

Original Research Article

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Biochemical Response of Mungbean [*Vigna radiata* (L.) Wilczek] Genotypes under Terminal Heat Stress at Reproductive Stage

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ABSTRACT

The investigation was conducted on three mungbean [*Vigna radiata* (L.) Wilczek] genotypes namely MH-421, MH-318 and Basanti genotypes to study their biochemical, and yield response in relation to terminal heat stress tolerance. The plants were raised in earthen pots (30 cm diameter) filled with 5.5 kg of dune sand (*Typic Torrisspamments*) under screen house conditions. High temperature stress was given by manipulation of sowing dates i.e. normal sown (12th March, 2013) and late sown (29th March, 2013). The sampling was done at 3 and 7 days after exposure (DAE) to > 35°C temperature and the control readings were taken at the temperature below <35°C at reproductive stage. High temperature resulted in decreased water potential relative water content, relative stress injury and yield but increase in proline content, total soluble carbohydrates and lipid peroxidation Sensitive genotypes showed large reductions in aforementioned physiological traits. On the other hand, tolerant genotype (MH 421) maintained higher proline, total soluble content, relative stress injury and yield. After 7 days of exposure to high temperature under late sown, above parameters and yield losses in most sensitive genotypes MH 318 and Basanti.

Keywords

Mungbean, Relative water content, Proline, Yield, Total soluble carbohydrates

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Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is a short duration, warm season legume crop Northern region of India. India is the largest producer and consumer of mungbean and accounts for about 65% of the average and 54% of the world production of this crop

(Lambridg *et al.*, 2007). It is the third most important pulse crop in India, occupying nearly 3.72 million ha area with 1.56 million tons production with productivity 512 kg ha⁻¹ (Ali *et al.*, 2012). Whereas Haryana, the approximate values are 0.712 million ha, 0.341 million tonnes and 534 kg ha⁻¹ respectively. Mungbean is a main protein

source for the vegetarian diet. Mungbean may also be sown as an inter crop or as a green manure or cover crop. It fetches high price in the market and is a preferred pulse crop for the farmers (Kumar *et al.*, 2011; Mansoor and Naqvi 2013).

Heat stress can cause several alterations at cellular and sub-cellular levels and the response of the plants depends upon the growth stage, intensity, and duration of the exposure to heat stress (Karim *et al.*, 2003; Sung *et al.*, 2003; Mansoor and Naqvi, 2013). The direct effects of heat stress include denaturation of proteins and enzymes (Kepova *et al.*, 2005), damage to membranes while its indirect effects may include inactivation of enzymes present in the mitochondria and chloroplasts, impaired protein synthesis, degradation of proteins, and disruption of membrane integrity (Karim *et al.*, 2003; Howarth 2005; Kumar *et al.*, 2011). Accumulation of total soluble sugars (TSS) under heat stress has been implicated in the establishment and maintenance of thermotolerance (Rizhsky *et al.*, 2004; Wahid and Close 2007). Sugars serve as signalling molecules during abiotic stress in stress-tolerant phenotypes (Rosa *et al.*, 2009). Sugar signaling pathways interact with stress pathways in a complex network to modulate the metabolic responses of plants (Gill *et al.*, 2003). Starch metabolism is very sensitive to abiotic stresses generally leads to a depletion of starch content and to the accumulation of soluble sugars in leaves (Kaplan and Guy 2004; Basu *et al.*, 2007; Kempa *et al.*, 2008). Heat stress causes increased membrane damage due to lipid peroxidation (Amirjani 2012). Lipid peroxidation is a natural metabolic process under normal aerobic conditions and it is one of the most investigated consequences of ROS action on membrane structure and function (Blokhina *et al.*, 2003). Lipid peroxidation is a commonly utilized stress indicator of membrane damage.

Heat stress impairs mitochondrial functions thereby resulting in the induction of oxidative damage that manifests in lipid peroxidation, detected by malondialdehyde (MDA) content (Larkindale and Knight 2002; Vacca *et al.*, 2004).

