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Comparison of Leaf Characters of Summer Mungbean (*Vigna radiata* (L.) Wilczek) Genotypes under Water Deficit Stress

Nazir Ahmad Malla*, Rajwinder Kaur** and Pardeep Kaur***
**Department of Botany, Govt. Degree College, Bijbehara (J&K), INDIA*
***Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (Punjab)-141004, INDIA*
****Department of Plant Pathology, Punjab Agricultural University, Ludhiana (Punjab)- 141004, INDIA*

(Corresponding author: Pardeep Kaur)
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ABSTRACT: Present investigation was undertaken to study various leaf characters as visual markers for screening of sensitive and tolerant genotypes of mungbean against drought tolerance. Two sensitive mungbean genotypes- SML-1082 and SML-1147 and two tolerant genotypes- SML-1168 and SML-1151 were used in field studies. Under field conditions, plants were stressed by withholding irrigation once at vegetative stage, flowering stage and at podding stage while plants grown under normal irrigation regime served as control plants. The data on various morphological characters in normal and stressed plants were recorded at vegetative, flower initiation and podding stages. Sensitive genotypes exhibited higher number of leaves, leaf area and leaf area index under normal conditions as compared to tolerant genotypes. A significant decline in all these parameters was observed when water stress was given. The plants of tolerant genotypes performed better under conditions of water deficit stress as compared to sensitive genotype. Under water stress conditions, tolerant genotypes maintained higher leaf area than that of the sensitive genotypes at all growth stages. Tolerant genotypes maintained lesser negative value of leaf water potential, indicating higher water status under the stress conditions. Pubescence on the leaves of tolerant genotypes were long and dense, while in the sensitive ones, pubescence was very less with short length of hair. Stomatal frequency was more in the sensitive genotypes as compared to tolerant ones. Out of the three developmental stages, moisture stress given at vegetative stage of growth was more sensitive to leaf growth and development.

Key words: Mungbean, moisture stress, leaf characters, tolerant and sensitive genotypes.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is the third most important pulse crop after chickpea and pigeon pea grown in India. Economically it is the most important crop of the *Vigna* group, being rich in quality protein, minerals and vitamins and is also used as animal fodder and green manure. It contains isoflavonoids having antioxidant activities. On account of short duration and photo-thermo insensitivity, mungbean is considered excellent crop for crop intensification and diversification. Although climatic and edaphic factors in our country are highly suitable for mungbean cultivation, yet it faces a number of physiological constraints for low productivity *viz*. excessive vegetative growth, indeterminate growth habit, flower and fruit drop, poor source-sink relationship, poor harvest index and lack of moisture stress tolerance.

Among the above constraints, water stress is considered principal environmental factor limiting growth and yield. Depending upon the system, soil moisture stress occurs due to either early cessation of rain, or low rainfall and poor soil moisture and erratic or insufficient irrigation. Moisture stress during the crop growth period becomes as one of the major production constraints in pulses. Occurrence of dry spell of different magnitudes during the crop growth period affect number of physiological and metabolic processes like germination, growth, photosynthesis, respiration, nutrient metabolism etc, consequently, leading to poor growth and yield. However, stress response depends upon the intensity, rate and duration of exposure and the stage of the crop. Therefore there is a great need for evolving drought resistant varieties which could withstand limited soil moisture and produce better yield.

MATERIAL AND METHODS

The present investigation was undertaken to evaluate leaf characters of drought sensitive and tolerant genotypes of summer mungbean (*Vigna radiate* (L.) Wilczek) under normal as well as stressed conditions. Seeds of summer mungbean genotypes- SML-1082, SML-1147, SML-1168 and SML-1151 were procured from the Department of Plant Breeding and Genetics (Pulses section), Punjab Agricultural University, Ludhiana. The plants of these genotypes were raised in the field area of Department of Plant Breeding and Genetics, in three replicates in plots measuring 4.2 × 3.0m, which were further divided into 12 beds, in a split plot design (SPD) in two replications. Each bed consisted of 4 rows covering 1 genotype with row to row spacing of 45 cm and in each row, spacing between two plants was 30 cm. The main plots were given following irrigation treatments.

-Control- Without stress, given irrigation at 5 days regular intervals.

