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EFFECT OF EXOGENOUS APPLICATION OF MANNITOL ON MORPHOLOGICAL, BIOCHEMICAL AND YIELD TRAITS OF HEAT STRESSED MUNGBEAN (*Vigna radiata* L.)

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Abstract

Mungbean is the major legume as well as cash crop after chickpea in the legume family. In changing climatic scenarios, heat stress has become a major challenge in mungbean yield and productivity. Research was laid down to understand the effect of exogenously applied mannitol on terminal heat stressed mungbean. The experiment was performed under greenhouse conditions. Completely Randomized Design (CRD) with split arrangement having five replications was selected to conduct the experiment. Heat stress was applied at flower initiation and pod formation stages and different levels (0 mg/L, 100 mg/L, 200 mg/L and 300 mg/L) of mannitol were foliar applied. Morphological, physiological, and yield traits were focused. Significance was determined by ANOVA and means were compared by using Tukey's test. Heat reduced all the parameters significantly and interaction of mannitol with heat improved all the morphological parameters (shoot and root length (cm), shoot fresh weight (g/ plant), root fresh and dry weight (g/ plant) significantly as the dose of mannitol was increased. All the biochemical parameters (chlorophyll a and chlorophyll b contents) were increased under heat stress. Mannitol worked as a compatible solute and as an antioxidant that help the plant to overcome the ROS species and showed significant interaction with heat at 300 mg L^{-1}. All yield parameters (number of seeds/pod, number of pods/plant, pod length (cm), 100 seed weight (g) and seed yield (g/plant) were also significantly improved due to interactive effect of mannitol and heat.

Key words: Heat stress, Mannitol, Mungbean, Green gram, Growth

Introduction:

Mungbean (*Vigna radiata* L.) also commonly called as green gram, is a leguminous crop. It is an annual grain legume crop (Parihar *et al*., 2017). Mungbean has a high range of storage protein (22%-27%) with sugar, minerals and soluble dietary fibers (Alom *et al*., 2014). The high variability in climatic conditions including rising temperature and unpredictable water deficit environments during its cropping season cause drastic reduction in mungbean productivity (Singh *et al*., 2016). Several abiotic stresses such as heat, salinity, water-logging and drought highly affects the growth and development in mungbean (Zandalinas *et al*., 2017). Among various factors, global temperature rise is the major challenge in legume crop production. The erratic and low rainfall, soil desertification, evolving new races of pest and pathogens are some other problems associated with the global temperature rise, which are adversely impacting crop production across the globe. Legumes including mungbean, which are grown in warm-humid climatic conditions are more affected by high temperature. Various studies have demonstrated that under heat stress, significant yield losses occur in mungbean at reproductive stage of plant (Priya *et al*., 2020). The constant higher atmospheric temperature for longer duration is highly detrimental for the growth and physiological functions of various food crops (Cao *et al*., 2011). Severity of the crop damage varies with the timing, duration and magnitude of the elevated temperature, as well as the genotype specific defense response. In mungbean, during the summer season where temperature rise above 40°C causes terminal heat stress during reproductive stage of the plants which is a major concern in mungbean productivity because it results in impaired anthesis, loss of pollen viability, reduced flower fertilization, increased flower drop and shortened period for grain filling (Basu *et al*., 2019). In mungbean, high temperature cause flower shedding as high as 79% (Kumari and Varma, 1983). However, the genotypic variability in mungbean germplasm is observed attributing to specific or combination of heat stress tolerance mechanisms (Sharma *et al*., 2016). The effect of heat stress in mungbean is not thoroughly investigated yet and it needs more in-depth research (Kaur *et al*., 2015). In higher plants, osmolytes and solutes that are responsible for resistance against a wide range of abiotic stresses include alditols and mannitol (Hema *et al*., 2014). Mannitol is a six carbon liquid sugar that is abundantly found in plants and fungi. Mannitol is present widely in plant species in every way and found in more than 70 families (Ruijter *et al*., 2003). Major functions in physiology of plants that include storage of carbon and the defense mechanism against environmental stress are carried out by mannitol (Patel and Williamson, 2016). Mannitol certainly has the ability to perform its role as compound which is compatible with cellular mechanism, act as osmoprotectant, protect from heat and avoid oxidation. It is also stated that mannitol has central importance in decreasing the osmotic stress and the stresses caused by the salts in majority of plant species (Bhauso *et al*., 2014). In the symplast of expanding tissues, there is 64% decline in potassium amount during salinity stress (Shahzad *et al*., 2012). According to an observation, in sandy soils, there is a decrease in height of plant, elongation of root, biomass of shoot and root, harvest index, area of leaf, mitotis, photosynthesis and the yield of straw and grains of maize plants per pot affected by salinity stress (El Sayed, 2011). It has been observed that in maize plants that are under salt stress, photosynthetic pigments and biomass level increases by the foliar application of mannitol (Kaya *et al*., 2013). Keeping in view about facts present study will be conducted to fulfill following aims; i) to examine the effect of heat stress on mungbean, ii) to examine the response of heat stressed mungbean to foliar applied mannitol.

