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EFFECT OF GA_3 PRETREATMENTS ON THERMOTOLERANCE IN MUNG BEAN (*VIGNA RADIATA* (L.) WILCZEK)

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ABSTRACT

High temperature is one the major abiotic stress that are principal cause of reduced crop yield world wide. Four mung bean genotypes (NM 19-19,NM 20-21, NM121-123 and NCM 89) were tested against high temperature treatments with and without GA_3.
When mung bean seedlings were exposed to 50°C for 2 hours (C) lethality occurred within 72 hours. The effect of lethal temperature was reduced by the mild temperature treatment of 40°C for 1 hour prior to 50°C for 2 hours (B) and most of the seedlings improved in their length and fresh weight. This effect is further improved by the exogenous application of 100 uM GA_3 either as treatment B1or treatment B2 . The effect of GA_3 showed positive effect on seedling length, fresh weight and heat stress tolerance index (HST), however treatment B2 was better than treatment B1. Mean seedling length, fresh weight and heat stress tolerance index was increased during GA_3 application. More improvement was seen in NM 19-19 than in NM 20-21.

Key words: Mung bean, Genotypes, Gibberellic acid, Thermotolerance, Seedling length, Fresh weight

INTRODUCTION

Mung bean is the major Kharif pulse crop grown in Pakistan in irrigated as well as rain fed areas (Khattak *et al*., 2004). Increase in temperature enhances transpiration and evaporation which causes deficiency of water in plants (Gates, 1968). In mung bean, high temperature causes drastic reduction in seed yield due to high flower shedding (Khattak *et al.,* 2009).

Hong *et al*., (2000), reported that the ability of organisms to acquire thermotolerance to normally lethal temperature is an ancient conserved adaptive response. Acquisition of thermotolerance is important to plants that experience daily temperature fluctuations and are unable to escape to more favorable environment. This thermotolerance could be inherent or acquired. Inherent thermotolerance relates to the ability of an organism to withstand, up to a certain degree, a rapid change in temperature above the optimum. Acquired thermotolerance means the level of protection beyond the inherent thermotolerance that results from prior exposure to elevated, non lethal temperature or by exogenous application of biomolecules (Howarth and Ougham, 1993).

The high temperature reduces the endogenous levels of cytokinins, gibberellins and auxins (Royal, 1981; Yakushikina and Tarasov, 1982). It is possible that exogenous GA_3 counteracts the reduction in the endogenous levels of plant hormones that promote growth and increase endogenous level of plant hormones that inhibits growth. It is reported that gibberellins can reduce the adverse effect of high temperature stress during seed germination (Kaufmann and Ross, 1970; Cavusoglu and Kudret, 2007).

Current study is an effort to find out the effect of GA_3 in the elevation of thermotolerance in four mung bean 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 with 1% sodium hypochlorite (common bleach) solution for 2 minutes, rinsed four times with distilled water.
Forty sterilized seeds (genotype^{-1} treatment^{-1}) were imbibed in distilled water for 5 hours, incubated at 30^{o}C in petri plates for 24 hours. Temperature treatments were given in the presence of incubating buffer (0.001M Sodium Phosphate buffer pH 6.0 and 1% Sucrose solution) with or without 100 μM GA_3 (Chen *et al.,* 1986) as shown in Table 1. Length of five and fresh weight of three seedlings were recorded after various time intervals. Percent promotion/inhibition was calculated by the formula; Percent 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 separately for each harvest by using a computer program SPSS 11. Means were compared using Duncan's multiple range test (DMRT) ($P \leq 0.05$) (Steel and Torrie, 1980).

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

Abbreviations for treatments	Temperature treatments
A	$30^{\circ}C$(d/w)→$30^{\circ}C$ (Inc.buf)→$30^{\circ}C$(d/w)
A1	$30^{\circ}C$(d/w)→$30^{\circ}C$ (Inc.buf+ GA_3)→$30^{\circ}C$(d/w)
B	$30^{\circ}C$(d/w)→$40^{\circ}C$(1hr,Inc.buf))→$50^{\circ}C$(2hr,Inc.buf))→$30^{\circ}C$(d/w)
B1	$30^{\circ}C$(d/w)→$40^{\circ}C$(1hr,Inc.buf+GA_3))→$50^{\circ}C$(2hr,Inc.buf))→$30^{\circ}C$(d/w)
B2	$30^{\circ}C$(d/w)→$40^{\circ}C$(1hr,Inc.buf))→$50^{\circ}C$(2hr,Inc.buf+GA_3))→$30^{\circ}C$(d/w)
C	$30^{\circ}C$(d/w)→$50^{\circ}C$(2hr,Inc.buf))→$30^{\circ}C$(d/w)

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

RESULTS

Effect of heat shock and GA_3 on seedling length

Table of ANOVA (Table 2) exhibited highly significant differences for temperature, genotypes, harvests, where as first degree interactions were either significant or highly significant however second degree interaction was non significant (Table 2).