-WS_V - Stressed by withholding irrigation once at vegetative stage (15 to 25 DAS) for 10 days and then watering at regular intervals.

-WS_F - Stressed by withholding irrigation once at flowering stage (25 to 35 DAS) for 10 days and then watering at regular intervals.

-WS_P - Stressed by withholding irrigation once at pod initiation stage (35 to 45 DAS) for 10 days and then watering at regular intervals.

The data on various morphological characters (Number of leaves plant^{-1} , Leaf area (cm^2), Leaf area index (LAI), in normal and stressed plants were recorded at vegetative, flower initiation and podding stages. Five plants of each replication were tagged randomly in order to record the data at different growth phases *viz.* vegetative stage (30 days after sowing DAS), flower initiation stage (40 DAS) and podding stage (50 DAS) in both the genotypes of mungbean and average of these observations was recorded.

Number of leaves per plant: Number of leaves was counted from the tagged plants and average of the replications was calculated.

Leaf area: The leaf area per plant was measured by making outline drawing of all the leaves of a plant on a plain paper. The paper was cut in the shape of leaf drawn. Weight of the paper was taken and thus, leaf area calculated as follows:

$$\text{Leaf area (cm}^2\text{)} = \frac{\text{Weight of paper cut in shape of leaf}}{\text{Weight of 1 cm}^2\text{ of that same paper}}$$

Leaf area index (LAI): LAI was calculated according to the formula

$$\text{LAI} = \frac{\text{Leaf area plant}^{-1}}{\text{Number of plants m}^{-2}}$$

Stomatal Frequency, Leaf Pubescence (SEM images) and Leaf Orientation

RESULTS AND DISCUSSION

A. Number of leaves

Leaf is the source of photosynthates, thus the number of leaves has a direct bearing on the capacity of sink and hence determines the yield of the plant. Inhibition of leaf is a primary whole plant response to moisture stress. The number of leaves (Table 1) in general depicted change from 30 to 50 days after sowing (DAS) with moisture stress in four mungbean genotypes (Table 1). Maximum number of leaves at 30 DAS were recorded in SML-1151(4.0) followed by SML-1168 (3.6) under control conditions. Moisture stress significantly reduced the leaf initiation in all the tested four mungbean genotypes at 30 DAS. Genotypes subjected to WS_V exhibited suppression in number of leaves amounting to 44.44% (SML-1168) followed by 42.85% (SML-1147 and SML-1082) and 35% (SML-1151) in comparison to that of control.

Maximum number of leaves at 50 DAS were observed in SML-1147(12) followed by SML-1151 (11.5), SML-1082 (10.2) and SML-1168 (9.7) under control conditions. At 50 DAS reduction in number of leaves with WS_P was recorded to be lesser than WS_V and WS_F. Percent reduction in number of leaves with WS_P was 2.06, 5.02, 5.08 and 5.88 in comparison to 10.30, 17.3, 5.25 and 19.60 with WS_F and 12.37, 30.43, 41.66 and 31.37 with WS_V at 50 DAS in mungbean genotype SML-1168, SML-1151, SML-1147and SML-1082. Genotypes subjected to moisture stress at vegetative stage resulted in most severe suppression in number of leaves, whereas least reduction in number of leaves was observed with WS_P. Sensitive genotypes recorded more reduction in number of leaves as compare to tolerant genotypes.

Table 1: Effect of water stress at vegetative (WS_V), flower initiation (WS_F) and pod initiation (WS_P) on number of leaves at various growth stages (30 DAS, 40 DAS and 50 DAS) of mungbean genotypes (30 DAS).

