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INDUCTION OF THERMO-TOLERANCE THROUGH SALICYLIC ACID IN MUNG BEAN (*VIGNA RADIATA* L.) SEEDLINGS

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ABSTRACT

High temperature is most important abiotic stress which causes morphological and physiological changes in plants. It is needed to screen those genotypes that can perform well under elevated temperatures. Developing plants with improved thermotolerance can mitigate the adverse effects of heat stress. There are certain hormones or hormone like substances like salicylic acid (SA) that can enhance stress tolerance in plants. During current investigations, seeds of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were imbibed in 0.1 and 1.0 mM SA prior to lethal temperature stress (50°C for 2h). Seedling length, fresh weight and dry weight were reduced remarkably under lethal stress but the pretreatment of SA before lethal stress caused significant improvement in these parameters at $P<0.05$ level of significance for all genotypes. Heat stress tolerance index (HSTI) is used as an effective tool for the detection of stress tolerance in plants. It was observed that the HSTI values reduced considerably at lethal temperature, however pretreatment of salicylic acid improved HSTI. The performance of NM 19-19 was best and NM 20-21 was poor amongst all genotypes by considering these morphological parameters as well as HSTI values.

Key words: mung bean; seedling length; fresh weight; thermotolerance; salicylic acid

INTRODUCTION

Mung bean, (*Vigna radiata* L. Wilczek) is an excellent pulse crop because of easy digestibility and palatability with relatively high protein content. Mung bean is the major kharif pulse crop grown in Pakistan, on irrigated as well as rainfed areas (Khattak *et al.*,2004). Optimum temperature is the temperature best suitable for the growth of any crop and when temperature exceeds the optimum temperature for biological purposes, crops often respond negatively with steep down with net growth and yield (Mearns *et al*.,1984 ; Wolfe,1994). High temperature causes oxidative stress and effect the germination of mung bean, and reduced subsequent growth and hypocotyl elongation. (Thind and Chanpreet , 1997). High temperature in mung bean causes drastic reduction in seed yield due to high or otherwise complete flowers shedding (Khattak *et al.*, 2009).

Salicylic acid (SA), a plant phenolic is now considered as a hormone-like endogenous regulator, and its role in the defense mechanisms against abiotic stresses has been well documented (Yalpani *et al*., 1994; Szalai *et al* .,2000). Salicylic acid has been reported to cause a multitude effects on the morphology and physiology of plants (Pierpoint, 1994; Pancheva *et al.*, 1996). Treatment of bean and tomato plants with SA increased their tolerance against heat, chilling and drought stress (Senaratna *at al.,* 2000). The effect of SA on heat tolerance showed that the heat tolerance of mustard plants was improved by spraying with SA (Dat *et al*.,1998). SA was shown to play a role in the induction of thermotolerance in Arabidopsis plants (Larkindale and Knight.,2002). Application of Salicylic acid has also reported to increase heat tolerance in creeping bent grass (Larkindale and Bingru, 2004). High temperature reduced growth but exogenous application of SA during temperature improved growth in beans and tomato (Senaratna *et al.,* 2000).

MATERIALS AND METHODS

Mung bean seeds of genotype NM 19-19, NM 20-21, NM 121-123 and NCM 89 were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan. Seeds were sterilized with 1% sodium hypochlorite solution for 5 min, rinsed with distilled water (d/w) several times. Seeds were divided into three sets, the first set was soaked in d/w, second set was soaked in 0.1mM Salicylic acid (SA), while the third set in 1.0 mM SA for 8 h. Healthy and uniformed sized seeds were allowed to germinated for 48 h at 30°C in Petri dishes, lined with two layers of filter paper moisten with d/w. Forty eight h old seedlings of second and third sets were then exposed to 50°C (lethal temp) for 2 h and first set with 30°C for 2h which served as control. Seedlings were transferred back to 30°C and allowed to grow for further 24h. Imbibition of seeds in SA was also performed by Barba-Espin *et al.*, (2011). Eighty hours old etiolated mung bean seedlings were harvested for seedling length, fresh weight (FW), dry weight (DW).