A A1 B B1 B2 C

Fig.1. Effect of different temperature treatments on mung bean seedlings on growth and development.

The difference in mean seedling length amongst all treatments at 0 hour was not very prominent for all genotypes. However for rest of the harvests the hierarchy in growth promotion mostly was A1>A>B2>B1>B>C for all genotypes (Fig1 and Table 3). Similarly inhibition in seedling length was lesser in B<B1<B2 and highest in C, where as A1 showed promotion in seedling length when compared with control (A). It was also observed that in NM 19-19 inhibition was less than in any other genotype and NM 20-21 showed highest inhibition for any treatment at 72 hours (Fig 2).

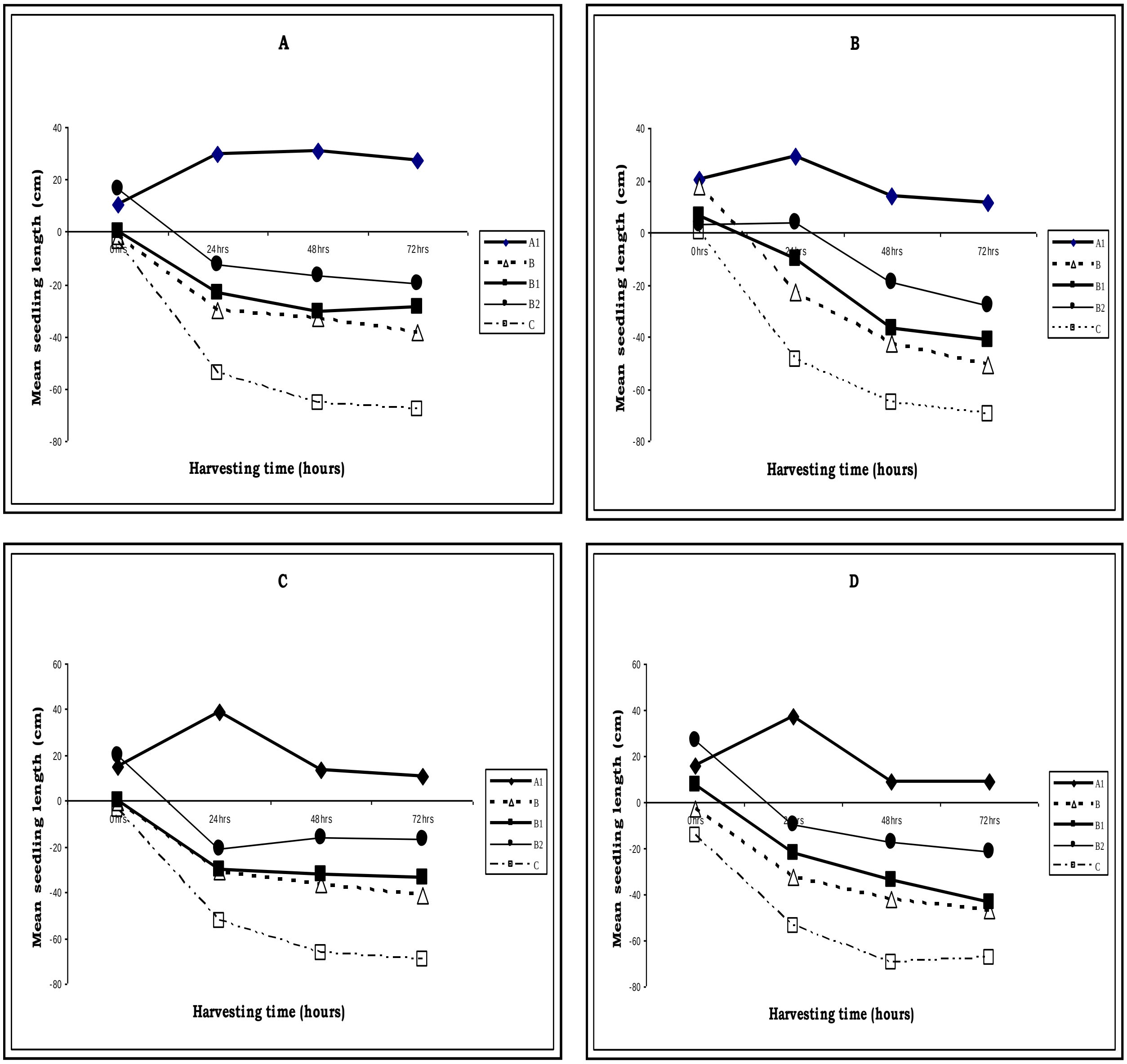

Fig.2. Percent inhibition/ promotion in mean seedling length harvested after various temperature treatments. A-genotype NM 19-19, B-genotype NM 20-21, C- genotype NM 121-123 and D- genotype NCM 89. A1→30°C(GA_3); B- 30°C→40°C(1hr)→50°C(2hr); B1- 30°C→40°C(1hr+GA3)+50°C(2hr), B2-30°C+40°C(1hr)+50°C(2hr+GA3), C-30°C →50°C(2hr)→30°C.

Table 2. Mean squares from analysis of variance for seedling length and fresh weight of four mung bean genotypes as affected by various temperature treatments.

Sources of Variation	df	Seedling length		Fresh Weight	
		MS	F Value	MS	F Value
Temperature (T)	5	906.95	503.8**	0.175	519.2**
Genotype (G)	3	124.53	69.18**	0.0095	28.18**
Harvest (H)	3	2221.4	1234.1*	0.544	1614.2**
T X G	15	4.69	2.60*	0.00133	3.94**
T X H	15	119.96	66.64**	0.0258	79.55**
G X H	9	25.24	14.02**	0.00429	12.72**
T X G X H	45	2.331	1.29ns	0.000624	1.85**
Error	190	1.8		0.000337	

*= significant ;**= highly significant at P< 0.01; ns = non-significant; df = degrees of freedom.

Table 3. Mean values of mung bean seedling length, harvested after various temperature treatments

Harvesting time	Treatments	Genotypes			
		NM 19-19	NM 20-21	NM 121-123	NCM 89