Materials and Methods:

A pot trial was laid down under completely randomized design and replicated five times to study the effect of mannitol on terminal heat stressed mungbean.

1.1. Plant material:

The seed of mungbean variety NM-16 was obtained from Nuclear Institute of Agriculture and Biology, Faisalabad.

1.2. Experimental site:

The experiment was conducted at glass house, Faculty of Agriculture, University of Agriculture Faisalabad.

1.3. Physiochemical traits of experimental site:

Soil samples were randomly collected before sowing and after harvesting from the collected soil. Analyzed the samples to quantify different physiochemical attributes (ICARDA, 2013). Soil analysis was carried out in Environmental Sciences lab, Institute of soil and environmental science, UAF (Table 1).

1.4. Weather elements:

Weather data were collected from metrological observatory cell, Department of Crop Physiology, University of Agriculture; Faisalabad situated at latitude 31° north, longitude 73° east and at altitude of 184.4 meter are presented graphically (Figure 1). The experimental site was semiarid with annual mean rain fall of 375 mm.

1.5. Treatments:

The experiment was comprised of two variables in which mannitol was applied with various concentrations (0, 100, 200 & 300 mg L^{-1}) to heat stressed mungbean at flowering initiation (H_1) and pod formation (H_2) and compared with control (H_0) where no heat stress was applied.

1.6. Experimental design:

The experiment was laid out in completely randomized design (CRD) with factorial arrangement having four replications.

1.7. Imposition of treatments:

Mannitol was applied at flower initiation and pod formation stages of heat stressed mungbean.

1.8. Statistical analysis:

The recorded data was analyzed by Fisher's analysis of variance (ANOVA) technique. All treatments mean was compared by honestly significant difference (HSD) test at 1% level of probability.

1.9. Agronomic practices:

The experiment was conducted in pots. Pots were filled with fine soil and five seeds of mungbean were sown in each pot at field capacity. After twelve days of emergence thinning was done to maintain three plants in each pot. Irrigation was applied as and when required. All other agronomic practices were kept normal and uniform for all the treatments.

1.10. Observations recorded:

Morphological traits

Soot and root length were taken after harvesting the selected plants and soaked in water and then washed to remove the soil. Length was taken with the help of meter rule manually and then averaged. Soot and root fresh weight were also noted after weighing on electronic balance of the freshly harvested sample and dry weights of shoot and root were recorded after sundry for 5 days then oven dried at 75^{o}C till constant weight.

Biochemical parameters:

The samples of 0.5 g from each pot were soaked overnight in 80% acetone and recorded absorbance at 663 and 645 nm using ELISA plate. Chlorophyll a and b contents (mg g^{-1}) were determined using formulae given by Arnon (1949):

$$Chl\ a = (12.7 \times A663 - 2.69 \times A645) \times V/ 1000 \times W$$

$$Chl\ b = (22.9 \times A645 - 4.68 \times A663) \times V/ 1000 \times W$$

Where "A" indicates absorbance (nm), "V" volume of the extract (mL) and "W" weight of the fresh leaf tissue (g)

Yield and yield components:

Number of pods per plant, seeds per pod and pod length were noted manually of the selected plants and then averaged. Hundred seeds were randomly counted from seed pod of each pot and then weighing on machine. Seed were collected from all plants after harvesting and threshing and weighed on weighing balance. Then calculate per plant yield.