Heat stress tolerance index (HSTI) of all the parameters were calculated by the method of Porch (2006) using following formula;

Heat stress tolerance index (HSTI) = cont x stress/ (control mean of all genotypes)^2

Statistical Analysis

Experiment was performed in factorial as CRD with three replications by taking genotypes as factor A and treatment as factor B. Analysis of Variance was performed using a computer program SPSS version 11. Comparative analysis of means was performed by Duncan's Multiple range test at P< 0.05 level of significance (Steel and Torrie, 1980)

RESULTS

Table 1 demonstrated the mean sum of square for seedling length, fresh weight (FW) and dry weight (DW) of four mung bean genotypes, harvested after lethal temperature and SA pretreatment effect before lethal stress. It was observed that there was significant differences for treatment, genotypes and interaction for all parameters with non significant interaction for DW.

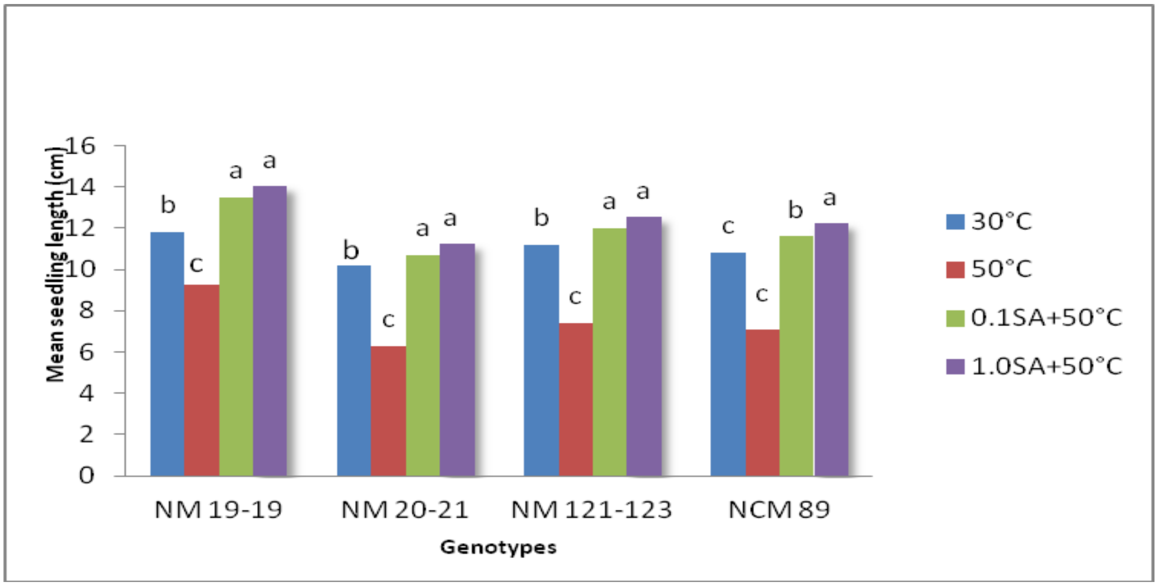

Fig 1. Mean seedling length of four mung bean (*Vigna radiata*) genotypes after lethal temperature and SA pretreatment. Similar alphabets represent homogenous means for each genotype.

Mean comparisons for seedling length, FW and DW was done to detect the effect of lethal temperature (50°C 2h) and pretreatment effect of salicylic acid (0.1 and 1.0 mM) on lethal temperature on mung bean seedlings. There was negative effect of lethal temperature on seedling length of mung bean. Seedling length was inhibited to 21.6% for NM 19-19, 38.2% for NM 20-21, 33.7% for NM 121-123 and 34.2% for NCM 89 at lethal temperature (Fig. 1). However there was significant promotion in mean seedling length when a mild concentration of SA (0.1mM) was applied prior to lethal temperature which further improved when SA concentration was raised to 1.0 mM as compared to control for all genotypes.

Mean FW of four mung bean genotypes reduced under lethal stress, but improved in SA pretreated samples (Fig 2). Mean FW for control sample in NM 19-19 was 0.32 g which inhibited to 10.9% for lethal temperature, however there was 21.87% promotion in 0.1mM SA pretreatment which further promoted to 34.37% for 1.0mM SA pretreatment. This pattern was also same for all genotypes.

Fig. 3, showed mean DW of seedlings of four mung bean genotypes, harvested after lethal temperature and pretreatment of SA prior to lethal stress along with control. Pattern was similar as that of fresh weight with variable response for different genotypes, however it was prominently higher in NM 19-19 and lowest in NM 20-21.