0 hrs	A	$2.46^{b}\pm0.115$	$2.28^{c}\pm 0.083$	$2.75^{bc}\pm 0.251$	$2.62^{c}\pm 0.114$
	A1	$2.72^{ab}\pm0.126$	$2.74^{a}\pm0.088$	$3.16^{ab}\pm0.145$	$3.04^{ab}\pm0.092$
	B	$2.42^{b}\pm0.151$	$2.68^{ab}\pm0.151$	$2.74^{bc}\pm 0.151$	$2.54^{cd}\pm0.115$
	B1	$2.46^{b}\pm0.081$	$2.42^{abc}\pm0.107$	$2.76^{bc}\pm0.077$	$2.82^{bc}\pm0.101$
	B2	$2.87^{a}\pm0.052$	$2.35^{bc}\pm0.073$	$3.3^{a}\pm0.130$	$3.32^{a}\pm0.224$
	C	$2.36^{b}\pm0.143$	$2.28^{c}\pm 0.149$	$2.63^{c}\pm0.113$	$2.23^{d}\pm 0.080$
24 hrs	A	$6.29^{b}\pm 0.307$	$4.84^{b}\pm0.388$	$5.96^{b}\pm 0.501$	$5.44^{b}\pm0.253$
	A1	$8.16^{a}\pm0.280$	$6.24^{a}\pm 0.243$	$8.28^{a}\pm 0.350$	$7.46^{a}\pm0.330$
	B	$4.28^{d}\pm0.147$	$3.74^{c}\pm0.138$	$4.11^{c}\pm0.268$	$3.58^{d}\pm0.238$
	B1	$4.82^{d}\pm0.172$	$4.34^{bc}\pm 0.217$	$4.17^{c}\pm0.181$	$4.23^{c}\pm0.159$
	B2	$5.5^{c}\pm 0.220$	$5.02^{b}\pm 0.330$	$4.70^{c}\pm 0.169$	$4.92^{b}\pm0.113$
	C	$2.90^{e}\pm 0.099$	$2.47^{d}\pm 0.135$	$2.84^{d}\pm0.169$	$2.54^{e}\pm0.127$
48 hrs	A	$10.12^{b}\pm0.539$	$8.65^{b}\pm 0.330$	$9.67^{b}\pm0.184$	$9.36^{b}\pm0.181$
	A1	$13.24^{a}\pm0.600$	$9.84^{a}\pm0.279$	$11.0^{a}\pm0.475$	$10.38^{a}\pm0.263$
	B	$6.79^{d}\pm0.179$	$4.94^{d}\pm 0.200$	$6.14^{d}\pm0.268$	$5.28^{d}\pm0.119$
	B1	$7.02^{d}\pm0.280$	$5.50^{d}\pm0.134$	$6.53^{d}\pm0.352$	$5.54^{d}\pm0.286$
	B2	$8.42^{c}\pm0.682$	$7.02^{c}\pm0.314$	$8.12^{c}\pm0.155$	$7.02^{c}\pm0.251$
	C	$3.52^{e}\pm0.098$	$2.95^{e}\pm0.116$	$3.24^{e}\pm0.145$	$3.30^{e}\pm0.113$
72 hrs	A	$12.80^{b}\pm0.671$	$10.27^{b}\pm0.20$	$11.12^{b}\pm0.305$	$10.64^{b}\pm 0.305$
	A1	$16.29^{a}\pm0.677$	$11.45^{a}\pm0.435$	$12.28^{a}\pm0.452$	$11.80^{a}\pm0.378$
	B	$7.85^{d}\pm0.424$	$5.1^{e}\pm 0.188$	$6.52^{e}\pm 0.184$	$5.89^{d}\pm 0.077$
	B1	$9.12^{cd}\pm0.102$	$6.0^{d}\pm0.140$	$7.4^{d}\pm 0.133$	$6.11^{d}\pm 0.156$
	B2	$10.28^{c}\pm0.284$	$7.41^{c}\pm0.240$	$9.25^{c}\pm0.204$	$8.54^{c}\pm0.353$
	C	$4.12^{e}\pm 0.110$	$3.07^{f}\pm0.107$	$3.37^{f}\pm 0.125$	$3.3^{e}\pm0.147$

