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Original Research Paper

Effect of Drought Stress on Chlorophyll Content and Anti-oxidant Enzymes of Green Gram Genotypes (*Vigna Radiata* L.)

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ABSTRACT

Green gram (*Vigna radiata* L.) Wilczek, chiefly grown as a post rainy season crop, faces water stress situation. A complex response, in terms of biochemical and molecular level is shown by plants, when exposed to drought, and depending on that, plants show differential adaptation and tolerance mechanisms. In the studied biochemical parameters, proline, catalase and peroxidase showed increased activity due to water stress and negatively correlated with seed yield and correlation was significant for catalase and peroxidase, while the total chlorophyll content decreased due to water stress and it was positively and significantly correlated with the seed yield. All the biochemical parameters recorded higher values in genotype WGG 37, whereas, lowest total chlorophyll and leaf proline were recorded in MGG 348 and lowest catalase and peroxidase activity in MGG 347. Highest seed yield was recorded by the genotype WGG 37 (1058.71 kg ha^{-1}), followed by WGG 42 (1052.22 kg ha^{-1}), while the lowest seed yield was recorded in MGG 348 (951.42 kg ha^{-1}). Thus indicating the role of the biochemical parameters and total chlorophyll content in stress mitigation.

INTRODUCTION

Pulses play an important role in meeting our dietary requirement of protein. India is the largest producer of pulses in the world. Green gram is an important short-duration legume crop with wide adaptability and low input requirement. It also plays a vital role in sustainable agriculture, as mixed crop, inter crop and rotational crop which improves the nitrogen status of soil and can also be used as nutritious green fodder for livestock.

The major factor limiting the crop yield is the amount of moisture available to the crop during the growing season. In India, about 68% of net sown area (140 million hectares) is reported to be vulnerable to drought conditions. Mung bean when cultivated in post rainy season faces water stress at various stages of crop growth. When the plant comes across the water stress, it produces some anti-oxidants (enzymes) to mitigate the water stress.

MATERIALS AND METHODS

Five green gram genotypes were used in the present study to study the response of genotypes to water stress. The crop was sown in Rabi, 2013-14 at College of Agriculture, Hyderabad and suitable management practices were followed to get a good crop. An experiment was laid out in split plot design with three irrigation levels as main treatments (T1- irrigated control and irrigated throughout the growth period, T2- water stress treatment, irrigated at flowering stage and T3- water stress treatment, irrigated at flowering and pod filling stages) and five genotypes as sub treatments. The experiment was replicated thrice and observations were recorded at maximum vegetative stage and 5 days after irrigation at flowering. Fresh leaves were collected from the field, both from well irrigated and the water stress treatments (at two stages i.e., 30 and 47 DAS) and biochemical parameters like total chlorophyll, leaf proline, catalase and peroxidase were estimated in the Crop Physiology Laboratory, College of Agriculture, Hyderabad. Seed yield was calculated from net plot.

Total chlorophyll: Total chlorophyll content was determined by following DMSO method (Hiscox & Israeltam 1979) at 30 DAS and 47 DAS. The absorbance of the leaf extract was measured at 645 nm, and 663 nm in a UV- Vis Spectrophotometer (Elico, SL - 159) and the total chlorophyll content was calculated by using the following formula and expressed in mg g^{-1} fresh weight.

$$\text{Total Chl.} = 20.2(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

Leaf proline: Leaf proline was estimated by Bates et al.

Table 1: Total chlorophyll content (mg chl. g^{-1} fr.wt) of green gram genotypes with different irrigation treatments.

Genotypes	30 DAS				47 DAS			
	T1	T2	T3	Mean	T1	T2	T3	Mean
MGG 295	1.776	0.846	0.836	1.153	2.096	1.790	1.816	1.901
MGG 347	1.800	0.870	0.886	1.186	2.143	1.90	1.863	1.969
MGG 348	1.753	0.826	0.803	1.128	2.053	1.613	1.636	1.768
WGG 37	1.916	0.923	0.963	1.268	2.293	1.973	2.033	2.10
WGG 42	1.850	0.903	0.910	1.221	2.246	1.940	1.913	2.033
Mean	1.819	0.873	0.880	1.190	2.166	1.843	1.852	1.954
C.D (p=0.05)(T)		0.025				0.071		
C.D (p=0.05) (G)		0.029				0.071		
TXG		0.052				0.124		

Table 2: Leaf proline content (µg g^{-1} fr.wt) of green gram genotypes with different irrigation treatments.

Genotypes	30 DAS				47 DAS			
	T1	T2	T3	Mean	T1	T2	T3	Mean
MGG 295	2.25	8.95	8.87	6.69	2.46	2.95	2.90	2.77
MGG 347	2.16	6.22	8.75	5.71	2.31	2.86	2.95	2.71
MGG 348	2.04	8.60	8.65	6.43	2.10	2.75	2.85	2.57
WGG 37	2.75	9.85	9.90	7.50	2.80	3.50	3.75	3.35
WGG 42	2.54	9.10	9.04	6.89	2.60	3.10	3.60	3.10
Mean	2.35	8.54	9.04	6.64	2.45	3.03	3.21	2.90
C.D(p=0.05)(T)		1.04				0.30		
C.D(p=0.05)(G)		1.120				0.363		
TXG		0.96				0.63		

(1973) method. In this procedure acid ninhydrin is used as reagent and toluene helped to develop colour. The developed colour was read in spectrophotometer (Elico, SL-149) at 520 nm. The proline concentration was determined from the standard curve and expressed in microgram of proline per gram fresh weight of the leaf.