HSTI was calculated for mean seedling length, FW, DW of seedlings of four mung bean genotypes after lethal temperature and pretreatment of SA prior to lethal temperature, hierarchy for the value of HSTI was

50°C+1.0SA>50°C+0.1SA >50°C. It was further noticed that the HSTI values were highest for NM 19-19 and lowest for NM 20-21.

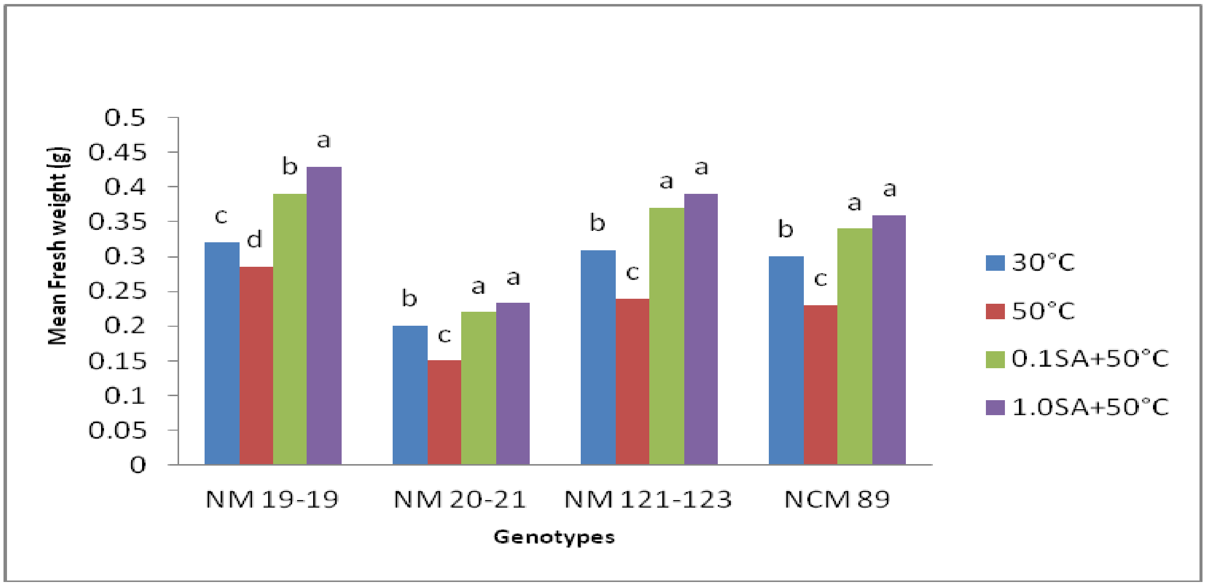

Fig 2. Mean fresh weight of seedlings of four mung bean (*Vigna radiata*) genotypes after lethal temperature and SA pretreatment. Similar alphabets represent homogenous means for each genotype.

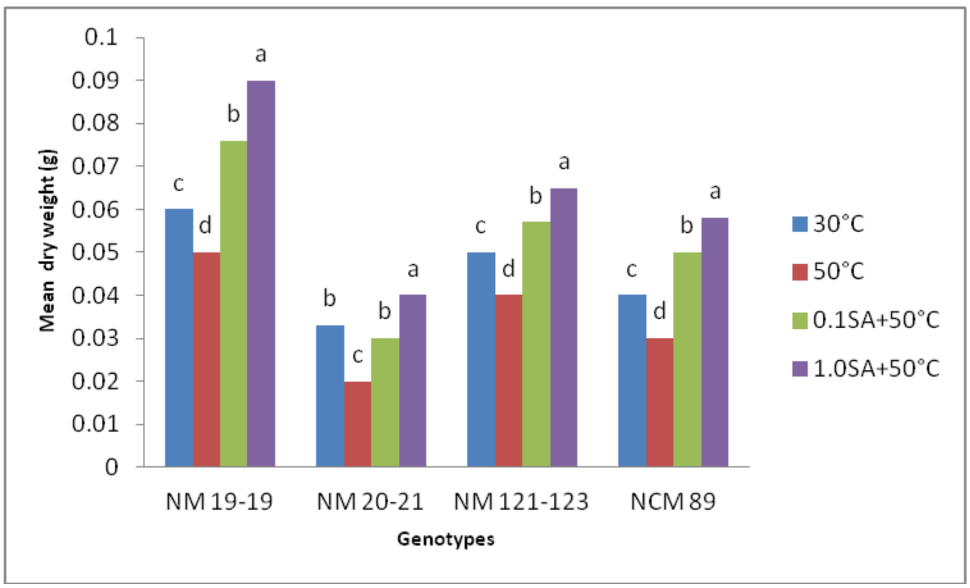

Fig 3. Mean dry weight of seedlings of four mung bean (*Vigna radiata*) genotypes after lethal temperature and SA pretreatment. Similar alphabets represent homogenous means for each genotype.