High temperature during the reproductive stage in chickpea is a major cause of yield loss by reducing pod formation and seed set (Paulsen 1994; Wang *et al.*, 2006; Basu *et al.*, 2009; Kumar *et al.*, 2012; Thangwana and Ogola 2012). Devasirvatham *et al.*, (2010) found that pollen sterility is one of the reasons for lower pod set during pre-anthesis high temperature stress. Recently, heat tolerant genotypes were identified from field screening in India (Krishnamurthy *et al.*, 2011; Upadhyaya *et al.*, 2011). Poor pod set and grain yield in chickpea can result from the high temperature stress during pre-and/or post-anthesis development. The negative effect of high temperature on grain yield is expected to increase due to global warming. A minimum decrease of 53 kg/ha of chickpea yield was observed in India per 1°C increase in seasonal temperature (Kalra *et al.*, 2008).

Materials and Methods

Plant material and growth condition

In this study Mungbean genotypes namely MH – 421, MH – 318, and Basanti were raised in earthen pots (30 cm diameter) filled with 5.5 kg of dune sand (*Typic Torrisspammments*) under screen house conditions. Before sowing, the seeds were surface sterilized with 0.1 % HgCl₂ for two minutes and washed with distilled water twice. Then seeds were inoculated with culture (*Rhizobium leguminosarum*, S-24). After thinning two plants were retained in each pot. Nitrogen free nutrient solution (Wilson and Reisenauer 1963) was given at required time intervals.

Heat stress imposition

Destructive sampling will be done at 3 to 7 days (DAT) after the plants experiencing temperature above 35°C.

Water potential (Ψ_w) of leaf

Water potential of leaf was measured with the help of Pressure Chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) between 8 AM to 10 AM.

Relative water content (RWC %) of leaf

The RWC was calculated using the formula (Weatherley, 1950).

The relative stress injury (RSI %)

The relative stress injury (RSI %) in leaves was evaluated by (Sullivan 1972). The relative stress injury (RSI) was calculated as follows: $RSI (\%) = 1 - EC_a / EC_b \times 100$.

Biochemical analysis

Proline content

Proline content was estimated by using the method of Bates *et al.*, (1973). Standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. The proline content was expressed as $\mu \text{ mol g}^{-1}$ FW.

Total soluble carbohydrates

Total soluble carbohydrates were determined with the method of Yemm and Willis (1954) using anthrone reagent. Standard curve was prepared using graded concentration of D-glucose and the data were expressed as g FW g⁻¹. The equation of the standard curve was $y = 1.222x - 0.001$, $R^2 = 0.9944$.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in leaf tissues. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968).

The Heat Susceptibility Index (HSI)

It was estimated for seed yield using the formula suggested by Fischer and Maurer (1978).

$$HSI = \frac{1 - [YL/YN]}{1 - [XL/XN]}$$

Where,

YL = Mean seed yield of a genotype under late sown condition

YN = Mean seed yield of a genotype under normal sown condition

XL = Mean seed yield of all genotype under late sown condition

XN = Mean seed yield of all genotype under normal sown condition

The yield stability ratio (YS)

Total soluble carbohydrates was calculated as per Lewis (1954).

$$YS = \frac{\text{Seed yield under late sown condition}}{\text{Seed yield under normal sown condition}} \times 100$$

Statistical analysis

For each parameter, three pots having three plants per pot were sampled at a time which comprises of three replicates. Data were analyzed using Completely Randomized Block Design (CRBD) for two factors.

Treatments were compared using critical difference (CD) at 5 % level of significance. Data were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar 125 004, Haryana, India).

Results and Discussion

Plant water status

Water potential (ψ_w) of leaf

With the increase in period of DAE to high temperature ($>35^\circ\text{C}$) a significant decline in ψ_w (-MPa) of leaves was observed in all three genotypes, the mean values of leaf water potential were -0.45, -0.92 and -1.71 at control, 3, and 7 DAE, respectively (Table 1). In normal sown genotype MH 421 (-1.14) showed more negative values followed by MH 318 (-1.01) and Basanti (-0.93). Under late sown condition leaf water potential (ψ_w) followed the same trend as in normal sown condition and genotype MH 421 showed highest negative values of ψ_w (-1.59) .

Relative water content (RWC %) of leaf

Table 2 showed that mean values of RWC of leaves in all three mungbean genotypes significantly decreased from 74.55% to 56.69% with increasing the period of DAE to high temperature ($>35^\circ\text{C}$) from control to 7 DAE. Maximum RWC was noticed in MH 421 (72.40%) followed by MH 318 (66.51%) and minimum in Basanti (62.90%) in normal sown and significantly decreased from 73.65% to 63.41% with increasing the period of DAE to high temperature ($>35^\circ\text{C}$) from control to 7 DAE. Maximum RWC was noticed in MH 421 (70.35%) at par with MH 318 (70.34%) and minimum in Basanti (65.22%) in late sown.