Effect of heat stress and GA_3 on fresh weight

Highly significant differences were observed for temperature, genotypes, harvests and interactions (Table 2).

Mean fresh weight was highest for treatment A1 and lowest at treatment C for all genotypes at all harvests except for 0 hour (Table 4). It was further noted that mean fresh weight was higher in GA_3 treated samples as compared to their controls for all harvest except at 0 hour. However B1 showed less mean fresh weight than in certain harvests and genotypes.

Heat stress tolerance index (HST) for mean seedling length and fresh weight

Heat stress tolerance (HST) index on mean seedling length and fresh weight under temperature stress and GA_3 treatments is represented in Table 5. HST index values showed variations in mean seedling length among genotypes as well as treatments. It is clear from the values that lowest HST index for mean seedling length as well as fresh weight were obtained in treatment C, increased in B and improved by the application of GA_3 in B1, which was further enhanced in B2, but highest HST index was found when seedlings were grown at optimum temperature along with GA_3 (treatment A1). It indicated that high temperature stress imposed negative effect and caused significant reduction in seedling length, but GA_3 exhibited positive effect on seedling length and highest tolerance was seen to be possessed by NM 19-19, whereas NM 20-21 exhibited lowest HST.

Table 4. Mean values of mung bean fresh weight, harvested after various temperature treatments.