DISCUSSIONS

Optimum temperature is the most suitable temperature which gives best growth performance. Different researchers reported different optimum temperature for mung bean with the range between 25-30°C, the difference could be due to temperature variations in these regions. According to Chen *et al*., (1986), 25°C was the optimum temperature for mung bean. We found that germination as well as growth of our four mung bean genotypes was best at 30°C, and when mung bean seeds were exposed to temperature 20°C higher than optimum (50°C for 2 h) caused significant inhibition in seedling length. Kaur *et al.*, (2009), reported that high temperature (45°C for 3 h) when given to Brassica species, reduced seedling length and decreased FW and DW, therefore high temperature is an important factor that can strike crop at any time and impose many limitations on growth and development of crop. Current results showed the decrease in seedling length, fresh weight and dry weight of all four mung bean genotypes. These findings were supported by Thind and Chanpreet (1997), they exposed mung bean seedlings at 45°C for 3, 5 and 7 days, found decrease in seedling length and dry weight as compared to plants in control.

Table 1. Mean squares of various morphological parameters of 80 h etiolated mung bean seedlings harvested after lethal temperature and SA pretreatments.

Sources of Variations	df	MS		
		Length	FW	DW
Genotypes(G)	3	50.9*	0.0205*	0.0031*
Treatments(T)	3	528.9*	0.0357*	0.0018*
G X T	9	3.09*	0.0018*	0.000189ns
Error	32	1.121	0.0006	0.00016

* Significant at $P<0.05$, ns non significant

Table 2. Heat stress tolerance index (HSTI) for seedling length, FW and DW of four mung bean genotypes after lethal temperature and pretreatment of salicylic acid.

Genotype	Treatment	**HEAT STRESS TOLERANCE INDEX (HSTI)**		
		Seedling length	FW	DW
NM 19-19	50°C	0.059	0.0714	0.089
	0.1SA+50°C	0.086	0.0977	0.1361
	1.0SA+50°C	0.0899	0.1077	0.1612
NM 20-21	50°C	0.035	0.0234	0.0197
	0.1SA+50°C	0.059	0.0344	0.0295
	1.0SA+50°C	0.0622	0.0364	0.0394
NM 121-123	50°C	0.045	0.0582	0.059
	0.1SA+50°C	0.0732	0.089	0.0851
	1.0SA+50°C	0.0762	0.0946	0.0978
NCM 89	50°C	0.041	0.054	0.0358
	0.1SA+50°C	0.068	0.0798	0.0597
	1.0SA+50°C	0.0717	0.0845	0.0692

Thermotolerance was developed by the pretreatment of salicylic acid prior to lethal temperature stress. This was manifested by improvement in seedling length, FW, DW and HSTI values. Sakhabutdinova *et al*., (2003) also reported that imbibitions of wheat seeds with salicylic acid help them to survive at high temperature. It was suggested that lethal temperature stress caused oxidative damage in all four mung bean genotypes which was reduced by pretreatment of salicylic acid. It is well documented that SA has a role in defence mechenisms against many biotic and abiotic stresses (Yalpani *et al*., 1994; Szalai *et al*., 2000). Exogenous application of SA to beans and tomatoe plants, increased their tolerance against heat, chilling, and drought (Senaratna *et al.*, 2000). Similarly the effect of SA on heat tolerance showed that the heat tolerance of mustard plants was improved by spraying plants with SA (Dat *et al.*, 1998). It was also reported that when 1.0 mM SA was sprayed to cucumber plants, induced thermotolerance (Shi *et al.*, 2006).