Month	Temperature (^{O}C)			RH (%)	Rain fall (mm)	Pan Evaporation (mm)	Sunshine (h)	ET (mm)	Wind speed (km ha^{-1})
	Max	Min	Avg.						
Aug-17	38.1	28.6	33.4	68.9	66.0	05.4	07.9	**03.8**	04.9
Sep-17	36.7	24.4	30.5	67.7	35.6	04.2	08.8	02.9	03.3
Oct-17	35.0	19.2	27.1	68.2	0.0	02.9	00	02.2	03.6
Nov-17	24.1	11.8	18.0	84.6	1.5	01.1	03.7	00.8	01.9

Table 1: Monthly weather data for full crop period of mungbean

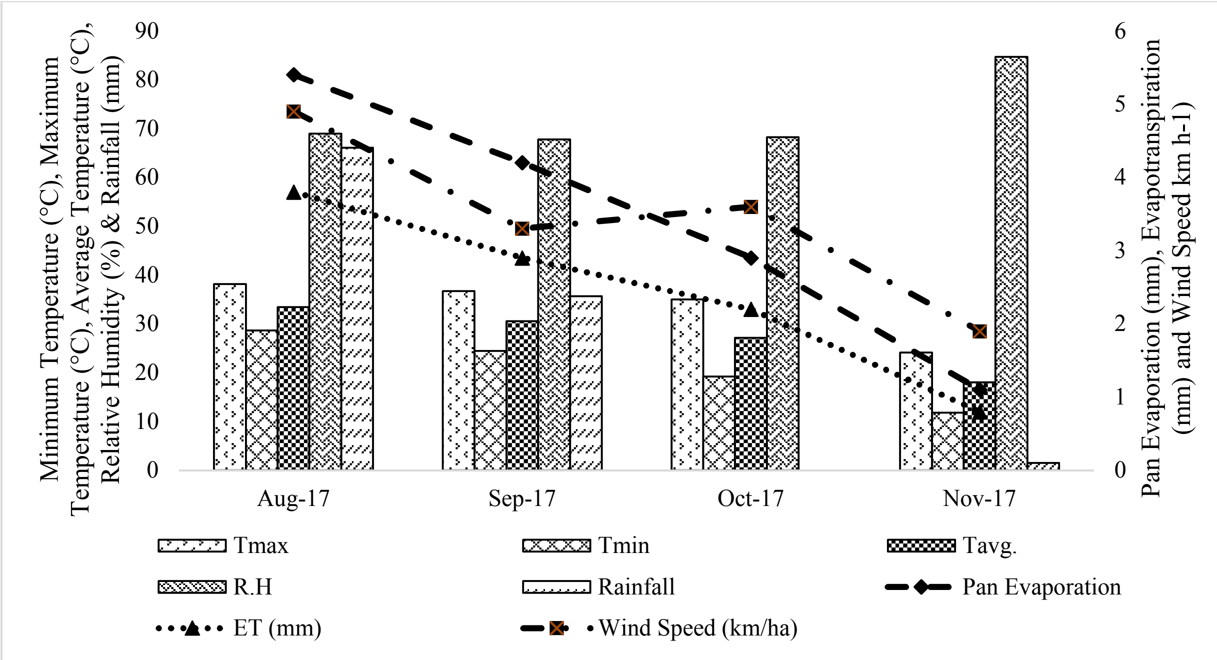

Figure 1: Weather condition of experimental cite during study period

Results:

Shoot and root lengths are the biomass of the plant that supports the plant for its growth and completes its life cycle. In heat stress shoot, root lengths, shoot fresh weight and root fresh weight were drastically affected. Heat and mannitol showed significant effects on shoot, root lengths and shoot and root fresh weight of the mungbean crop. Interaction is also depicted as significant. The treatment H_0 (control) showed significantly minimum shoot length (37.00 cm), root length (26.92 cm), shoot fresh weight (8.08 g/plant) and root fresh weight (3.59 g/plant) were observed at M_0 (no mannitol applied), while maximum shoot length (45.60 cm), root length (29.82 cm), shoot fresh weight (10.68 g/plant) and root fresh weight (3.90 g/ plant) were observed at M_3 (300 mg L^{-1}). In H_1 (heat applied at flower initiation stage) and H_2 (heat applied at pod initiation stage), minimum shoot, root lengths, shoot fresh and root fresh weight were recorded in control and maximum with 300 mg L^{-1}. Interaction also depicted as significant for these traits.