Genotypes Treatments	SML-1168	SML-1151	SML-1147	SML-1082	Mean
Control	3.6	4.0	3.5	3.5	3.6
WS_V	2.0	2.6	2.0	2.0	2.1
Mean	2.8	3.3	2.7	2.7	
LSD 0.05	**V=0.018, T=0.016, VXT=0.023**				
(40 DAS)					
Control	5.3	5.6	5.7	5.5	5.5
WS_V	4.3	4.7	4.6	4.2	5.0
WS_F	5.1	5.3	5.4	5.1	5.2
Mean	5.1	5.3	5.3	5.2	
LSD 0.05	**V=NS, T=NS, VXT=0.386**				
(50 DAS)					
Control	9.7	11.5	12	10.2	10.85
WS_V	8.2	8.0	7.0	7	7.62
WS_F	8.7	9.5	9.0	8.2	8.85
WS_P	9.5	10.9	11.8	9.6	10.45
Mean	9.1	9.97	9.9	8.7	
LSD 0.05	**V=0.023, T=0.014, VXT=0.029**				

Reduction in number of leaves under water stress is due to drop in relative water content, which reduces leaf turgor. Moreover increase in level of ABA due to water stress acts as antagonist of auxins and cytokines thus increasing bud dormancy and reducing development of new leaves and also causes decrease in leaf size (Taiz and Zeiger 1998). Reduction in number of leaves in stressed plants is in agreement with the results observed by Lobato *et al* (2009) in soybean plants.

B. Leaf area

Drought stress adversely affects leaf growth and expansion. Leaf development is highly affected at vegetative stage than at flowering or pod formation stage. Highest leaf area per plant was recorded in control plants at all the growth stages (Table 2).

Table 2: Effect of water stress at vegetative (WS_V), flower initiation (WS_F) and pod initiation (WS_P) on leaf area/plant ($cm^2 plant^{-1}$) at various growth stages (30 DAS, 40 DAS and 50 DAS) of mungbean genotypes (30 DAS).

Genotypes Treatments	SML-1168	SML-1151	SML-1147	SML-1082	Mean
Control	6.57	7.70	7.70	8.62	7.64
WS_V	3.10	4.75	3.42	3.82	3.77
Mean	4.83	6.22	5.56	6.22	
LSD 0.05	**V=NS, T=1.19, VXT= NS**				
(40 DAS)					
Control	445.61	465.01	479.05	498.01	471.92
WS_V	389.02	365.64	318.05	354.61	356.83
WS_F	409.31	398.61	365.01	393.34	391.56
Mean	414.64	409.75	387.37	415.32	
LSD 0.05	**V=NS, T=10.23, VXT=1.02**				
(50 DAS)					
Control	863.12	786.30	897.01	891.21	859.41
WS_V	647.23	642.10	489.10	501.13	569.89
WS_F	727.54	662.01	589.01	573.20	637.94
WS_P	794.69	729.20	699.20	721.20	736.02
Mean	758.14	704.90	668.58	671.63	
LSD 0.05	**V=0.576, T=1.03, VXT= 2.06**				

Maximum leaf area per plant was registered in genotype SML-1082 (8.62 cm^2) followed by SML-1151 (7.70 cm^2), SML-1147 (7.70 cm^2) and SML-1168 (6.57 cm^2) under control conditions. Significant reduction in leaf area per plant was observed with WS$_V$ at 30 DAS and the percent reduction was maximum (55.68%) in sensitive genotype SML-1082 and least (38.31%) in tolerant genotype SML-1151. Moisture stress significantly reduced the leaf area per plant in all the four mungbean genotypes under WS$_F$ (40 DAS). Reduction in leaf area was recorded more in sensitive genotypes SML-1147 (23.80%) and SML-1082 (21.01%), whereas tolerant genotypes SML- 1151(14.27%) and SML-1168 (8.14%), showed lesser reduction in leaf area.

Similar results were obtained with WS$_P$ (50 DAS). Among the four mungbean genotypes SML-1082 again recorded maximum (891.21cm^2) and SML-1151 showed minimum (786.30 cm^2) leaf area per plant under control conditions at 50 DAS. Tolerant genotypes SML-1168 and SML-1151 maintained higher leaf area (758.14 and 704.90 cm^2, respectively) per plant even under stressed conditions as compared to sensitive genotypes SML-1147(668.58 cm^2) and SML-1082 (671.63 cm^2).

Low assimilation of water and nitrogen compounds, leaf rolling, and death of whole leaf under water stressed result in lower or void extension in rate of leaf area. Comparable results were obtained by De and Kar (1995) recording that leaf area of mungbean leaves declined with water stress. Mahajan and Tuteja (2005) also concluded that reduction in leaf expansion and leaf growth occurred under water deficit condition.