Relative stress injury (RSI %) (Membrane stability) of leaf

Data presented in Table 3 shows the effect of high temperature ($>35^\circ\text{C}$) on leaf membrane stability of mungbean genotypes. RSI increased significantly with increase in DAE to high temperature in all three genotypes *i.e.* from 21.40 to 34.69%. The maximum increase in RSI (32.17 to 41.34%) was observed in Basanti followed by MH 318 (29.24 to 40.72%) and minimum was noticed in MH 421 (26.80 to 34.88%).

Biochemical studies

Proline content

The data showed significant differences in proline content of leaves and it increased from 3.11 to 17.77 with increased DAE to high temperature (Fig. 1). The highest proline content (19.83) was observed in MH 421 followed by MH 318 (17.57) and lowest in Basanti (15.91) at 7 DAE as compared to control under normal sown. Whereas under late sown it increased from 4.06 to 23.72 with increased DAE to high temperature. The highest proline content (25.21) was observed in MH 421 followed by MH 318 (23.75) and lowest was in Basanti (22.19) at 7 DAE as compared to control.

Total soluble carbohydrates (TSC)

The changes in the levels of TSC with increasing DAE to high temperature in leaves of mungbean genotypes are shown in Fig. 2. The TSC increased significantly with every increment of exposed day to high temperature ($>35^\circ\text{C}$) *i.e.* from 23.58 to 33.65. The genotype MH 421 maintained higher (31.85) TSC followed by MH 318 (27.20) and minimum in Basanti (26.56) in normal sown. Similarly, in late sown the TSC increased *i.e.* from 24.49 to 35.15. The genotype MH 421

maintained higher (33.38) TSC followed by MH 318 (30.08) and minimum in Basanti (28.26).

Lipid Peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content. MDA content showed increasing trend from 21.75 to 34.44 with the increased DAE to high temperature (>35°C) in all genotypes. Fig. 3 showed that the maximum MDA content was measured in Basanti (30.66) as compared to MH 318 (28.97) and minimum in MH 421 (28.26) under normal sown. In late sown also the maximum MDA content was measured in Basanti (35.84) as compared to MH 318 (34.81) and minimum in MH 421 (29.60).

Seed yield plant⁻¹

The mean seed yield plant⁻¹ of late sown treatment was less than the mean seed yield of normal sown due to high temperature (>35°C) i.e. it was 2.86 g per plant in normal sown,

while it was 1.93 g in late sown (Table 4). The genotypes showed significant differences for seed yield in both normal and late sown experiments. The mean seed yield was highest in MH 421(3.11g) compared to MH 318(2.21) and Basanti (1.86 g). The maximum reduction in seed yield was found in Basanti (44.4%) followed by MH 318 (36.7%) and minimum in MH 421 (21.5%). The overall interaction values were statistically significant for test weight in both normal and late sown experiments

Heat Susceptibility Index (HSI) and yield stability (YS %)

The HSI and YS were calculated for both genotypes (Table 4). The mean HSI value was low while YS value was high in MH 421 i.e. 0.66 and 78.51, respectively. In genotype MH 318 their values were 1.16 and 63.33 and in genotype Basanti were 1.38 and 55.64, respectively. The results were statistically significant for yield plant⁻¹.

Table.1 Changes in water potential (ψ_w) of leaves in mungbean genotypes when exposed to high temperature

Genotypes	ψ_w (-MPa)							
	Normal sown				Late sown			
	Control < 35°	>35°C Days of exposure (DAE)			>35°C Days of exposure (DAE)			
		Control	3	7	Mean	Control	3	7
MH 421	-0.54	-1.09	-1.79	-1.14	-0.74	-1.80	-1.96	-1.59
MH 318	-0.43	-0.89	-1.69	-1.01	-0.69	-1.78	-1.96	-1.48
Basanti	-0.37	-0.77	-1.64	-0.93	-0.54	-1.68	-1.77	-1.33
Mean	-0.45	-0.92	-1.71		-0.66	-1.76	-1.97	
<i>C.D. at 5%</i>	<i>Genotypes</i> =0.11				<i>Genotypes</i> =0.06			
	<i>Temperature</i> =0.11				<i>Temperature</i> =0.06			
	<i>Genotypes X Temperature</i> = NS				<i>Genotypes X Temperature</i> =NS			