Maximum shoot dry weight (2.83 g/plant) and root dry weight (1.88 g/plant) in H_0 (control). In H_1 (heat applied at flowering initiation) shoot dry weight (1.47 g/plant) was lower and statistically at par with H_2 (heat applied at pod initiation) that showed (1.52 g/plant). Mannitol increased the shoot dry weight and significantly maximum dry weight (2.19 g/plant) was recorded in pots where mannitol was applied at 300 mg L^{-1} and lowest value of dry weight of shoot (1.73

g/plant) was observed at M_0 (no mannitol applied). Heat applied at pod initiation (H_2) showed root dry weight was 1.41 g/plant and minimum value of root dry weight (1.31 g/plant) was recorded in H_1 (heat applied at flowering initiation). Mannitol at 300 mg L^{-1} showed maximum root dry weight (1.70 g/plant) and minimum root dry weight (1.39 g/plant) was recorded in M_0 (no foliar mannitol applied). The interactive effect was, non-significant for shoot and root dry weights.

Chlorophyll a, chlorophyll b and carotenoids are main pigments of photosynthesis that take part in photosynthesis. Imposition of heat (H), application of mannitol (M) and their interaction (H×M) showed significant effect on chlorophyll a and b contents. Treatments' means showed that maximum chlorophyll a contents (2.02 mg g^{-1}) and chlorophyll b contents (0.960 mg g^{-1}) under H_0 (control) were studied when mannitol was applied at 300 mg L^{-1}, and minimum chlorophyll a value (1.89 mg g^{-1}) and chlorophyll b value (0.876 mg g^{-1}) were observed with no mannitol application. In H_2 (heat applied at pod initiation) and H_1 (heat applied at flowering initiation) maximum chlorophyll a and chlorophyll b at 300 mg L^{-1}, and lowest value of chlorophyll a and chlorophyll b in M_0 (no foliar mannitol applied).

Pods are the yield determining components of the mungbean crop. Significant effect of heat and mannitol on number of pods/ plant and number of seeds per pod however the interaction was non-significant. Treatments' means indicated that maximum number of pods per plant (12.95) and number of seeds per pod (8.00) were studied under H_0 (control) and minimum number of pods per plant (8.05) and seeds per pod (6.30) were counted in H_1 (heat applied at flowering initiation). Mannitol showed maximum value of number of pods per plant (12.00) and seeds per pod (7.93) with foliar spray of 300 mg L^{-1} mannitol and minimum number of pods per plant (9.13) and seeds per pod (6.53) were counted in M_0 (control).

Significant effect of heat (H) and mannitol (M) on pod length, 100 seed weight, seed yield and their interaction (H×M) was also significant results. Treatments' means show that minimum pod length, 100 seed weight and seed yield were observed at M_0 (control). In H_2 (heat applied at pod initiation) maximum pod length and seed yield with foliar of mannitol at 200 mg L^{-1} which was statistically at par to its spray at 300 mg L^{-1}, for 100 seed weight all levels of mannitol performed equally well and least under M_0 (control). In H_1 (heat applied at flowering initiation) maximum pod length was measured at 200 mg L^{-1}, 100 seed weight at 300 mg L^{-1} which is statistically similar results to other lower levels of mannitol and seed yield at 300 mg L^{-1} but it was statistically similar to its lower dose (200 mg L^{-1}) and minimum was recorded at M_0 (control).