C. Leaf area index

Leaf area index (LAI) defines an important structural property of a plant canopy. Leaf area index was found higher in control leaves than moisture stressed leaves (Table 3). Leaf area index was recorded maximum in SML-1082 (0.0206) followed by SML-1151 and SML-1147 (0.0184) and SML-1168 (0.0105) under control condition at 30 DAS. A gradual reduction in leaf area index was observed with water stress in all the mungbean genotypes. With WS$_V$ maximum percent reduction in leaf area index was recorded in SML-1082 followed by SML-1147, SML-1168 and SML-1151.

WS$_V$ submitted genotypes recorded recovery in leaf area index at 40DAS as compared to 30 DAS. Leaf area index was recorded 1.010, 0.933, 0.789 and 0.955 in mungbean genotypes SML-1168, SML-1151, SML-1147 and SML-1082 respectively with WS$_F$ at 40DAS.

Reduction in leaf area index was more pronounced with WS$_V$ followed by WS$_F$ and WS$_P$ respectively at 50 DAS. The percent reduction in leaf area index varied between 43.70% (SML-1082) to 21.57% (SML-1151) with WS$_V$, 35.60% (SML-1082) to 15.70% (SML-1151) with WS$_F$ and 26.30% to 8.01% (SML-1168) with WS$_P$.

Table 3: Effect of water stress at vegetative (WS$_V$), flower initiation (WS$_F$) and pod initiation (WS$_P$) on leaf *area index* at various growth stages (30 DAS, 40 DAS and 50 DAS) of mungbean genotypes (30 DAS).

Genotypes **Treatments**	**SML-1168**	**SML-1151**	**SML-1147**	**SML-1082**	**Mean**
Control	0.0157	0.0184	0.0184	0.0206	0.0182
WS$_V$	0.0074	0.0114	0.0082	0.0091	0.0090
Mean	0.0115	0.0149	0.0133	0.0148	
LSD 0.05	**V=0.003, T=.0051, VXT=.0072**				
(40 DAS)					
Control	1.195	1.123	1.208	1.329	1.213
WS$_V$	0.876	0.765	0.710	0.643	0.748
WS$_F$	1.010	0.933	0.789	0.955	0.921
Mean	1.027	0.940	0.902	0.975	
LSD 0.05	**V=0.0018, T=0.0012, VXT=0.0020**				
(50 DAS)					
Control	2.071	1.91	2.152	2.138	2.067
WS$_V$	1.552	1.498	1.173	1.202	1.356
WS$_F$	1.744	1.589	1.415	1.375	1.530
WS$_P$	1.905	1.749	1.629	1.574	1.714
Mean	1.818	1.686	1.592	1.572	
LSD 0.05	**V=0.005, T=0.0010, VXT=0.0021**				

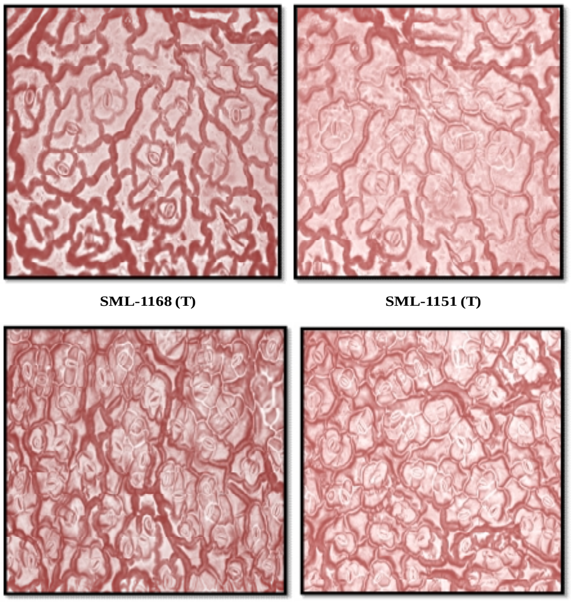
Mungbean genotypes SML-1147 and SML-1082 registered higher value of percent reduction at all the stages and the water stress treatments over mungbean genotypes SML-1168 and SML-1151, conforming that SML-1168 and SML-1151 are more tolerant to moisture stress than SML-1147 and SML-1082.In the present study maximum reduction in leaf area index occurred when moisture stress was given at vegetative stage of growth. Similar results were observed by De Costa *et al* (1999) stating vegetative stage to be more sensitive for the leaf area index and other leaf growth parameters in mungbean.

