Yield and physiological responses of mungbean *Vigna radiata* (L.) Wilczek genotypes to high temperature at reproductive stage

G. Chand*, A.S. Nandwal, N. Kumar, Sarita Devi and S. Khajuria

Department of Botany and Plant Physiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar-125 004, Haryana, India.

Received: 18-04-2017  Accepted:09-03-2018  DOI: 10.18805/LR-3795

**ABSTRACT**

A study was conducted to examine the physiological responses and yield of contrasting mungbean genotypes *viz.*, MH 421, MH 318 and Basanti differing in their sensitivity to high temperature raised in earthen pots (30 cm diameter) filled with 5.5 kg of dune sand (*Typic Torrispamments*) under screen house conditions. High temperature stress was given by manipulating sowing dates i.e. normal (12th March, 2013) and late (29th March, 2013) sown. Samplings were done at 3 and 7 days after exposure (DAE) of temperature above 35°C at reproductive stage. Sampling below 35°C temperature was considered as control. High temperature resulted in decreased chlorophyll stability index, chlorophyll and carotenoid contents, relative stress injury and yield. Sensitive genotypes showed large reductions in aforementioned physiological traits. On the other hand, tolerant genotype (MH 421) maintained higher chlorophyll stability index, chlorophyll and carotenoid contents, relative stress injury and yield. After 7 days of exposure to high temperature under late sown, a significant decrease was noticed in genotypes MH 318 and Basanti.

**Key words:** Chlorophyll and carotenoid contents, Chlorophyll stability index, High temperature, Relative stress injury, *Vigna radiata* (L.) Wilczek.

**INTRODUCTION**

Mungbean [*Vigna radiata* (L.) Wilczek] is a short duration, warm season legume crop grown in Northern region of the country. India is the largest producer of mungbean and accounts for about 65% of the average of India and 54% of the world production (Lambrides *et al.*, 2007). Mungbean is one of main protein sources 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. Mungbean crop grown in warm seasons confronts with high temperature at different growth stages in different seasons. The variation in temperature requirements and temperature extremes varies widely for different cultivars of the same species, and among difference species. The reproductive phase of many crop species is relatively more sensitive to heat stress than the vegetative phase (Martiniello and Teixeira da Silva, 2011).

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) and 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).

High temperature affects photosynthetic functions of plants by its effect on the rate of chemical reactions and on structural organization. The physicochemical properties as well as the functional organization of thylakoid membrane are changed reversibly or irreversibly by high temperature (Berry *et al.*, 1980; Karim *et al.*, 1999). PSII is the most heat-sensitive component of the photosynthetic (Berry *et al.*, 1980; Mamedov *et al.*, 1993). High temperature inhibited net photosynthetic and stomatal conductance significantly in many plant species (Morales *et al.*, 2003; Ashraf and Hafeez 2004; Prasad *et al.*, 2006). High temperature decreased photosynthetic pigments (Todorov *et al.*, 2003), enhanced chlorophyll a/b ratio and declined chlorophylls to carotenoids ratio (Wahid 2007). Furthermore, under high temperatures, degradation of chlorophylls *a* and *b* was more pronounced in developed leaves compared to developing leaves (Karim *et al.*, 1997)

High temperature during the reproductive stage in chickpea is a major cause of yield loss by reducing pod formation and seed set (Kumar *et al.*, 2012). Drastic reductions in chickpea seed yields were observed when plants at flowering and pod development stages were exposed to...
high (35°C) temperatures (Summerfield et al., 1984). Wang et al. (2006) exposed various chickpea lines at pre-flowering and pod formation stage under higher temperature and observed maximum yield damage by 59% due to higher temperature at pod formation stage as compared to 34% yield reduction at pre-flowering stage. 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). In this context, the present study was designed to screen mungbean genotypes for high temperature tolerance at reproductive stage and observe physiological growth/yield performance of selected genotypes under high temperature conditions.

