temperature treatments. The induction temperature treatments, gradual temperature increase from 30°C to 40°C for 3h and 30°C to 42°C for 3h were imposed and subsequently the induced seedlings were exposed to the challenging temperature, 45°C for 3h at 60% RH. Based on the per cent seedling survival and the maximum seedling recovery growth, gradual temperature increase from 30°C to 40°C for 3h was considered to be the optimum induction temperature.

Recovery Growth: After subjecting the seedlings to different challenging temperatures and also to induction followed by challenging temperature, seedlings were

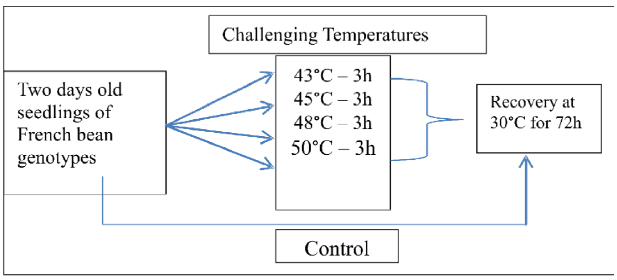


Fig 1: Steps involved in standardization of challenging temperature

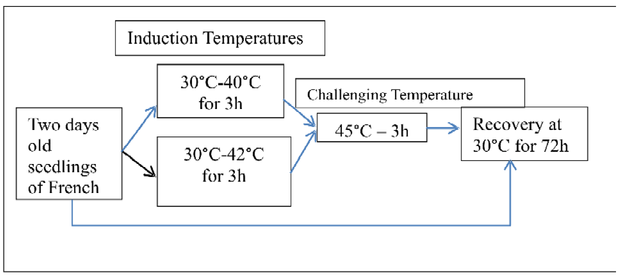


Fig 2: Steps involved in standardization of induction temperature

maintained at 30°C for 72 h for recovery growth. A set of seedlings maintained at 30°C throughout the experimental period were considered as control. At the end of recovery period the percent seedling survival and growth of the surviving seedlings were recorded.

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

$$\text{Per cent Reduction in growth} = \frac{(\text{Growth of control seedlings after 72 h recovery} - \text{Growth of treated seedlings after 72 h recovery})}{\text{Growth of control seedlings after recovery}} \times 100$$

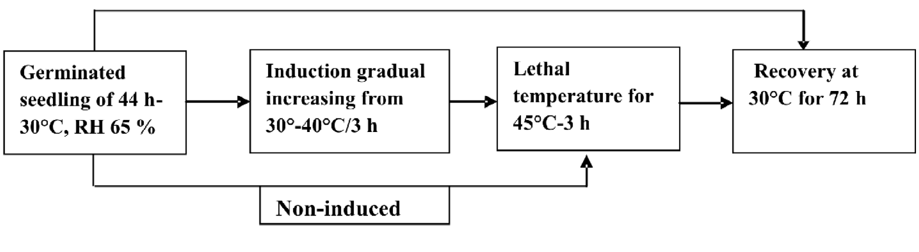
Results and Discussion

Various mechanisms enable plants to adapt to high temperature stress (Rampino et al. 2009). The capability to survive at high temperatures is inherent in plants and acquired thermo tolerance could be induced through either exposure to sub lethal temperatures (De Klerk et al. 2012) or to a gradual temperature increase to lethal levels (Larkindale and Vierling 2008). Evaluation for high temperature stress tolerance needs to reflect traits that control basal as well as acquired thermo-tolerance (Gomathi et al. 2014), as observed in studies on pea and sunflower (Amutha et al. 2007). Temperature Induction Response (TIR) is a powerful technique that has been employed to screen seedlings for high temperature stress tolerance (Senthil-Kumar 2001). In this technique, the plantlets are exposed to an optimum induction temperature before being exposed to a severe challenging temperature and subsequently allowed to recover at room temperature. The genotypes that show the maximum survivability and growth at the end of the recovery period are selected as thermotolerant (Srikanthbabu et al. 2002; Kheir et al. 2012). Hence, these screening studies for high temperature tolerance take in to consideration the survival and recovery growth of plantlets/seedlings on exposure to high temperature stress upon acclimation. In the present study as the challenging temperature increased from 43°C to 50°C the reduction in percent seedling survival was observed in French bean cv. Arka Sharath at the end of recovery period (72h) (Fig 4A). The seedling recovery growth also decreased as the challenging temperature increased from 43°C to 50°C. Based on the percent seedling survival and recovery growth, the challenging temperature, 45°C for 3 hrs at which 11% of seedlings survived with 8% recovery growth over control, was identified as the challenging temperature for further screening of French bean genotypes (Fig 4B). The challenging temperature for a crop is determined based on least survival of seedlings and the challenging temperatures vary for different crops. The temperature of 48°C for 1 h was identified for pea (Srikanthbabu et al. 2002) and 55°C from 1 to 3 h for sunflower (Kumar et al. 1999).