Table.2 Changes in relative water content (RWC %) of leaves in mungbean genotypes as affected by high temperature

Genotypes	Relative water content (RWC %)							
	Normal sown				Late sown			
	Control < 35°	>35°C Days of exposure (DAE)			>35°C Days of exposure (DAE)			
		Control	3	7	Mean	Control	3	7
MH 421	75.83	71.58	69.79	72.40	73.34	70.27	67.41	70.35
MH 318	75.02	70.81	53.71	66.51	74.52	71.32	65.18	70.34
Basanti	72.79	69.36	46.56	62.90	74.00	64.91	57.66	65.22
Mean	74.55	70.58	56.69		73.65	68.84	63.41	
<i>C.D. at 5%</i>	<i>Genotypes</i> =5.59				<i>Genotypes</i> =1.28			
	<i>Temperature</i> =5.59				<i>Temperature</i> =1.28			
	<i>Genotypes X Temperature</i> = 9.61				<i>Genotypes X Temperature</i> =2.23			

Table.3 Changes in relative stress injury (RSI %) of leaves in mungbean genotypes as affected by high temperature

Genotypes	Relative stress injury (RSI %)							
	Normal sown				Late sown			
	Control < 35°	>35°C Days of exposure (DAE)			>35°C Days of exposure (DAE)			
		Control	3	7	Mean	Control	3	7
MH 421	18.54	21.81	27.04	22.46	26.80	30.53	34.88	30.74
MH 318	21.12	24.24	37.43	27.60	29.54	33.08	40.72	34.45
Basanti	24.54	30.82	39.30	31.55	32.17	37.53	41.34	37.01
Mean	21.40	25.62	34.59		29.50	33.71	38.98	
<i>C.D. at 5%</i>	<i>Genotypes</i> =4.61				<i>Genotypes</i> =4.13			
	<i>Temperature</i> =4.61				<i>Temperature</i> =4.13			
	<i>Genotypes X Temperature</i> = NS				<i>Genotypes X Temperature</i> =NS			

Table.4 Yield plant⁻¹, Heat susceptibility index (HSI) and yield stability (YS) of mungbean genotypes under normal sown (NS) and late sown (LS) conditions

Genotypes	Yield plant ⁻¹ (g)				HSI	YS (%)
	NS	LS	Mean	Reduction (%)		
MH 421	3.49	2.74	3.11	21.5	0.66	78.51
MH 318	2.70	1.71	2.21	36.7	1.16	63.33
Basanti	2.39	1.33	1.86	44.4	1.38	55.64
Mean	2.86	1.93				
<i>C.D. at 5%</i>	Genotypes				=0.41	
	Temperature				=0.50	
	Genotypes × Temperature				=NS	

Fig.1 Proline content mungbean leaves after exposure to high temperature under normal sown (NS) and late sown (LS) conditions. Vertical bars indicate ± SE mean