MATERIALS AND METHODS

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 Torripsammments) under screen house conditions. During 2014-2015 at Division of Plant Physiology, COBS & H, CCS, Haryana Agricultural University, Hisar Haryana, India. Before sowing, the seeds were surface sterilized with 0.1 % HCl, for two minutes and washed with distilled water twice. Seeds were inoculated with culture Rhizobium leguminosarum (S-24) and sown in pots. After thinning two plants were retained in each pot. Nitrogen free nutrient solution (Wilson and Reisenauer 1963) was given at required time intervals. Destructive sampling was done at 3 to 7days after the plants experiencing temperature (DAT) above 35°C at reproductive stage.

Chlorophyll Stability Index (CSI %) : Chlorophyll was extracted by the non-destructive method described by Gunes et al. (2007) using dimethyl sulphoxide (DMSO). For estimation of photosynthetic pigments, 30 mg of freshly harvested young fully expanded leaf (3rd) from top was taken in test tube containing 3 ml of DMSO. These tubes were then placed at room temperature till the tissue became chlorophyll free (12-16 h). In another set, 30 mg of above mentioned leaves were taken separately in test tubes containing 10 ml of de-ionized water, and heated at 65°C for 30 min in a water bath. After cooling, leaves were taken out of water, blotted dry and transferred to test tubes containing 3 ml of DMSO and processed for extraction of pigments as explained above. After incubation, the extract was transferred to a graduated tube and absorbance was read at 665, 645 and 454 nm as described by Kaloyereas (1958) on a computer aided spectrophotometer (Systronic India Spectrophotometer 117) running a multiple wave length programme. DMSO was used as blank.

Calculations for different pigments were made according to the formulae (Lichtenthaler, 1987) given below:

Chlorophyll \( a \) (mg/g FW) = \( (11.75 \times A_{665} - 2.35 \times A_{454}) / X \times 3/30 \)

Chlorophyll \( b \) (mg/g FW) = \( (18.61 \times A_{665} - 3.96 \times A_{645}) / X \times 3/30 \)

Carotenoid (mg/g FW) = \[ (1000 \times A_{645} - (2.27 \times \text{chl a}) / (81.4 \times \text{Chl b})/227] / X \times 3/30 \]

Quantities of these pigments were calculated in mg g\(^{-1}\) tissue fresh weight.

Total chlorophyll = Chla + Chlb

CSI=Total chlorophyll of non-heated samples – Total chlorophyll of heated samples.

Chlorophyll stability index (CSI %) = \[
\frac{\text{Total Chlorophyll Content (Treated)}}{\text{Total Chlorophyll Content (Control)}} \times 100
\]

Relative stress injury (RSI %): The relative stress injury (RSI %) in leaves was evaluated by (Sullivan(1972). The third fully expanded leaf from the top was collected and kept in 20 ml vials containing 10 ml de-ionized water at 25°C. After 4 h, the electrical conductivity (EC) of the solution was measured by the Water Analysis Kit (Naina, India Ltd., NDC 732) and designated as EC\(_a\). Then the samples were kept in boiling water bath for 50 min to achieve total killing of the tissue. After cooling, the EC of the solution was again measured and designated as EC\(_b\).

The relative stress injury (RSI) was calculated as follows:

\[
\text{RSI} (\%) = 1 - \frac{\text{EC}_a}{\text{EC}_b} \times 100
\]

Heat Susceptibility Index (HSI):

The Heat Susceptibility Index (HSI) was estimated for seed yield using the formula suggested by Fischer and Maurer (1978).

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

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

Yield stability ratio (YS):

The yield stability ratio (YS) was calculated as per Lewis (1954).

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

RESULTS AND DISCUSSION

The total chlorophyll content showed a decline in all three genotypes with increase in DAE to high temperature (>35°C) (Fig. 1). The total chlorophyll content varied from...
Changes in total chlorophyll content of leaves in mungbean genotypes as affected by high temperature.