Subsequent to standardization of challenging temperature the induction temperature was identified using the two day old French bean Cv. Arka Sharath seedlings. The seedlings were subjected to two induction temperature regimes, a gradual temperature increase from 30°C to 40°C for 3h and 30°C to 42°C for 3h, followed by standardized challenging temperature of 45°C for 3h. The exposure to induction temperature of gradual increase from 30°C to 42 °C for 3h caused 100 per cent mortality. The exposure to induction temperature of 30°C to 40°C for 3h followed by 45°C for 3h showed 60% seedling survival. Hence, the induction temperature treatment of exposing the two day old seedlings from 30°C to 40°C for 3 h was identified as optimum induction temperature. Based on the two experiments the exposure of two day old seedlings to induction temperature of 30°C to 40°C for 3h and subsequently followed by challenging temperature of 45°C for 3h was standardized for further screening of French bean genotypes. Exposure of seedlings to induction temperature before subjecting to challenging temperature enhances seedling survival. Studies have revealed the activation of heat shock proteins and subsequent trigger of stress associated gene expression (Prandl et al. 1998).The previous studies on standardization of induction temperature identified gradual temperature increase from 28°C to 40°C over 4 h in cotton (Kheir et al. 2012). Based on higher recovery growth induction temperature from 35°C to 45°C for 4 h was identified for sunflower (Amutha et al. 2007).

Standardized protocol for TIR:

Evaluation of French bean genotypes for high temperature stress tolerance: The evaluation of ten different French bean genotypes using the standardized TIR technique showed significant differences in terms of percent seedling survival after 72 h of recovery period (Fig 6). Among the genotypes Arka Anoop showed 100 percent seedling survival followed by genotype (IC-525224 x IC-525239) IPS-1 and (IC-525224 x IC-525239)1-12 which showed 90% seedling survival. The least percent survival of 21.67% was observed in



(IC-525224 x IC-525239)-12 (IC-525224 x IC-525239) IPS-1

Arka Sharath Arka Anoop

Fig 3: Comparison among the genotypes

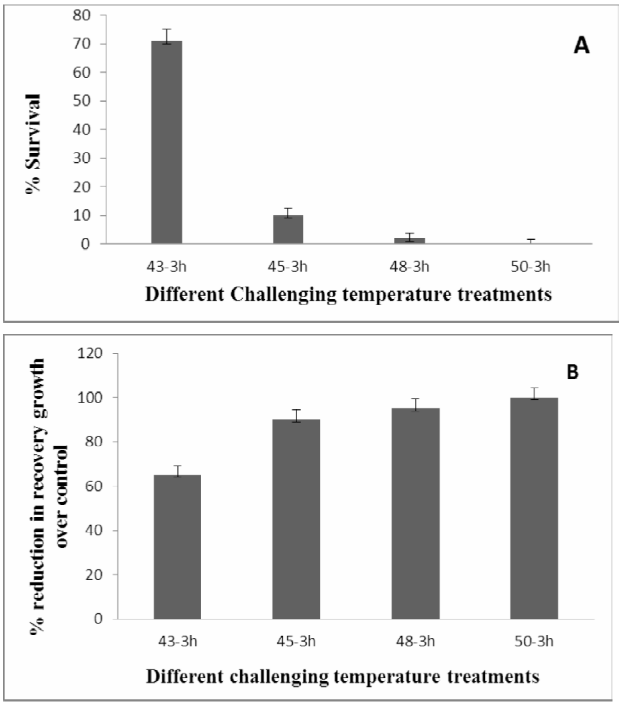


Fig 4: Per cent survival of French bean cv. Arka Sharath seedlings (A) and per cent reduction in recovery growth over control (B) after exposure to different challenging temperatures. Bars represent the std. error of means at 5% level.

genotype (IC-525224 x IC-525239)1-5. The differences in percent reduction in seedling growth were also observed among the genotypes during recovery period over control seedlings grown at 30°C temperature. The genotypes, (IC-525224 x IC-525239) IPS-1, (IC-525224 x IC-525239) 1-12 and Arka Anoop, with 60%, 65.27 %, 66.30% respectively the reduction in recovery growth relative to seedlings maintained at ambient temperatures, showed tolerance to high temperature stress.

