

EFFECT OF WATER STRESS ON MOTH BEAN [*VIGNA ACONITIFOLIA* (JACQ.) MARECHAL] GENOTYPES

B.K. GARG*, U. BURMAN AND S. KATHJU

Division of Soil-Water-Plant-Relationships, Central Arid Zone Research Institute, Jodhpur 342 003

SUMMARY

Six genotypes of moth bean [*Vigna aconitifolia* (Jacq.) Marechal] viz., RMO-40, RMO-257, CZM-99 (early flowering) and CAZRI moth-1, Maru moth, Jawala (late flowering) were subjected to water stress till wilting by withholding of water at the vegetative and flowering stages in a pot culture trial. Water stress decreased the plant water potential (Ψ_{plant}) and relative water content (RWC) in all the genotypes. The Ψ_{plant} ranged from -1.75 to -1.97 MPa at the vegetative and from -1.77 to -2.10 MPa at the flowering stage in the water stressed plants as compared to -0.40 to -1.07 MPa in the control. Though water stress significantly reduced the contents of total chlorophyll, starch and soluble protein and increased those of reducing sugars and free amino acids at both the growth stages, but the effects of water stress were more pronounced at the flowering stage. Rates of net photosynthesis and nitrate reductase activity were also reduced drastically at permanent wilting point (PWP). Early flowering genotypes consistently displayed less metabolic alterations than late genotypes for all the aforesaid parameters at both the growth stages. However, RMO-40 among the early and Jawala among the late flowering genotypes displayed better drought tolerance than other genotypes. The maintenance of higher photosynthetic rates and better metabolic efficiency in early genotypes under water stress at either growth stages led to significantly less reduction in their seed yield and dry matter production as compared to late flowering genotypes.

Key words : Chlorophyll, nitrate reductase, photosynthesis, water potential.

INTRODUCTION

Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] is an important arid legume tolerant to drought and high temperature (Kharb *et al.* 1987, Kumar and Singh 2002). In sandy soils, where moisture retention is poor, the development of stress is rapid and soil moisture deficit adversely influences the metabolism, growth and yield of crops. Vyas *et al.* (1996) reported that increasing water stress at the critical pre-flowering stage progressively decreased the activities of nitrate reductase, glutamine synthetase and glutamate synthase, and soluble protein content in moth bean leaves. Garg *et al.* (2001) also

found that increasing water stress progressively decreased plant water potential, leaf area, net photosynthetic rate, starch and soluble protein contents and nitrate reductase activity in moth bean genotypes. In several arid zone crops genotypic differences in response to water stress exist (Garg *et al.* 1998, Kuhad and Sheoran 1986). But information on the differences in physiology of the early and late flowering genotypes of moth bean is meagre (Garg and Burman 2002). Therefore, an attempt was made to investigate the effects of water stress at vegetative and flowering stages on water relations, net photosynthesis and biochemical changes in selected early and late flowering genotypes of moth bean.

*Corresponding author

MATERIALS AND METHODS

The present investigation was conducted in the net house with six elite moth bean genotypes [*Vigna aconitifolia* (Jacq.) Marechal] including three early flowering (30-31 days) viz., RMO-40, RMO-257 and CZM-99 and three late flowering genotypes (42-45 days) viz., CAZRI moth-1, Maru moth and Jawala. Experimental plants were raised in earthen pots (2 plants per pot) containing 10 kg loamy sand soil (typic camborthids having 7.1% clay, 5.6% silt, 63.1% fine sand and 24.1% coarse sand). The soil contained 0.28% organic carbon, 0.023% total nitrogen, 80 kg ha⁻¹ available N, 12 kg ha⁻¹ available P₂O₅ and 120 kg ha⁻¹ available K₂O. All pots were given a basal dose of 17.9 mg P kg⁻¹ soil as super phosphate and 8.9 mg K kg⁻¹ soil as KCl at the time of sowing. The plants were subjected to water stress by withholding of water (for 9 days) till wilting symptoms appeared at vegetative (25 days after sowing) and flowering (40 days after sowing) stages in different sets of pots. Control plants were watered at regular intervals.