Vertical bars indicate ± SE mean

Fig. 1: Changes in total chlorophyll content of leaves in mungbean genotypes as affected by high temperature. At 7 DAE and minimum decline was observed in MH 421 (12.67 mg g⁻¹ DW) followed by MH 318 (11.14 mg g⁻¹ DW) and maximum decline in Basanti (10.12 mg g⁻¹ DW) over their respective control. Under high temperature condition the genotype MH 421 has maintained higher photosynthetic pigments than MH 318 and Basanti. Similar results were observed in wheat by Almeselmani et al. (2006) under changes in irradiance and high temperature in all the genotypes, however, tolerant genotypes maintained comparatively higher chlorophyll content and showed less reduction compared to other genotypes under increasing temperature. A decrease of total chlorophyll content with high temperature implies a lowered capacity for light harvesting (Karim et al., 2003; Afzal et al., 2014). Chlorophyll stability index (%) decreased with increased DAE to high temperature (>35°C) in all three genotypes and the values varied from 81.01 to 67.36. Under late sown conditions the mean values of CSI in leaves were significantly higher in the genotype of MH 421 (78.60) as compared with MH 318 (72.15) and Basanti (69.98) as showed in Table 1. At 7 DAE, maximum CSI was observed in MH 421 (70.81) followed by MH 318 (67.30) and minimum in Basanti (63.99) over their respective control. CSI follows the similar trend as that of the total chlorophyll content. Differences in genotypes and temperature were significant. Data presented in Fig. 2 shows that carotenoid content significantly declined (4.61 to 2.13 mg g⁻¹ DW) with increase in DAE to high temperature (>35°C) in all genotypes. The maximum carotenoid was observed in MH 421 (2.33) followed by MH 318 (2.15) and minimum in Basanti (1.91) at 7 DAE over to their control. The genotypic and temperature difference were statistically significant.

Table 1: Changes in chlorophyll stability index (CSI) in mungbean genotypes as affected by high temperature.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Normal sown</th>
<th>Late sown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>&gt;35°C Days after exposure (DAE)</td>
</tr>
<tr>
<td>MH 421</td>
<td>87.93</td>
<td>82.21 71.62 80.59 85.57 79.42 70.81</td>
</tr>
<tr>
<td>MH 318</td>
<td>83.73</td>
<td>75.81 67.30 75.62 78.97 70.18 67.30</td>
</tr>
<tr>
<td>Basanti</td>
<td>81.23</td>
<td>74.84 65.41 73.83 78.48 67.48 63.99</td>
</tr>
<tr>
<td>Mean</td>
<td>84.30</td>
<td>77.62 68.11 81.01 72.36 67.36</td>
</tr>
</tbody>
</table>

C.D. at 5% Genotypes = 3.28
Temperature = 3.28
Genotypes X Temperature = NS

Volume 41 Issue 4 (August 2018)
Table 2: Changes in relative stress injury (RSI %) of leaves in mungbean genotypes as affected by high temperature.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Normal sown</th>
<th>Late sown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after exposure (DAE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>&lt; 35°C</td>
</tr>
<tr>
<td>MH 421</td>
<td>18.54</td>
<td>21.81</td>
</tr>
<tr>
<td>MH 318</td>
<td>21.12</td>
<td>24.24</td>
</tr>
<tr>
<td>Basanti</td>
<td>24.54</td>
<td>30.82</td>
</tr>
<tr>
<td>Mean</td>
<td>21.40</td>
<td>25.62</td>
</tr>
</tbody>
</table>

Genotypes = 4.13

Table 3: Yield and yield parameters of mungbean genotypes under normal sown (NS) and late sown (LS) conditions.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of pods plant⁻¹</th>
<th>No. of seeds pod⁻¹</th>
<th>100 seed weight (g)</th>
<th>Yield plant⁻¹ (g)</th>
<th>Yield Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>LS</td>
<td>NS</td>
<td>LS</td>
<td>NS</td>
</tr>
<tr>
<td>MH 421</td>
<td>10.00</td>
<td>9.33</td>
<td>9.15</td>
<td>8.61</td>
<td>4.00</td>
</tr>
<tr>
<td>MH 318</td>
<td>8.00</td>
<td>7.35</td>
<td>8.75</td>
<td>8.12</td>
<td>3.27</td>
</tr>
<tr>
<td>Basanti</td>
<td>7.33</td>
<td>6.55</td>
<td>8.00</td>
<td>6.93</td>
<td>3.17</td>
</tr>
<tr>
<td>Mean</td>
<td>8.44</td>
<td>7.74</td>
<td>8.63</td>
<td>7.89</td>
<td>3.48</td>
</tr>
</tbody>
</table>