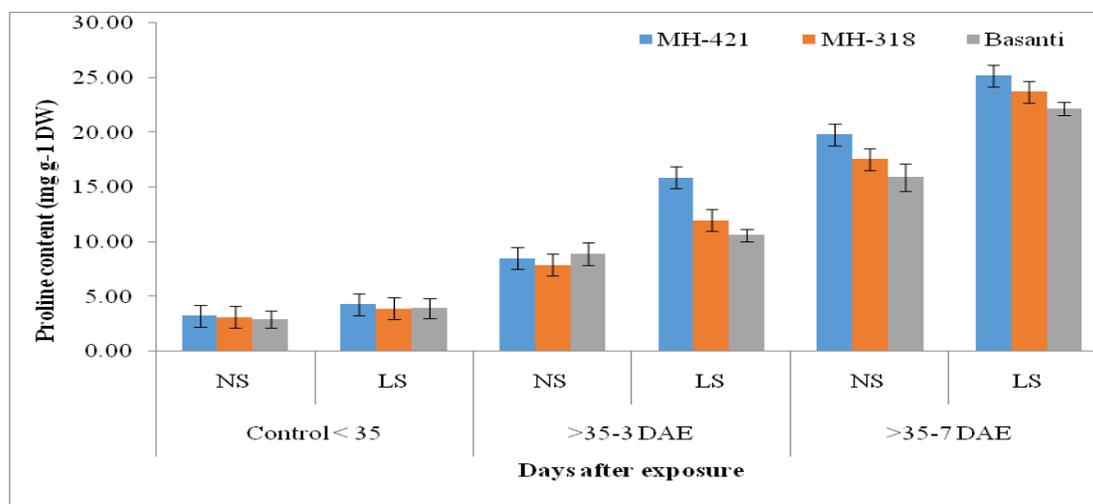


Fig.2 Total soluble carbohydrates(TSC)in mungbean leaves after exposure to high temperature under normal sown (NS) and late sown (LS) conditions. Vertical bars indicate ± SE mean

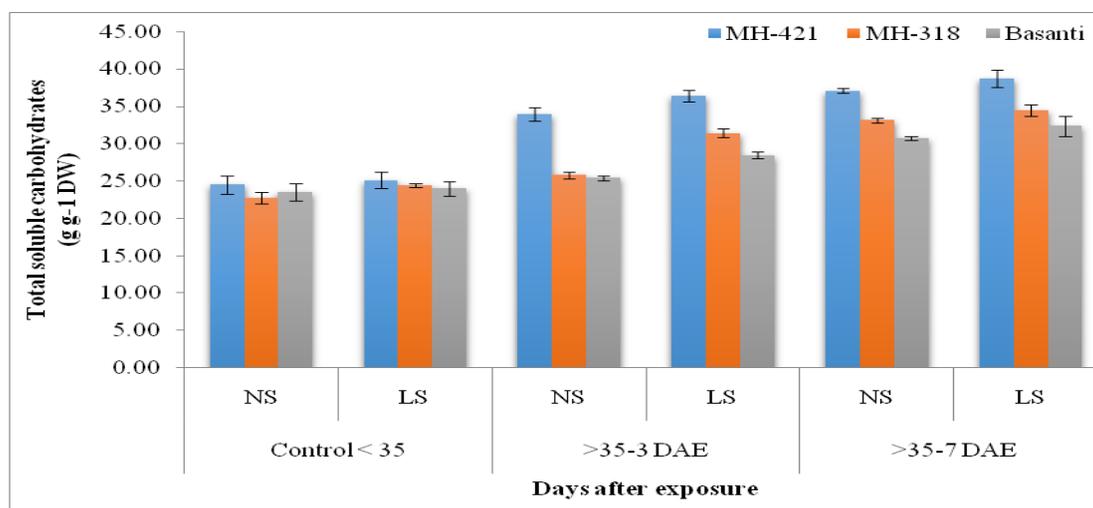
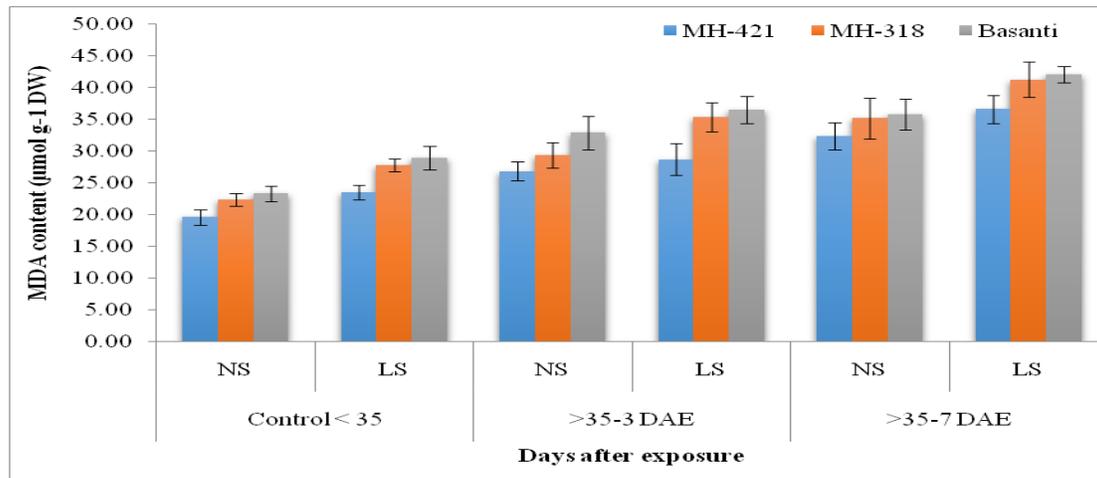