Stomatal frequency, leaf pubescence and orientation

Plate I shows the distribution of stomata on adaxial surface of leaves of tolerant and sensitive mungbean genotypes under control conditions. Stomatal frequency was recorded higher in leaves of sensitive genotypes, SML-1082 (142 mm^{-2}) and SML-1147 (155 mm^{-2}) than tolerant genotypes SML-1168 (111 mm^{-2}) and SML-1151 (132 mm^{-2}). Significant differences have been observed with regard to stomatal frequency among leaves of tested tolerant and sensitive mungbean genotypes. These stomatal features are commonly related to ability of plants to tolerate water stress tolerance. The leaf modifications in response to stressful environment include the number of stomata (Kozlowski and Pallardy 2002). Kauser *et al* (2006) suggested that low stomatal frequency might be a drought resistance mechanism in canola. Comparative studies on different plant species have shown that xeric species have higher stomatal density than mesic species (Carpenter and Smith 1975).

The leaf pubescence was also observed under control conditions in the tolerant and sensitive genotypes. Plate II shows the difference in hair distribution in tolerant and sensitive genotypes. The hair density was higher in tolerant genotypes, SML-1168 and SML-1151 as compared to the sensitive ones, SML-1082 and SML-1147. Leaf pubescence has adaptive value because the hairs allow the leaf to avoid potentially lethal loss of water. Azmat *et al* (2009) studies with *Phaseolus mungo* and *Lens culinaris* suggested that stress tolerant plants tend to possess leaves that have more hairs than sensitive plants. Several possibilities exist for the function of leaf pubescence in plants from arid habitats.

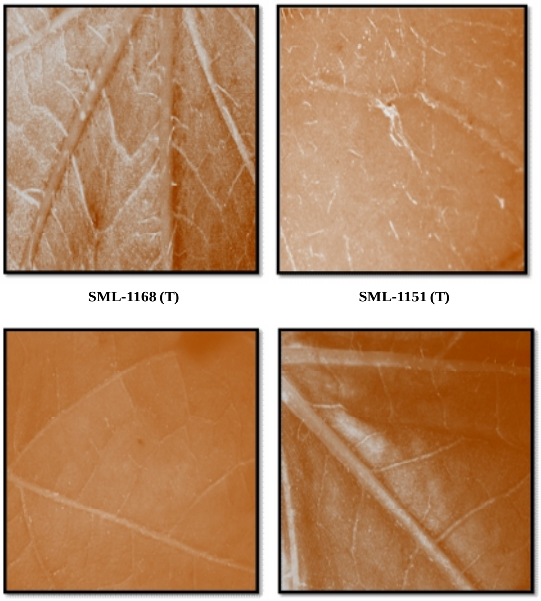
These include reduction of light absorption during conditions of high temperature and water stress conditions.



SML-1168 (T) SML-1151 (T)

SML-1147 (S) SML-1082 (S)

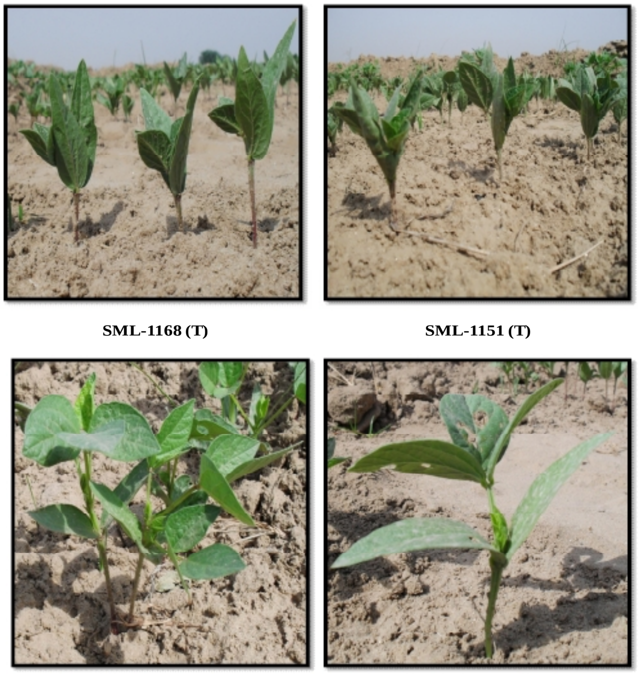
Plate I :comparison of stomatal frequency on the adaxial surface in tolerant (T) and sensitive (S) mungbean genotypes under control conditions.