Genotypes = 4.61

Table 3 depicts the number of seeds pod⁻¹ in two dates of sowing. The mean number of seeds pod⁻¹ in NS was 8.64, the difference was significant. The maximum number of mean of seeds per pod was in MH 421 (8.61) followed by MH 318 (8.12) and lowest in Basanti (6.93). The maximum reduction in number of seeds pod⁻¹ was found in Basanti (13.38%) followed by MH 318 (7.20%) and minimum in MH 421 (5.90%). 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 3). The genotypes showed significant differences for seed yield in both normal and late sown experiments. The mean seed yield reduction was 11.12% and 26.82% in normal and late sown, respectively.

Both genotypes i.e. from 29.50 to 38.98%. 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%) Table 2. The results of RSI for genotypes and temperature were statistically significant.

The reduced chlorophyll stability index more destruction of chlorophyll under high temperature and poor translocation of photosynthates from the sink to the source in the present study could be the other reasons for decrease in seed yield (Table 3) in the present investigation. The results were in agreement with those of Subrahmanyan and Rathore (1994) who observed that high temperature during reproductive stage significantly inhibited the import of photosynthates by both upper and lower pods of terminal raceme and thereby reduced sink strength. The high temperature significantly reduced the mean number of pods plant⁻¹ in all the three tested genotypes. The mean number of pods plant⁻¹ in LS was 7.74 as against 8.44 in NS condition (Table 3). The normal sown experiment produced higher mean pod number compared to late sown experiment. Maximum numbers of pods were observed in MH 421 (9.35) followed by MH 318 (7.35) and minimum was in Basanti (6.55). The maximum reduction in number of pods plant⁻¹ was found in Basanti (10.64%) followed by MH 318 (8.13%) and minimum in MH 421 (6.70%).

Table 3 depicts the number of seeds pod⁻¹ in two dates of sowing. The mean number of seeds pod⁻¹ in NS was 8.64, the difference was significant. The maximum number of mean of seeds per pod was in MH 421 (8.61) followed by MH 318 (8.12) and lowest in Basanti (6.93). The maximum reduction in number of seeds pod⁻¹ was found in Basanti (13.38%) followed by MH 318 (7.20%) and minimum in MH 421 (5.90%). 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 3). The genotypes showed significant differences for seed yield in both normal and late sown experiments. The mean seed yield reduction was 11.12% and 26.82% in normal and late sown, respectively.

Both genotypes i.e. from 29.50 to 38.98%. 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%) Table 2. The results of RSI for genotypes and temperature were statistically significant.

The reduced chlorophyll stability index more destruction of chlorophyll under high temperature and poor translocation of photosynthates from the sink to the source in the present study could be the other reasons for decrease in seed yield (Table 3) in the present investigation. The results were in agreement with those of Subrahmanyan and Rathore (1994) who observed that high temperature during reproductive stage significantly inhibited the import of photosynthates by both upper and lower pods of terminal raceme and thereby reduced sink strength. The high temperature significantly reduced the mean number of pods plant⁻¹ in all the three tested genotypes. The mean number of pods plant⁻¹ in LS was 7.74 as against 8.44 in NS condition (Table 3). The normal sown experiment produced higher mean pod number compared to late sown experiment. Maximum numbers of pods were observed in MH 421 (9.35) followed by MH 318 (7.35) and minimum was in Basanti (6.55). The maximum reduction in number of pods plant⁻¹ was found in Basanti (10.64%) followed by MH 318 (8.13%) and minimum in MH 421 (6.70%).