Catalase: Catalase activity was estimated as per the method given by Teranishi et al. (1974). The absorbance of leaf extract was recorded at 240 nm and expressed as units g^{-1} fr.wt min^{-1}. One unit is defined as the change in absorbance per gram fresh weight per minute.

Peroxidase: The estimation was carried out as per the method given by Lowenstein & Linsey (1961). As per this procedure leaf extract absorbance was recorded at 436 nm and expressed as units g^{-1} fr.wt min^{-1}. One unit is defined as change in absorbance per gram fresh weight per minute.

RESULTS AND DISCUSSION

Total chlorophyll content (mg Chl. g^{-1} fr.wt): Water stress effect on the total chlorophyll content is presented in Table 1 and significant difference observed at maximum vegetative stage (stage 1) (30 DAS) and at flowering stage (stage 2) (47 DAS). At maximum vegetative stage, the total chlorophyll content decreased in T2 (0.873 mg chl. g^{-1} fr.wt) and T3 (0.880 mg chl. g^{-1} fr.wt) treatments as against T1 (1.819 mg chl. g^{-1} fr.wt), while in flowering stage, the decrease in the total chlorophyll content in the treatments T2 (1.843 mg chl. g^{-1} fr.wt) and T3 (1.852 mg chl g^{-1} fr.wt) was less as compared to T1 (2.166 mg chl. g^{-1} fr.wt). Water stress at maximum vegetative stage decreased the total chlorophyll content by 52 percent, whereas at the stage 2 (flowering stage), decreased by 14.50 percent compared to irrigated control. Water stress affected the chlorophyll content, which ultimately affects the seed yield. Such a decrease in total chlorophyll content due to drought stress was also reported in mung bean (Lalinia et al. 2012). The highest total chlorophyll content was recorded by WGG 37 (1.268 and 2.10 mg chl. g^{-1} fr.wt) and lowest by MGG 348 (1.128 and 1.768 mg chl. g^{-1} fr.wt) at stage 1 and stage 2, respectively. Total chlorophyll content significantly and positively correlated with seed yield at 30 (r=0.933) and 47 (r=0.904) DAS. Correlation coefficient among biochemical parameters (at different stages) and yield of green gram genotypes are given in Table 6.

Leaf proline (µg g^{-1} fr.wt): Table 2 shows that water stress significantly affected the leaf proline at 30 and 47 DAS. The leaf proline content in water stress treatments T2 (8.54 µg g^{-1} fr.wt) and T3 (9.04 µg g^{-1} fr.wt) was high as compared

Table 3: Catalase activity (Units g^{-1} fr.wt min^{-1}) of green gram genotypes with different irrigation treatments.

Genotypes	30 DAS				47 DAS			
	T1	T2	T3	Mean	T1	T2	T3	Mean
MGG 295	0.381	0.926	0.916	0.741	0.501	0.589	0.591	0.560
MGG 347	0.368	0.987	0.895	0.750	0.492	0.571	0.574	0.546
MGG 348	0.376	0.821	0.913	0.703	0.496	0.590	0.587	0.558
WGG 37	0.391	0.957	0.963	0.770	0.515	0.601	0.609	0.575
WGG 42	0.389	0.931	0.928	0.749	0.506	0.595	0.593	0.565
Mean	0.381	0.924	0.923	0.742	0.502	0.589	0.591	0.561
C.D (p=0.05)(T)		0.053				0.033		
C.D (p=0.05)(G)		0.036				0.032		
TXG		0.062				0.055		

Table 4: Peroxidase activity (Units g^{-1} fr.wt min^{-1}) of green gram genotypes with different irrigation treatments.

Genotypes	30 DAS				47 DAS			
	T1	T2	T3	Mean	T1	T2	T3	Mean
MGG 295	1.418	2.500	2.491	2.136	1.807	1.921	1.920	1.883
MGG 347	1.374	2.380	2.296	2.017	1.684	1.873	1.896	1.818
MGG 348	1.390	2.417	2.365	2.057	1.718	1.901	1.909	1.843
WGG 37	1.471	2.726	2.690	2.296	1.845	1.972	2.011	1.943
WGG 42	1.452	2.673	2.675	2.267	1.823	1.954	1.946	1.908
Mean	1.421	2.539	2.503	2.154	1.775	1.924	1.936	1.878
C.D (p=0.05)(T)		0.166				NS		
C.D (p=0.05)(G)		0.155				NS		
TXG		NS				NS		

Table 5: Seed yield (kg ha^{-1}) of green gram genotypes with different irrigation treatments.

