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HEAT STRESS AND ACQUISITION OF THERMOTOLERANCE IN MUNG BEAN (*VIGNA RADIATA* (L.) WILCZEK)

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ABSTRACT

Mung bean (*vigna radiata*) is one of the most popular pulse crop in Asia. It is cultivated biannually in large areas of Pakistan during warm seasons and is a good source of protein. Optimum temperature for all four mung bean genotypes (NM 19-19, NM 20-21, NM121-123 and NCM 89) was found to be 30°C. Lethal temperature was 50°C (2 hours) as severe growth retardation was seen but better growth performance was found when a pretreatment of 40°C (1 hour) prior to 50°C (2 hours) was given as visualized by improvement in seedling length and heat stress tolerance index (HST). Mean seedling length and heat stress tolerance index was reduced under heat stress however more reduction was seen in NM 20-21 while less in NM 19-19.

Key words; Mung bean, genotypes, thermotolerance, seedling length, fresh weight

INTRODUCTION

Germination, growth and number of physiological processes depend upon certain chemicals, water and climate. As plants are such organisms that cannot move for shelter, they exposed to number of environmental stresses like water logging, salinity, drought and temperature. Plants have evolved mechanisms to cope with the problems caused by high temperature because the most typical kind of stress plants received from it surrounding is temperature (Larkindale and Knight, 2002; Iba, 2002). Heat stress due to increased temperature is an agricultural problem in many areas of world. High temperature is responsible for number of morpho-anatomical, physiological and biochemical changes in plants, which effect plant growth and development and may lead to drastic reduction in economic yield (Wahid *et al*., 2007). Under increasingly stressful conditions, plant experience progressively more abnormal, impaired or dysfunctional cellular processes, ultimately leads to death (Fitter and Hay, 1981)

It is well documented that pretreatment with a mild temperature treatment prior to lethal temperature allows plants to tolerate higher temperature than non-pretreated plants and such plants are termed as thermo-tolerant (Howarth and Ougham 1993; Burke 2001). Therefore pretreatment with temperature acclimation has been used as an affective method of enhancing temperature stress resistance in various plants (Wang *et al.*, 2003).

Current research is an effort to find out optimum and lethal temperature and also to evaluate the effect of mild temperature pretreatment prior to lethal temperature in the acquisition of thermotolerance hence to screen out the most and least thermo-tolerant genotypes.

MATERIALS AND METHODS

Four genotypes of mung bean (*Vigna radiata* (L.) Wilczek), NM 19-19, NM 20-21, NM 121-123, and NCM 89 were obtained from Pakistan Agricultural Research Council, Islamabad, Pakistan. Seeds were sterilized in 1% sodium hypochlorite (common bleach) solution for 2 minutes, rinsed four times with distilled water, imbibed in distilled water for 5 hours.

Twenty seeds were incubated in petri plate for each genotype and temperature (25°C and 30°C), lined with two layers of filter papers soaked with distilled water(d/w). Seedling length (cm) was recorded after 24, 48, 72 and 96 h.

ACQUISITION OF THERMOTOLERANCE

Thermotolerance was detected following the method of Chen *et al*., (1986) with minor modifications. Forty sterilized seeds (genotype^{-1} treatment^{-1}) were exposed to temperature treatments as given in Table 1. Seedling length (cm) was taken 24, 48 and 72 hour after treatment.

% promotion/inhibition was calculated by the following formula,

% promotion/inhibition= treatment-control÷control x 100

Heat stress tolerance (HST) index was calculated by the method of Porch (2006)

STATISTICAL ANALYSIS:

Data was analyzed by using analysis of variance in three factorial as CRD, taking temperature as factor A, genotypes as factor B and harvest as factor C. Experiment was repeated three times. Analysis of Variance was performed by a computer program SPSS 11. Means were compared using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980).

Table 1. Temperature treatments and abbreviations used during the experiment.