SML-1168 (T) SML-1151 (T)

SML-1147 (S) SML-1082 (S)

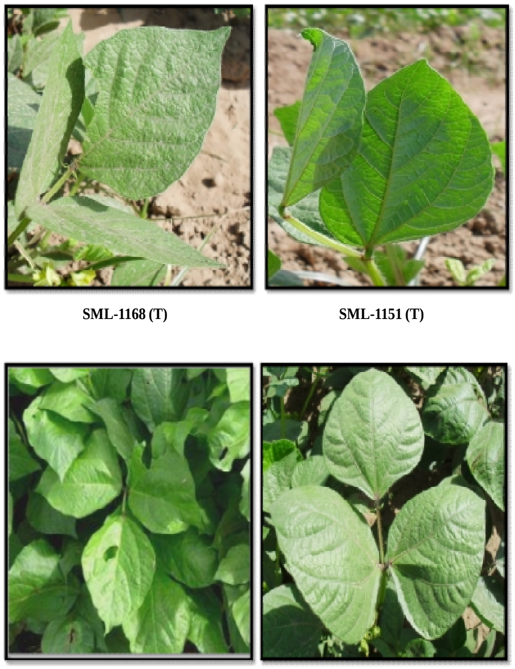
Plate II: comparison of leaf pubescence ion adaxial surface n tolerant (T) and sensitive (S) mungbean genotypes under control conditions.



SML-1168 (T) **SML-1151 (T)**

SML-1147 (S) **SML-1082 (S)**

Plate III(a): Leaf orientation in tolerant (T) and sensitive (S) mungbean genotypes under water stress conditions at 30 DAS.



SML-1168 (T) **SML-1151 (T)**

SML-1147 (S) **SML-1082 (S)**

Plate III(b): Leaf orientation in tolerant (T) and sensitive (S) mungbean genotypes under water stress conditions at 40 DAS.

Lindsay *et al* (2007) reported in three perennial legumes, *Medicago sativa, Dorycnium hirsutum* and *Dorycnium rectum* that leaf hairs increase reflection of radiation and reduce water conductance through the leaf boundary layer, thus reducing water loss and moderating leaf temperature.

Leaf morphological adaptations to water stress were observed in the tolerant as well as sensitive mungbean genotypes. Both SML-1168 and SML-1151 leaves responded to water stress by changing the orientation of leaves and reducing radiations interception. Leaflets of both the tolerant genotypes were folded to form a cup and were oriented parallel to incoming solar radiation under water stress conditions, where as in sensitive mungbean genotypes (SML-1082 and SML-1147) no such morphological adaptation was observed. The leaf angle of the tolerant genotypes was ranging between 60° to 90° where as in the sensitive genotypes it was recorded lesser than 50° (Plate III(a) and Plate III(b)). Orientation of the leaves parallel to the coming radiations has been described as a stress avoidance mechanism. Leaves move by means of turgor pressure changes at the pulvinus at the base of each lamina. In beans, leaf movement is modulated by leaf water status and temperature. The extent of leaf movement was increased as the water potential drops, thus reducing the irradiance under conditions of stress, thus reducing transpiration and leaf temperature (Wang *et al* 2004). The phenomenon of leaf cupping has also been documented by Lindsay *et al* (2007) in *Medicago sativa* and *Dorycnium rectum* with midday decline in leaf water potential.

CONCLUSION

Soil water content either directly or indirectly influences plant growth. The present results of leaf characteristics highlighted the fact that water stress significantly influenced all the leaf growth parameters. All the leaf parameters viz. number of leaves, leaf area, leaf area index, stomatal frequency, leaf pubescence and leaf orientation, adversely influenced by the water stress and the plants subjected to moisture stress at vegetative stage of growth observed maximum sensitivity.

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