Genotypes	T1	T2	T3	Mean
MGG 295	1173.62	966.12	976.58	1038.77
MGG 347	1100.86	896.68	911.73	969.75
MGG 348	1085.58	876.70	891.97	951.42
WGG 37	1200.73	976.08	999.32	1058.71
WGG 42	1190.22	973.76	992.68	1052.22
Mean	1150.20	937.87	954.46	1014.18
C.D (p=0.05) (T)		78.443		
C.D (p=0.05) (G)		NS		
TXG		NS		

to irrigated control (2.35 µg g^{-1} fr.wt). While at stage 2 also the proline content increased in T2 and T3 as compared to control, but this increment was low in stage 2 as compared to stage 1. Proline concentration increased enormously during water stress and it acts as osmolyte and protect the plants from reactive oxygen species (ROS). This was in conformity with the finding of Sonali et al. (2014), who reported that water stress at an early growth stage increased the free proline content in black gram. Significant differences were observed among genotypes at both the growth stages. A negative correlation was observed at both stages between leaf proline content and seed yield.

Catalase activity (units g^{-1} fr.wt min^{-1}): At maximum vegetative stage the catalase activity was significantly high in T2 (0.924 units g^{-1} fr.wt min^{-1}) and T3 (0.923 units g^{-1} fr.wt min^{-1}) compared to the irrigation control (T1) (0.381 units g^{-1} fr.wt min^{-1}) presented in Table 3. Similarly, in stage 2 the catalase activity was significantly high in T2 (0.589 units g^{-1} fr.wt min^{-1}) and T3 (0.591 units g^{-1} fr.wt min^{-1}) as against T1 (0.502 units g^{-1} fr.wt min^{-1}) but this increase was less as compared to stage 1. At both the stages, T2 and T3 treatments were on par with each other. Catalase activity increased by 142.5 and 137.53 percent at maximum vegetative stage in water stress treatments T2 and T3 respectively, while at stage 2 the catalase activity increased by 17.72 and 17.73 percent in T2 and T3 treatments respectively, compared to irrigation control. Increase in catalase activity during drought conditions were earlier reported in cowpea (Nair et al. 2008). Significant and negative correlation was observed with the seed yield in the present investigation with respect to catalase activity.

Peroxidase activity (units g^{-1} fr.wt min^{-1}): The peroxidase activity recorded at maximum vegetative stage (stage 1) increased significantly with water stress, which was 2.539 units g^{-1} fr.wt min^{-1} in T2 and 2.503 units g^{-1} fr.wt min^{-1} in T3 as against 1.421 units g^{-1} fr.wt min^{-1} in irrigated control

Table 6: Correlation coefficient among biochemical parameters (at different stages) and yield of green gram genotypes.

	CHL 30DAS	CHL 47DAS	PRO 30DAS	PRO 47DAS	CAT 30DAS	CAT 47DAS	POD 30DAS	SEED YIELD
CHL 30DAS	1.000	0.482	-0.949	-0.640	-0.977	-0.951	-0.950	0.933
CHL 47DAS		1.000	-0.715	-0.218	-0.719	-0.701	-0.672	0.904
PRO 30DAS			1.000	0.797	0.954	0.988	0.978	-0.811
PRO 47DAS				1.000	0.751	0.815	0.823	-0.404
CAT 30DAS					1.000	0.968	0.976	-0.871
CAT 47DAS						1.000	0.990	-0.807
POD 30DAS							1.000	-0.805

shown in Table 4. This statement is agreement with the study carried out by Dutta (2007) who reported the increase in peroxidase activity during water stress in mung bean cultivars. High peroxidase activity was observed in WGG 37 (2.296 units g^{-1} fr.wt min^{-1}) genotype, followed by WGG 42 (2.267 units g^{-1} fr.wt min^{-1}) and least activity seen in MGG 347 genotype (2.017 units g^{-1} fr.wt min^{-1}). At stage 1, peroxidase activity was negatively and significantly correlated with seed yield, while at stage 2 (47 DAS), the correlation was non significant with seed yield.

Seed yield (kg ha^{-1}): Water stress decreased the seed yield significantly in both the water stress treatments, i.e. T2 (937.87 kg ha^{-1}) and T3 (954.46 kg ha^{-1}) compared to irrigated control (1150.20 kg ha^{-1}) as shown in Table 5. Data on seed yield revealed that, water stress decreased the seed yield by 18.46 percent in T2 and by 17.01 percent in T3 over irrigated control. Drought stress during vegetative and reproductive stages reduced the yield by 9 and 49% respectively over the control (Moradi et al. 2008). Though genotype WGG 37 maintained numerical superiority over other genotypes, but the differences were statistically non significant at harvest.

CONCLUSION

In the present study, it was observed that the green gram cultivars studied, were significantly affected by drought stress in terms of reduction in total chlorophyll content. Increased leaf proline, catalase and peroxidase activity indicate a protective mechanism as they act as antioxidants in plant tissue. Among the five genotypes under this study, WGG 37 recorded high total chlorophyll content, proline, catalase and peroxidase activity under drought stress conditions and recorded high yield compared to other genotypes. The drought stress response on the high total chlorophyll, proline, catalase and peroxidase activity helped WGG 37 to maintain high yield even under stress conditions.

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