The genetic variability of ten French bean genotypes to high temperature tolerance was assessed by plotting the

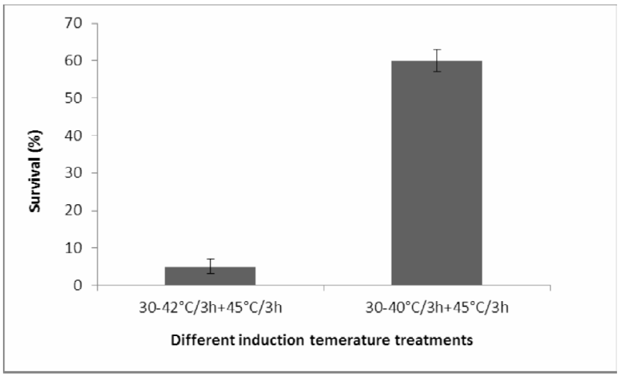


Fig 5: Per cent survival of French bean cv. Arka Sharath seedlings under two levels of induction temperature followed by challenging temperature. Bars represent the std. error of means at 5% level.

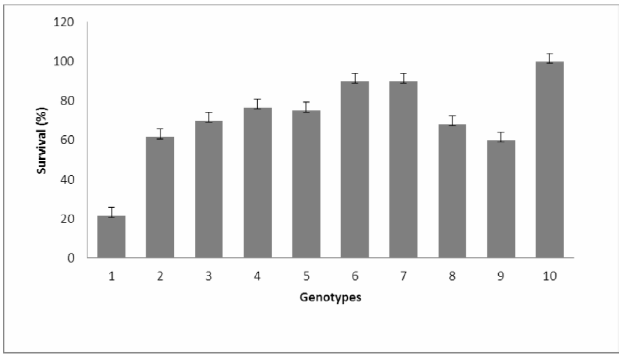


Fig 6: Per cent seedling survival of French bean genotypes exposed to induction followed by challenging temperature stress.

Z distribution, based on percent reduction in recovery growth and percent seedling survival after recovery period. The French bean genotypes were classified into three different categories as tolerant, moderately tolerant and susceptible (Fig 7). The classification of tolerant, moderately tolerant and susceptible genotypes using the Z distribution plot was followed in cotton (Kheir et al. 2012) and in tomato (Vijayalakshmi et al. 2015).The

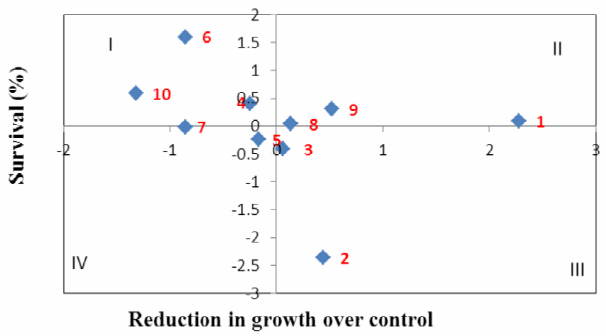


Fig. 7: Normal Z-distribution graph of French bean genotypes on per cent survival and per cent reduction in growth over control. Quadrant I has the highly tolerant genotypes and quadrant II and IV includes the moderately tolerant and quadrant III includes susceptible genotypes

Table 2: Genetic variability in ten French bean genotypes for high temperature tolerance

S No.	Genotypes	Root length of seedlings exposed to induction followed by challenging temperature after 72 hr recovery (cm)	Root length of control seedlings (cm)	Per cent reduction in root length over control
1	(IC-525224 x IC-525239)1-5	3.98	10.26	61.28
2	(IC-525224 x IC-525239)-5	2.50	10.77	76.83
3	(IC-525224 x IC-525239)-9	3.15	9.87	64.40
4	(IC-525224 x IC-525239) -12	4.94	12.05	59.33
5	(IC-525224 x IC-525239)- 2	4.46	12.30	63.37
6	(IC-525224 x IC-525239) IPS-1	4.60	9.64	51.73
7	(IC-525224 x IC-525239) 1-12	4.24	11.15	61.94
8	(IC-525224 x IC-525239)-1	3.47	9.15	61.57
9	Arka Sharath	4.70	11.89	59.91
10	Arka Anoop	4.34	10.51	58.17
	Mean	4.03	10.75	61.85
	CD at 5%	1.42	2.71	16.31
	CV(%)	20.45	14.67	15.38