Table 3 depicts the number of seeds pod⁻¹ in two dates of sowing. The mean number of seeds pod⁻¹ in NS was 8.64, the difference was significant. The maximum number of mean of seeds per pod was in MH 421 (8.61) followed by MH 318 (8.12) and lowest in Basanti (6.93). The maximum reduction in number of seeds pod⁻¹ was found in Basanti (13.38%) followed by MH 318 (7.20%) and minimum in MH 421 (5.90%). 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 3). The genotypes showed significant differences for seed yield in both normal and late sown experiments. The mean seed yield reduction was 11.12% and 26.82% in normal and late sown, respectively.
yield was highest in MH 421 (3.49 g) compared to MH 318 (2.70) and Basanti (2.39 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. Significant effect of high temperature was noticed on mean test weight (g) in both the dates of sowing. The mean test weight was more under normal sown (3.48) than late sown (2.83) (Table 3). A significant reduction in test weight was observed in all three genotypes, however more test weight was observed in MH 421 (3.68) followed by MH 318 (2.99) less in Basanti (2.80). The maximum reduction in 100 seed weight was found in Basanti (23.3%) followed by MH 318 (17.4%) and minimum in MH 421 (16.3%). The overall interaction was statistically significant for test weight in both normal and late sown experiments.

The HSI and YS were calculated for both genotypes (Fig 3). 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 there 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. Generally, high temperature reduces grain yield by reducing the crop cycle. This indicates that developmental stages of mungbean were reduced due to high temperature. Our findings showed that high temperature decreased seed yield (Table 3) in LS compare to NS, under LS condition maximum seed yield was observed in MH 421 followed by MH 318 and less in Basanti (Table 3).

Temperature at different developmental stages of mungbean played an important role in crop performance under heat stress. It is concluded that the genotype, MH 421 was identified as the most thermo-insensitive with better photosynthetic pigments, antioxidative defense mechanism and reproductive behavior under high temperature with low HSI value but high YS percentage as compared with MH 318 and Basanti. In the present investigation, grain filling period was most affected under high temperature, apart from seed yield, there was a significant reduction was observed in several yield components viz. number of seeds per pod (Table 3), 100 seed weight (Table 3) in late sown experiment in all three genotypes. Comparatively MH 421 showed better performance than MH 318 and Basanti. These results clearly indicated that there is genotype and temperature interaction in late sowing and it may always suitable for identification of high temperature tolerant genotypes. 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) (Fig 3) and yield stability (YS) (Fig 3) 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. The yield related traits most affected by temperature stress were pod number per plant (Table 3). 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 widely accepted that the genetic structure of grain yield can be better understood by studying linked quantitative traits (Guler et al. 2001), such as those identified in the current study.

CONCLUSION

The investigations were carried out on mungbean [Vigna radiata (L.) Wilczek] genotypes i.e. MH421, MH 318 and Basanti to study their physiological, biochemical and yield traits in relation to high temperature tolerance. High temperature reduced photosynthesis through disruptions in the structure and function of photosynthetic pigments by enhanced chlorosis of leaves. High temperature significantly decreased total chlorophyll pigment content with increasing period of DAE. At 7 DAE minimum decline was observed in MH 421 (12.67) followed by MH 318 (11.14) and maximum decline in Basanti (10.12) over their respective control. Decrease in carotenoid content from 4.61 to 2.13 (mg g\(^{-1}\) DW) was observed due to high temperature in all the genotypes, however, tolerant genotype MH 421 (2.33) followed by MH 318 (2.15) maintained comparatively higher carotenoid content and showed less reduction compared to Basanti (1.91) over to their respective control. A significant decrease in chlorophyll stability index (CSI %) was observed from control to 7 DAE in all genotypes. With the increasing period DAE to high temperature, the relative stress injury (RSI %) was increased from 21.40 to 34.69%. In three genotypes and maximum increase in RSI (32.17 to 40.72%) was observed in Basanti followed by MH 318 (29.24 to 41.34%) and minimum was noticed in MH 421 (26.80 to 34.88%) over to their respective control. Finally, it is concluded that high temperature stress significantly leads to the reduction was observed in seed yield plant\(^{-1}\) and yield components in late sown as compared to normal sown. Based on mean performance of mungbean genotypes for various morpho-physiological traits as well as yield and its components, the genotype MH 421 was found to have high values than MH 318 and Basanti under both the environments. MH 421 also showed low value of HSI, high value of YS in comparisons to MH 318 and Basanti.

REFERENCES


Prasad, P.V., Boote, K.J. and Allen, L.H. (2006). Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain sorghum (*Sorghum bicolor* L. Moench) are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agric Forest Meteor.* **139**: 237-251.