Abbreviations for treatments	Temperature treatments and pretreatments
A	30°C(d/w)→30°C (Inc.buff)→30°C(d/w)
B	30°C(d/w)→40°C(1hr,Inc.buf))→50°C(2hr,Inc.buf))→30°C(d/w)
C	30°C(d/w)→50°C(2hr,Inc.buf))+ →30°C(d/w)

Inc.buff = Incubating buffer; d/w = distilled water

RESULTS

ANOVA revealed highly significant differences for temperature as well as genotypes for all harvests except for 24 hours. The interaction of the factors for all harvests was non significant interaction (Table 2). Seedling length was larger at 30°C in all genotypes and harvests. Therefore 30°C may be considered to be an optimum temperature for mung bean growth (Fig 1). It was further noticed that NM 19-19 exhibited highest seedling length at 72 hours for both 25 and 30°C when compared with the rest of the genotypes

Table 2. Mean squares from analysis of variance for seedling length of four mung bean genotypes, harvested after germinated at 25 °C and 30°C

Sources of Variation	**df**	**MS**	**F Value**
Replication	2	1.65	0.665ns
Temperature (T)	1	440.2	179.52**
Genotype (G)	3	35.34	14.175**
Harvest (H)	3	1324.15	530.99**
T X G	3	0.399	0.16^{ns}
T X H	3	39.03	15.65**
G X H	9	6.38	2.56^{ns}
T X G X H	9	0.299	0.12^{ns}
Error	62	2.494	-
Total	95		

Table 3. Mean squares from analysis of variance for mean seedling length of four mung bean genotypes harvested after temperature treatments

Sources of Variation	**df**	**MS**	**F value**
Replication	2	1.97	0.922^{ns}
Temperature (T)	2	1031.43	480.72**
Genotype (G)	3	35.76	16.7**
Harvest (H)	3	698.75	325.10**
T X G	6	2.25	1.05^{ns}
T X H	6	165.63	76.72**
G X H	9	6.18	2.878*
T X G X H	18	0.903	0.418^{ns}
Error	94	2.171	-
Total	143		

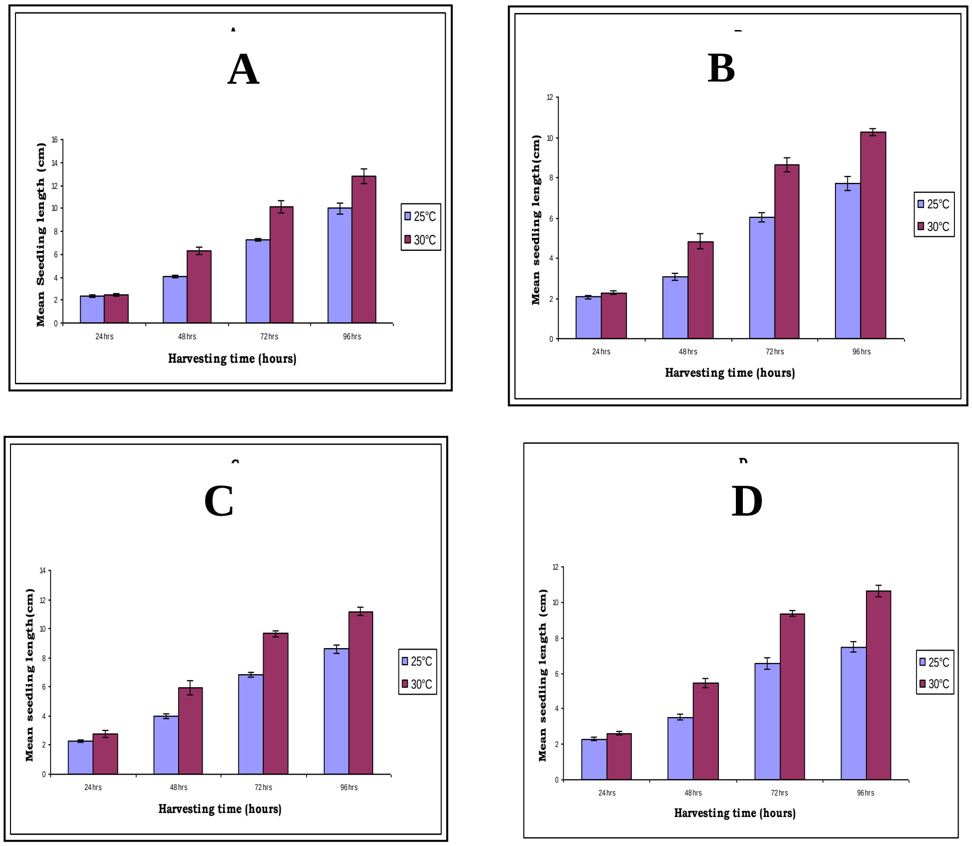

Fig.1: Mean seedling length (cm) in mung bean (*Vigna radiata*), harvested after germinating at 25°C and 30°C. A- NM 19-19; B- NM 20-21; C- NM 121-123 and D- NCM 89.