Harvesting time	Treatments	Genotypes			
		NM 19-19	NM 20-21	NM 121-123	NCM 89
0 hrs	A	$0.226^{bc}\pm 0.003$	$0.25^{b}\pm 0.005$	$0.21^{b}\pm 0.005$	$0.28^{c} \pm 0.005$
	A1	$0.28^{a}\pm 0.005$	0.24b± 0.010	$0.22^{b}\pm 0.005$	$0.313^{b}\pm 0.033$
	B	$0.22^{bc}\pm 0.005$	$0.226^{cd}\pm 0.003$	$0.2061^{b}\pm 0.006$	$0.246^{d}\pm 0.003$
	B1	$0.246^{ab}\pm 0.003$	$0.31^{a}\pm 0.010$	$0.296^{a}\pm 0.003$	$0.35^{a}\pm 0.015$
	B2	$0.266^{a}\pm 0.024$	$0.293^{a}\pm 0.003$	$0.286^{a}\pm 0.012$	$0.29^{bc}\pm 0.005$
	C	$0.21^{c} \pm 0.005$	$0.21^{d}\pm 0.005$	$0.21^{b} \pm 0.005$	$0.23^{d}\pm 0.008$
24 hrs	A	$0.31^{bc} \pm 0.018$	$0.303^{c}\pm 0.008$	$0.29^{c}\pm 0.005$	$0.34^{b}\pm 0.023$
	A1	$0.42^{a}\pm 0.003$	$0.366^{a}\pm 0.006$	$0.386^{a}\pm 0.003$	$0.41^{a}\pm 0.005$
	B	$0.283c^{cd}\pm 0.003$	$0.273^{d}\pm 0.003$	$0.28^{c}\pm 0.013$	$0.316^{b}\pm 0.008$
	B1	$0.266^{d}\pm 0.003$	$0.33^{b}\pm 0.005$	$0.31^{c}\pm 0.005$	$0.355^{b}\pm 0.010$
	B2	$0.31^{b}\pm 0.008$	$0.34^{b}\pm 0.010$	$0.336^{b}\pm 0.008$	$0.39^{a}\pm 0.012$
	C	$0.26^{d}\pm 0.011$	$0.246^{e}\pm 0.003$	$0.246^{d}\pm 0.003$	$0.25^{c}\pm 0.003$
48 hrs	A	$0.42^{b}\pm 0.011$	$0.4^{ab}\pm 0.003$	$0.43^{b}\pm 0.015$	$0.41^{ab}\pm 0.005$
	A1	$0.49^{a}\pm 0.005$	$0.43^{a}\pm 0.005$	$0.47^{a}\pm 0.015$	$0.45^{a}\pm 0.010$
	B	$0.37^{d}\pm 0.145$	$0.326^{d}\pm 0.045$	$0.34^{d}\pm 0.006$	$0.336^{c}\pm 0.027$
	B1	$0.386^{c}\pm 0.008$	$0.35^{cd}\pm 0.003$	$0.37^{cd}\pm 0.011$	$0.38^{c}\pm 0.015$
	B2	$0.41^{bc}\pm 0.005$	$0.37^{bc}\pm 0.003$	$0.4^{bc}\pm 0.005$	$0.403^{b}\pm 0.003$
	C	$0.276^{e}\pm 0.008$	$0.25^{e}\pm 0.008$	$0.275^{e}\pm 0.005$	0.273d±0.008
72 hrs	A	$0.546^{b}\pm 0.026$	$0.54^{b}\pm 0.012$	$0.54^{b}\pm 0.015$	$0.53^{b}\pm 0.005$
	A1	$0.65^{a}\pm 0.005$	$0.6^{a}\pm 0.005$	$0.61^{a}\pm 0.008$	$0.616^{a}\pm 0.003$
	B	$0.456^{c}\pm 0.012$	$0.413^{cd}\pm 0.028$	$0.42^{d}\pm 0.011$	$0.43^{cd}\pm 0.008$
	B1	$0.44^{c}\pm 0.011$	$0.386^{d}\pm 0.008$	$0.436^{cd}\pm 0.014$	$0.423^{d}\pm 0.008$
	B2	$0.476^{c}\pm 0.018$	$0.436^{c}\pm 0.008$	$0.46^{c}\pm 0.005$	$0.466^{c}\pm 0.017$
	C	$0.29^{d}\pm 0.006$	$0.27^{e}\pm 0.02$	$0.283^{e}\pm 0.012$	$0.29^{e}\pm 0.008$

DISCUSSION

Studies were conducted with the aim of removing the inhibitory effect of high temperature stress on growth and physiology by using growth regulators like GA_3. During present work, GA_3 in combination with temperature regimes was used to see the alleviating effect on seedling growth. Current results indicated that heat stress reduces seedling length but with the application of GA_3 seedling length enhances at optimum as well as at temperature treatments of all harvests. Akman (2009) reported that as temperature increased from 35-41°C, average time of germination delayed significantly however the application of GA_3 stimulated germination in rice as well in sorghum. Similarly Cavusoglu and Kudret (2007) also reported that pretreatment of GA_3 before heat stress can enhance shoot, radicle length of radish and barley.

Present investigations showed that when GA_3 applied during optimum temperature, seedlings length was increased. It is suggested that GA_3 promotes the growth of plant. Gibberellin production lies in its effect upon mobilization of food reserves from storage organ of seeds, which in turn supports growth of young seedlings (Marcus and Rodway, 1982). Current results indicated more inhibition in seedling length at lethal (C) or pretreatment (B) as compared to pretreatment B1 and B2. Actually high temperature reduces the endogenous levels of gibberllins (Yakushikina and Tarasov,1982) and exogenous application of GA_3 compensates endogenous levels of plant hormones which in turn promote growth (Mohsin and Naqvi, 1994).