Conclusions

Lethal temperature (50°C, 2h) was responsible for the drastic reduction in seedling length, FW, DW and HSTI values for all genotypes. Pretreatments of SA before lethal stress, showed improvement in these parameters, which was further improved by the increase in concentration of SA. By looking at the morphological performance as well as HSTI values, NM 19-19 was most thermotolerant and NM 20-21 was least thermotolerant genotype

REFERENCES

Barba-Espin, G., M.J. Clemente-Moreno, S. Alvarez, M.F. Garcia-Legaz, J.A. Hernandez and P. Diaz- Vivancos (2011). Salicylic acid negatively effect the response to salt stress in pea plants. *Plant Biology*, 12: 909-917.

Chen,Y.M., K.Seiichiro and Y.Masuda (1986). Enhancing effect of heat shock and gibberellic acid on the thermotolerance in etiolated *Vigna radiata*.L. Physiological aspects on thermotolerance. *Physiol.Plant*, 66: 595- 601.

Dat, J. F., C.H. Foyer and I.M. Scott (1998). Changes in salicylic acid and antioxidants during induction of thermotolerance in mustard seedlings. *Plant Physiology*, 118: 1455-1461.

Kaur,P., G.Navita and K.S.Manjeet (2009). Introduction of thermo- tolerance through heat acclimation and Salicylic acid in *Brassica* species. *African journal of Biotechnology*, 619-625.

Khattak, G.S., S. Iqbal and M. Tilla (2009). Flower shedding under high temperature in mung bean (*Vigna radiate* L. Wilczek). *Pak.J.Bot*. 41: 35-39.

Khattak, M.K., A. Shafqat and I.C Jamshed (2004). Varietal resistance of mung bean (*Vigna radiate L*.) against whitefly (*Bemisia tabaci Genn*), Jassid (*Amrasca devastans Dis*) and Thrips (*Thrips tabaci Lind*.) *Pak. Entomol*., 26: 9-12.

Larkindale, J. and M.R.Knight (2002). Protection against heat stress induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene and salicylic acid. *Plant Pysiol*., 128: 682-695.

Larkindale, J. and H. Bingru (2004). Effect of abscisic acid, salicylic acid, ethylene and hydrogen peroxide in thermotolerance and recovery for creeping bentgrass. *Plant Growth Regul*., 47: 17-28.

Mearns, L.O., R.W. Katz and S.R. Schneider (1984). Extreme high temperature events: change in their probabilities with changes in mean temperature. *J. of Clim. And App. Meteo*., 23: 1601-1613.

Pancheva, T.V., L.P. Popova and A.N. Uzunova (1996). Effect of salicylic acid on growth and photosynthesis in barley plants. *J.Plant Physiol.*, 149: 57-63.

Pierpoint, W.S. (1994). Salicylic acid and its derivatives in plants; medicines, metabolites and messenger molecules. *Bot.Res*., 20: 163-235.

Porch,T.G. (2006). Application of stress indices for heat tolerance screening of common bean. *J. Agronomy and Crop Science*, 192: 390-394.

Sakhabutdinova, AR., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. *J. Plant Physiology*, Special issue, 314-319.

Senaratna,T., D. Tuochell, T. Bunn and K. Dixon (2000). Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul*., 30: 157-161.

Shi, Q., Z. Bao, Z. Zhu, Q. Ying and Q. Qian (2006). Effects of different treatments of salicylic acid on heat tolerance, chlorophyll flurescence, and enzyme activity in seedlings of *Cucumis sativa* L. *Plant Growth Regul*., 48: 127-135.

Steel, R.G.D. and J.H.Torrie (1980). *Principles and procedures of statistics*. Mc Graw Hill Book Co-New York.

Szalai, G., I. Tari, T. Janda, A. Pestenacz and E. Paldi (2000). Effects of cold acclimation and salicylic acid on changes in ACC and MACC contents in maize during chilling. *Biol. Plant*, 43: 637-640.

Thind, S.K. and M. Chanpreet (1997). Effect of fluridone on free sugar level in heat stressed mungbean seedlings. *Plant Growth Regulation*, 22: 19-22.

Wolfe, D.W. (1994). Physiological and growth responces to atmospheric carbon dioxide. In: Pessarakli (Ed) *Handbook of plant and crop physiology*. Marcel Dekker, Inc, New York.223-242.

Yalpani, N., A.J. Enyedi, J. Leon and I. Raskin (1994). Ultraviolet light and ozone stimulate accumulation of salicylic acid and pathogenesis related proteins and virus resistance in tobacco. *Planta*, 193: 373-376.

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