Fig.3 Malondialdehyde (MDA) content in mungbean leaves after exposure to high temperature under normal sown (NS) and late sown (LS) conditions. Vertical bars indicate \pm SE mean



High temperature disturbs the water relations and hydraulic conductivity of roots (Morales *et al.*, 2003). Our observations were in agreement with the earlier ones reporting reduction in RWC due to heat stress in mungbean (Sanjeev *et al.*, 2012) and wheat (Sairam *et al.*, 2000). Elevated temperature above 35°C significantly declined the water potential (Ψ_w) and relative water content (RWC) of plants. Our results showed that, in all three tested genotypes water status was affected by days after expose (DAE) to high temperature and it significantly lowered the Ψ_w of leaf (Table 1) and RWC (%) of leaf (Table 2) in all three genotypes of mungbean at reproductive stage.

In our investigations, leakage of electrolytes (Table 3) increased significantly from leaves was observed with increase DAE to high temperature in all three genotypes. Increased leakage from tissues is usually an expression of modification in the physical properties of cell membranes. The leakage being least in leaves of MH 421 than in Basanti and MH 318. The decrease in cellular respiration in heat-stressed plants is in agreement with the observations on mungbean (Sanjeev *et al.*, 2012).

The accumulation of proline in leaves under high temperature condition at flowering stage in the genotype MH 421 was more than MH 318 and Basanti (Fig. 1). Proline content increased at 35°C and above in this experiment is similar to the observations of (Sairam and Tyagi 2004; Zuther *et al.*, 2007; Verbruggen and Hermanna 2008; Hossain *et al.*, 2012). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Proline accumulation is believed to play an adaptive role in plant stress tolerance mechanisms (Verbruggen and Hermans, 2008).

The influence of high temperature increases the total soluble carbohydrates TSC in leaves of mungbean (Fig. 2). Similar to proline, the overall accumulation of TSC was more in leaves of MH 421 at 7 DAE to high temperature, leading to maintenance of higher RWC and thus better plants water status. The amount of TSC increased rapidly to the increasing high temperature, this result agrees with the result of some researchers who indicated that drought (Jebory 2012, Naresh *et*

al., 2013) and salinity stress induced soluble carbohydrates accumulation in chickpea (Nandwal *et al.*, 2007; Kukreja *et al.*, 2010).

The content of MDA (Fig.3) has been considered as an indicator of oxidative injury (Mandhania *et al.*, 2006, Moller *et al.*, 2007). The results of this study show that high temperature caused negative effect on growth which could be due to the generation of high levels of ROS. Plants generate ROS during growth, but the generation of ROS significantly increases when the plant is under stressful conditions (Zhang *et al.*, 2006), which cause severe oxidative damage to different cell organelles and biomolecules (Amirjani 2012).

Reproductive duration and early maturity are the major adaptive traits for seed yield under high temperature stress. The present investigation also revealed that under late sown condition heat susceptibility index (HSI) (Table 4) and yield stability (YS) (Table 4) were 0.66, 78.51, 1.16, 63.33 and 1.38, 55.64 in MH 421, MH 318 and Basanti, respectively. The HSI was high in heat tolerant genotypes which have advantages in earliness and yield potential under stress. These observations support the findings of Krishnamurthy *et al.*, (2011). The advantage of earliness and the link between pod and seed number with eventual yield under heat stress suggests that manipulation of these traits will further improve yield in warmer environments.

It is concluded that high temperature adversely affected plant water status of three genotypes. The water potential (Ψ_w) of leaf became more negative with increased period DAE to high temperature. With the increasing period of DAE, relative water content [RWC (%)] of leaf declined significantly, whereas total soluble carbohydrates (TSC) content increased. The leakage of electrolytes from

leaf tissue showed a correlation with MDA content. Based on above biochemical behavior it is concluded that MH 421 showed better performance under high temperature than MH 315 and Basanti. These results suggest that above studied characters may helpful in further selection of heat tolerance genotypes of mungbean under crop improvement programme.

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