Discussion:

Heat severally reduced the reproductive stage as compared to vegetative growth in mungbean (Priya *et al*., 2020). Decrease in seed germination, length of root and shoot, seedling vigor and fresh mass might be due to heat stress that reduced the yield of *Vigna radiata* (Vollenweider and Günthardt-Goerg, 2005). Heat stress raises the temperature of surface that causes damage in rooting system and reduces root mass as well as root hairs. Abiotic stresses like salinity and heat decline root and shoot lengths in *Vigna radiata*. Reduction in dry weight of shoot, relative growth rate and net assimilation rate in *Zea mays*, *Pennisetum glaucum* and *Triticum* caused by high temperature (Wahid *et al*., 2007). Vegetative stages of the plant are responsive to heat stress and show different morphological symptoms like burning of leaves, twigs, leaf fall by abscission, decreased growth of shoot and root that lead to reduced yield with high exposure of temperature (Bita and Gerats, 2013). When plant is exposed to temperature extremes many times, it caused reduction in growth of root and shoots, number of roots and its diameter (Kaushal *et al*., 2016). Stress also leads to chlorosis, necrosis and decrease in chlorophyll and carotenoids. Alternation in pigment system content is used as a selection parameter for the crop under stress condition because it relates to photosynthetic process (Chand *et al*., 2018). The degradation of chl a and chl b amounts with use of sodium chloride was investigated in many plants such as Corn*,* Safflower*, Bean* and reason behind this is increasing activity of destructive enzymes called chlorophyllase (Sharma *et al*., 2016). Pods production from a plant is the essential selection criterion to increase potential yield in mung bean (Mason *et al*., 2013). It means that drastic reduction to reproductive traits reduced the potential yield in crop (Kaushal *et al*., 2016). Temperature extremes cause poor seed and seedling development in crops that alter it's functioning and reduce yield of the crops (Devasirvatham *et al*., 2012). Reduction in photo assimilates in plants due to heat stress causes lower weight of the seed. Fewer endosperm cells, less starch accumulation, less supply of assimilates, and disruption of synthetic process of starch are the primary reasons for the less seed weight that is affected by heat stress (Taiz *et al*., 2015). Reduction in yield might be caused by male and female gametophyte disturbance, poor viability of pollen grains, poor germination of pollens, stigma loss in receptivity, abortion of ovule and poor seed set, and these all effects are caused by heat stress (Patriyawaty *et al*., 2018). Mannitol controls the cell turgor that works under stress in intracellular spaces such as in hypertonic conditions. Mannitol controls the cell turgor that works under stress in intracellular spaces such as in hypertonic conditions (Siringam *et al*., 2011). Reduction in average number of lateral roots directly reduces the root biomass. Higher concentrations of mannitol were found in root system of water stressed maize (Canak *et al*., 2016). Seckin *et al*. (2009), investigated that mannitol showed significant interaction and alleviated the stress and enhanced root growth and dry mass of the root system. Exogenously applied mannitol was transported to shoot quickly without causing any toxicity and also stabilize the membrane (Bhauso *et al*., 2014). Mannitol was found more in stressed plant and increase the yield by increasing the yield components (Farooq *et al*., 2015).

Conclusion:

Heat caused damage to plant at its vegetative and reproductive stage. It has been concluded from all the physiological, biochemical and yield parameters drastically reduced all the parameters due to heat stress while, foliar application of mannitol showed increment in all parameters. Among levels of mannitol 300 mg L^{-1} of mannitol performed best under all heat stress treatment i.e. H_1 (control) and in stress conditions H_1 (heat applied at flower initiation) and H_2 (heat applied at pod initiation).

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Parameters	Source of variation				
	Heat (H)	**Error (I)**	**Mannitol (M)**	**H × M**	**Error (II)**
Shoot length	114.517**	11.183	106.511**	10.028*	3.869
Root length	183.624**	0.046	27.787**	0.230**	0.032
Shoot fresh weight	69.4781**	0.2436	25.0247**	0.7251*	0.2180
Root fresh weight	16.3230**	0.0013	0.1804**	0.0164**	0.0003
Shoot dry weight	11.3992**	0.0177	0.6339**	0.0166^{NS}	0.0165
Root dry weight	1.84953**	0.00695	0.27132**	0.00187^{NS}	0.00508
Chlorophyll a contents	0.50991**	0.00048	0.10324**	0.00842**	0.00034
Chlorophyll b contents	0.59899**	0.00013	0.01529**	0.00025**	0.00004
Number of pods per plant	124.867**	2.533	21.883**	0.867^{NS}	0.775
Number of seeds per pod	15.0500**	0.7792	5.6167*	0.0500^{NS}	0.3222
Pod length	23.9470**	0.1544	1.4318**	0.3796*	0.1335
100 seeds weight	2.53044*	0.50205	3.45643**	0.63813*	0.20839
Seed yield per plant	162.354**	0.816	38.477**	1.799**	0.252