Table 5. Heat stress tolerance (HST) index of mean seedling length and fresh weight for three trials under various temperature and pretreatments.

Harvesting time	Temp. treat	Genotypes							
		NM 19-19		NM 20-21		NM 121-123		NCM 89	
		Length	F.W	Length	F.W	Length	F.W	Length	F.W
0 hrs	A1	0.065	0.067	0.061	0.064	0.085	0.049	0.077	0.093
	B	0.058	0.053	0.059	0.060	0.073	0.046	0.065	0.073
	B1	0.059	0.059	0.063	0.083	0.074	0.066	0.072	0.105
	B2	0.069	0.064	0.052	0.078	0.088	0.064	0.085	0.087
	C	0.056	0.050	0.050	0.056	0.070	0.047	0.057	0.069
24 hrs	A1	0.100	0.086	0.061	0.072	0.085	0.072	0.071	0.090
	B	0.053	0.056	0.035	0.053	0.048	0.052	0.038	0.069
	B1	0.059	0.053	0.041	0.064	0.048	0.058	0.045	0.077
	B2	0.068	0.062	0.047	0.066	0.055	0.063	0.052	0.086
	C	0.035	0.052	0.023	0.048	0.033	0.046	0.026	0.055
48 hrs	A1	0.093	0.071	0.059	0.062	0.074	0.073	0.067	0.067
	B	0.048	0.053	0.029	0.048	0.041	0.053	0.034	0.050
	B1	0.049	0.058	0.033	0.050	0.044	0.057	0.036	0.056
	B2	0.059	0.062	0.042	0.053	0.054	0.062	0.045	0.060
	C	0.024	0.042	0.018	0.036	0.021	0.043	0.021	0.040
72 hrs	A1	0.103	0.076	0.058	0.069	0.067	0.070	0.062	0.70
	B	0.049	0.053	0.026	0.049	0.036	0.048	0.031	0.045
	B1	0.057	0.050	0.030	0.044	0.040	0.050	0.032	0.048
	B2	0.065	0.055	0.037	0.055	0.050	0.053	0.044	0.052
	C	0.026	0.034	0.015	0.031	0.018	0.032	0.017	0.033

Present results revealed that the application of 100 µM GA_3 enhanced seedling's weight during optimum temperature as well as during heat stress. It is reported by Chen *et al.*,(1986) and Cavusoglu and Kudret (2007) that heat shock response and GA_3 treatments enhance growth of seedlings and acquirement of thermotolerance in etiolated mung bean seedlings and also enhanced the subsequent growth of hypocotyls and fresh weight.

It was noticed that fresh weight increased with age for all genotypes and more inhibition was seen for lethal temperature. When 100 µM GA_3 was applied during pretreatment as B1 or B2, fresh weight was better than was seen in pretreatment B. Our findings are also supported by Cavusoglu and Kudret (2007), who reported an increase in fresh weight of barley seedlings when 900 µM GA_3 was applied during 35°C, could be because GA_3 counteracts with the high temperature on barley seedlings. High temperature interferes with seed germination by changing the water status of the seed so that water uptake is inhibited (Kabar and Baltepe, 1990) and when growth is retarded fresh weight will also retarded.

It is reported that endogenous levels of GA_3 and other plant hormones reduced under heat stress which can be overcome by exogenous application of GA_3 to reduce the adverse effect of high temperature stress during seed germination (Kaufman and Ross, 1970; Cavusoglu and Kudret, 2007). Therefore it may be suggested from present investigations as well that heat stress reduce fresh weight in all genotypes and application of 100 µM GA_3 improves fresh weight in all genotypes when compared with sample of heat stressed alone. It was further observed that the application of 100 µM GA_3 in pretreatment as B2, mean fresh weight was higher than the samples treated with 100 µM GA_3 in pretreatment as B1.

It is suggested that different genotypes respond differently at various environmental conditions, like Cavusoglu and Kudret (2007) reported variable response of barley and radish seedlings to GA_3 on fresh weight basis. By looking at the HST, NM 19-19 responded as most thermo-tolerant and NM 20-21 as the least thermo-tolerant genotype.

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