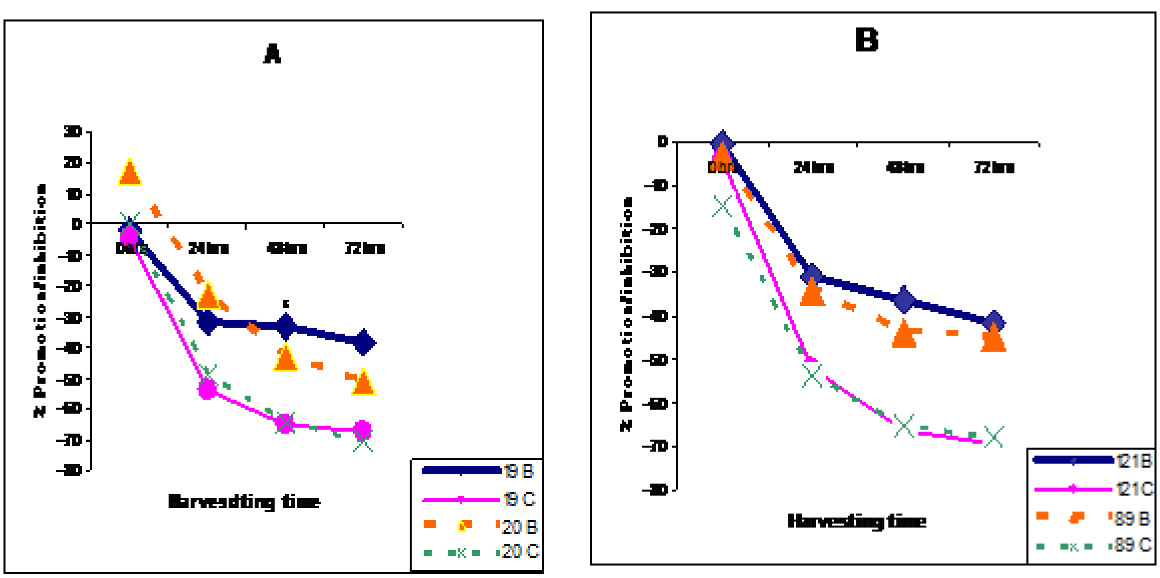

Fig.3. Percent inhibition / promotion in mean seedling length harvested after temperature treatments. A-genotype NM 19-19 and NM 20-21; B- genotype NM 121-123 and NCM 89

Acquisition of Thermotolerance

Mean sum of square is given in Table 3, demonstrating that in 0 hour harvested samples non significant differences were found for temperatures, genotypes as well as interactions. Highly significant differences were observed for temperature and genotypes with non significant interactions for 24, 48 and 72 hours harvested samples. It was noticed that mean seedling length of all genotypes taken at 0 hour after treatments exhibited unset pattern among treatments (Table 4).The differences became prominent at 72 hours. The lowest mean seedling length was found in treatment C (50°C) which was improved by the application of mild temperature of 40°C before 50°C (treatment B) but was still less than A(30°C) and lowest in treatment C (50°C). It was further noted that mean seedling length was highest in NM 19-19 and lowest in NM 20-21 for all treatments and harvests except for 0 hour. Percent inhibition was also less in NM 19-19 and more in NM 20-21 for treatment B and C (Fig.3)

Heat stress tolerance index (HST)

Table 4 represents heat stress tolerance index for mean seedling length for pretreatment and lethal treatments. All genotypes possessed highest HST index for pretreatment (B) and lowest for lethal temperature (C) for all harvesting time.

HST values for mean seedling length was lowest in NM 20-21 and highest in NM 19-19 for all harvests except at 0 hour. Low HST indicated marked reduction in mean seedling length. It reflected that great reduction took place at lethal temperature (C), which improved by the application of mild temperature treatment (B) ie 40°C for 1 hour prior to lethal shock.

Table 4. Mean values for mung bean seedling length and heat stress tolerance (HST) index of seedlings harvested after various temperature treatments