Table 1. Mean sum of square for heat stress and mannitol on morphological, biochemical yield and yield related traits in cotton

Where:

* = Significant, ** = Highly significant, NS = Non-significant

Treatments	Shoot dry weight (g)	Root dry weight (g)	Pods per plant	Seeds per pod
Heat Imposition				
No Heat	2.83 A	1.88 A	12.95 A	8.00 A
At Flowering Initiation	1.47 B	1.31 C	8.05 B	6.30 B
At pod initiation	1.52 B	1.41 B	11.35 B	7.45 A
Tukey's HSD at p≤0.05	**0.120**	**0.075**	**0.144**	**0.796**
Mannitol Application				
0 mg L^{-1}	1.73 C	1.39 D	9.13 C	6.53 B
100 mg L^{-1}	1.85 C	1.47 C	10.80 B	7.00 BC
200 mg L^{-1}	2.05 B	1.57 B	11.20 AB	7.53 AB
300 mg L^{-1}	2.19A	1.70 A	12.00 A	7.93 A
Tukey's HSD at p≤0.05	**0.126**	**0.070**	**0.866**	**0.558**

Table 2: Effect of mannitol application on morphological and yield components of mungbean

Any two means not sharing a letter in column differ significantly at 5% probability.

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Table 3: Effect of mannitol application on morphological, biochemical, yield and yield components of mungbean

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Chl *a* content (mg g^{-1})	Chl *b* content (mg g^{-1})	Pod length (cm)	100-Seed weight (g)	Seed yield (g/plant)
No Heat									
0 mg L^{-1}	37.00 b	26.92 d	8.08 c	3.59 d	1.89 c	0.876 d	8.43 a	4.69 b	10.11 d
100 mg L^{-1}	43.60 a	27.93 c	9.21 b	3.67 c	1.96 b	0.902 c	8.55 a	4.64 b	11.97 c
200 mg L^{-1}	44.00 a	28.89 b	9.61 b	3.75 b	1.98 b	0.938 b	8.55 a	5.19 ab	13.57 b
300 mg L^{-1}	45.60 a	29.82 a	10.68 a	3.90 a	2.02 a	0.960 a	8.71 a	5.96 a	15.18 a
At flowering initiation									
0 mg L^{-1}	36.60 a	20.56 d	4.64 c	1.87 b	1.55 c	0.562 c	6.23 b	3.67 b	5.78 b
100 mg L^{-1}	37.40 a	21.92 c	5.30 b	1.89 b	1.68 b	0.570 c	6.12 b	4.87 a	6.56 b
200 mg L^{-1}	38.60 a	22.80 b	5.72 b	1.96 a	1.69 b	0.592 b	7.00 a	4.48 a	7.75 a
300 mg L^{-1}	39.80 a	24.04 a	6.55 a	1.98 a	1.74 a	0.622 a	6.35 b	4.83 a	8.03 a
At pod formation									
0 mg L^{-1}	37.80 b	23.50 d	4.73 d	2.52 d	1.57 d	0.632 d	6.48 c	3.92 b	7.47 c
100 mg L^{-1}	42.40 a	24.75 c	6.38 c	2.67 c	1.67c	0.656 c	6.88 b	4.38 a	9.10 b
200 mg L^{-1}	42.20 a	26.16 b	7.36 b	2.75 b	1.75 b	0.684 b	7.70 a	5.05 a	9.96 b
300 mg L^{-1}	45.00 a	26.54 a	7.53 a	2.88 a	1.85 a	0.706 a	7.22 ab	4.89 a	11.32 a
Tukey's HSD at $p \leq 0.05$	**3.334**	**0.300**	**0.791**	**0.029**	**0.031**	**0.0107**	**0.619**	**0.774**	**0.851**

Any two means not sharing a letter in column differ significantly at 5% probability.