normal Z distribution of the genotypes studied showed that the genotypes, (IC-525224 x IC-525239)-12, (IC-525224 x IC-525239) IPS-1 and Arka Anoop were located in Quadrant I, which were classified as highly tolerant. The two genotypes, Arka Sharath and (IC-525224 x IC-525239)-2 were classified as moderately tolerant (Quadrant II and IV). The genotypes, (IC-525224 x IC-525239)-5 and (IC-525224 x IC-525239)-9 were grouped in Quadrant III, as susceptible. The genotypes falling in quadrants I and III are important because they distinctly differ in thermotolerance. The genotypic variability in high temperature stress tolerance has been assessed using the TIR techniques and contrasting cultivars in pea (Srikanthbabu et al. 2002), sunflower (Kumar et al. 1999) and tomato (Senthil Kumar et al. 2004) have been identified.

Though subjecting French bean seedling to challenging temperatures caused drastic reduction in seedling survival, the exposure to gradual induction temperature enhanced seedling survival through activation of inherent cellular tolerance mechanisms. Hence, for screening the French bean genotypes, the exposure to induction temperature followed by challenging temperature is required to assess the genetic potential of the genotypes. Each crop species may require different induction and challenging temperature requirements. Thus, for French bean, we standardized the induction temperature of gradual increase from 30°C - 40°C for 3 h followed by the challenging temperature of 45°C for 3 h in the present study. Using the standardized TIR technique three genotypes (IC-525224 x IC-525239)-12, (IC-525224 x IC-525239) IPS-1 and Arka Anoop were identified as tolerant to high temperature stress.

Acknowledgement

Authors are highly thankful to the Director of ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore and ICAR-Indian Institute of Vegetable Research, Varanasi, India for providing the support and all the facilities required for completion of the work.

सारांश

राजमा–फली एक शीतकालीन दलहनी सब्जी है, जिसके उत्तम विकास के लिये 17–28 डिग्री सेन्टीग्रेड का तापमान उचित है। सामान्यतः इस फसल की खेती खरीफ तथा रवी मौसम में की जाती है। ग्रीष्म काल में, अति उष्ण तापमान के चलते राजमा–फली की खेती बहुत कम की जाती है। जलवायु परिवर्तन के चलते राजमा–फली के फली उत्पादन में कमी होने की सम्भावना है। इसीलिये अधिक तापमान में खेती योग्य वंशक्रम की पहचान करना अनिवार्य हो गया है। टेम्परेचर इंडक्शन रिस्पॉस तकनीक (टीआइआर) में पौधों को सब लीथल तापमान में रखते हैं ताकि उसमें कोशिकाओं के स्तर पर सहनशीलता हो जाये और पौधे आगे आने वाले ऊष्ण तापमान को सह सके। दो दिन वाले राजमा–फली के पौधों को तीन घंटे के लिये 43–50 डिग्री सेन्टीग्रेड का तापमान पर रखा गया और 72 घंटों के बाद उन्हें रूम टेम्परेचर पर लाया गया। प्रतिलाभ के दौरान सबसे कम उत्तरजिविता (11 प्रतिशत) और सबसे ज्यादा विकास में कमी (92 प्रतिशत) के आधार पर 3 घंटों के लिये 45 डिग्री सेन्टीग्रेड का तापमान इसे चुनौती तापमान के रूप में पहचाना गया। साथ ही इंडक्शन तापमान की पहचान करने के लिये राजमा–फली के पौधों को विभिन्न तापमान वाले कक्षाओं में रखा गया। तीन घंटों के लिये 30–40 डिग्री सेन्टीग्रेड का ग्रेजुअल इंडक्शन टेम्परेचर, जिसमें प्रतिलाभ के दौरान 60 प्रतिशत पौधे जीवित रहे उसे उचित तापमान पहचाना गया। राजमा–फली की दस किस्मों को 30–40 डिग्री सेन्टीग्रेड के स्टैण्डर्ड इंडक्शन टेम्परेचर और उसके बाद 3 घंटे और 45 डिग्री सेन्टीग्रेड के उच्च तापमान पर परखा गया। सभी किस्मों के पौधों का प्रतिशत विकास कम प्रदर्शन रहा। उच्च तापमान में आईपीएस–1 (आईसी–525224 × आईसी–525224), 1–2 (आईसी–525224 × आईसी–525224) एवं अर्का अनूप में क्रमशः 60 प्रतिशत, 65.27 प्रतिशत और 66.30 प्रतिशत पौधों के विकास में कमी पायी गयी। इस प्रयोग के तहत राजमा–फली के लिये टीआईआर तकनीक का मानकीकरण किया गया, जिसके कारण अधिक तापमान सहनशील किस्मों की पहचान की जा सके।

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