Harvesting time	Treatments	Genotypes							
		NM 19-19		NM 20-21		NM 121-123		NCM 89	
		Length	HST	Length	HST	Length	HST	Length	HST
0 hrs	A	$2.46^{a}\pm0.115$	—	$2.28^{b}\pm0.083$	—	$2.75^{a}\pm0.251$	—	$2.62^{a}\pm0.114$	—
	B	$2.42^{a}\pm0.151$	0.058	$268^{a}\pm0.151$	0.059	$2.74^{a}\pm0.151$	0.073	$2.54^{a}\pm0.115$	0.065
	C	$2.36^{a}\pm0.143$	0.056	$2.28^{b}\pm0.149$	0.05	$2.63^{a}\pm0.113$	0.07	$2.23^{b}\pm0.08$	0.057
24 hrs	A	$6.29^{a}\pm0.307$	—	$4.84^{a}\pm0.388$	—	5.96 ± 0.501	—	$5.44^{a}\pm0.253$	—
	B	$4.28^{b}\pm0.147$	0.053	$3.74^{b}\pm0.138$	0.035	$4.11^{b}\pm0.268$	0.048	$3.58^{b}\pm0.238$	0.038
	C	$2.90^{c}\pm0.099$	0.035	$2.47^{c}\pm0.135$	0.023	$2.84^{c}\pm0.169$	0.033	$2.51^{c}\pm0.128$	0.026
48 hrs	A	$10.12^{a}\pm0.539$	—	$8.65^{a}\pm0.336$	—	$9.67^{a}\pm0.184$	—	$9.36^{a}\pm0.18$	
	B	$6.79^{b}\pm0.179$	0.048	$4.94^{b}\pm0.20$	0.029	$6.14^{b}\pm0.268$	0.041	$5.28^{b}\pm0.119$	0.034
	C	$3.52^{c}\pm0.098$	0.024	$2.98^{c}\pm0.116$	0.018	$3.24^{c}\pm0.145$	0.021	$3.3^{c}\pm0.113$	0.021
72 hrs	A	$12.80^{a}\pm0.671$	—	$10.34^{a}\pm0.2$	—	$11.18^{a}\pm0.3$	—	$10.64^{a}\pm0.305$	—
	B	$7.89^{b}\pm0.424$	0.049	$5.1^{b}\pm0.188$	0.026	$6.52^{b}\pm0.184$	0.036	$5.89^{b}\pm0.077$	0.031
	C	$4.13^{c}\pm0.116$	0.026	$3.07^{c}\pm0.107$	0.015	$3.324^{c}\pm0.125$	0.018	$3.3^{c}\pm0.147$	0.017

Similar alphabets represent homogeneous mean in each box with standard errors.

DISCUSSIONS

Temperature is one of the most important factor that effect seed germination, growth as well as physiology of any living organism like plants, and ultimately reduces crop yield. There is an optimum temperature for different organisms at which their morphological and physiological performance would be at their best levels. However genotypic variations can be seen in response to temperature stress.

When seeds of all four genotypes were allowed to germinate at 25°C and 30°C, growth in terms of seedling length was noted down and for all four genotypes 30°C was considered to be an optimum temperature.

Amal *et al*., (2007) reported that highest germination percentage of mung bean was obtained at 30°C, whereas Chen *et al.,* (1986), reported that 25°C is optimum temperature for mung bean. This variation could be due to the adaptation to various climatic conditions

During current work it was detected that the effect of 50°C for 2 hours was lethal but when seedlings were treated with relatively mild temperature of 40°C for 1 hour prior to 50°C for 2 hours, seedlings survived well. It indicated that mung bean seedlings survived well even at lethal temperature if exposed to comparatively mild temperature prior to lethal temperature exposure. However when compared to control seedlings, the growth of thermo-protected seedlings was significantly inhibited. These findings are in full agreement with Chen *et al*.,(1986), who reported that potentially lethal temperature for 32 hours old etiolated mung bean seedlings was 45°C for 2 hours, and seedlings became thermo-tolerant and survived a 2 hours 45°C treatment when they were pre-incubated for 1 hour at 40°C. It was also detected that seedlings transferred to the high temperature without pretreatment were injured more than pretreated seedlings of creeping bent grass (Gulen and Atilla, 2003). Larkindale *et al.*, (2005) reported that thermotolerance can also be induced by gradual increase in temperature to lethal temperature as would be experienced in natural environment. This thermotolerance is because of the synthesis of heat shock proteins (HSPs) (Burke, 2001). In higher plants, HSPs are generally induced by a short exposure to a temperature of 38°C-40°C (Iba, 2002).

By observing harvest data of 72 hours it was detected that mean seedling length was highest in genotype NM 19-19 and lowest in NM 20-21 in all treatments. Higher heat stress tolerance index values for seedling length was also obtained at lower temperature and lowest was achieved at lethal shock means pretreatment increases HST as compared to lethal shock. It is suggested that non lethal temperature treatment before lethal can produce thermotolerance in mung bean. Further more NM 19-19 responded better under temperature treatments as far as seedling length and HST index is concerned. On the basis of their growth performance it may be concluded that the most thermo-tolerant genotype was NM 19-19 and the least NM 20-21.

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