Just prior to termination of water stress at each of the two stages, observations were recorded, in triplicate, on plant water potential using pressure chamber (PMS Instrument Company, USA) and relative water content as per standard procedure (Slatyer and McIlroy 1961).

Two uppermost fully expanded leaves of six plants from each treatment were analysed for the estimation of total chlorophyll (Arnon 1949), starch (Yemm and Willis 1954), reducing sugars (Nelson 1944), soluble protein (Lowry *et al.* 1951), free amino acids (Yemm and Cocking 1955) and nitrate reductase activity (Jaworski 1971) at both the growth stages. At the same time rates of net photosynthesis were measured in two fully expanded upper most leaves of the remaining intact plants in the pots using LICOR-6200 portable photosynthetic system. These measurements were made on six plants under each treatment between 10.00 to 12.00 a.m at both the growth stages. Data on seed yield and dry matter of shoot were recorded at harvest from 10 pots from each treatment. The significance of data was adjudged through the analysis of variance adopting split plot design.

RESULTS AND DISCUSSION

The plant water potential (Ψ_{plant}) and relative water content (RWC) declined due to water stress in all the genotypes at both the growth stages (Table 1). The Ψ_{plant} of stressed plants varied between -1.75 to -1.97 and -1.77 to -2.10 MPa at the vegetative and flowering stages, respectively in different genotypes. However, in well watered control plants Ψ_{plant} remained between -0.40 to -1.07 MPa. Uniform withholding of water for

Table 1. Effect of moisture stress at vegetative and flowering stages on plant water potential and relative water content of moth bean genotypes.

Genotypes	Plant water potential (-MPa)				Relative water content (%)							
	Vegetative		Flowering		Vegetative		Flowering					
	C	D	C	D	C	D	C	D				
RMO-40	0.40	1.75	0.87	1.77	87.1	76.0	88.0	77.1				
RMO-257	0.50	1.80	1.05	1.80	86.9	73.5	83.4	73.4				
CZM-99	0.52	1.90	1.07	2.00	85.1	71.4	79.2	68.8				
CAZRI-moth-1	0.50	1.97	1.05	2.10	88.9	71.8	82.0	66.0				
Maru moth	0.65	1.85	0.90	2.05	84.9	73.1	81.7	65.0				
Jawala	0.60	1.85	0.85	1.95	88.2	75.2	81.2	68.4				
Mean	0.53	1.85	0.97	1.95	86.9	73.5	82.6	69.8				
LSD(0.05)	G	D	GxD	G	D	GxD	G	D	GxD	G	D	GXD
	0.08	0.05	NS	0.10	0.06	0.14	NS	3.1	NS	5.1	2.9	NS

C – Control plants, D – Stressed plants
G – Genotypes, NS – Non-significant

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nine days led to development of more plant water deficit in late flowering genotypes at both the growth stages. Under water stress the percent decline in plant water potential was minimum in early genotype RMO-257 at both the growth stages. Late flowering genotype CAZRI moth-1 at vegetative and Jawala at flowering stage showed maximum reduction. RWC also decreased significantly in water stressed plants at both vegetative and flowering stages. In general, early flowering genotypes maintained a higher RWC than late flowering genotypes under water stress condition. Similar genotypic variations have been reported earlier in clusterbean (Garg *et al.* 1998) and moth bean under moisture deficits (Garg *et al.* 2001).

Decrease in plant water status was associated with significant decline in net photosynthetic rates in all the genotypes at both the growth stages (Table 2). The decline was 52.4 to 70.0 % at the vegetative and 65.8 to 79.0 % at the flowering stage in different genotypes indicating higher sensitivity to water stress at the reproductive stage. Late flowering genotypes experienced more reduction in net photosynthesis than early flowering genotypes when subjected to stress at either growth stage. However, during water stress the photosynthetic rates were comparable in both early and late genotypes. The greater decline in late flowering genotypes was

mainly due to higher rate of photosynthesis in control plants maintained under well-watered condition. Decreased plant water potential and RWC under drought, leading to reduced leaf turgor and stomatal conductance (Sen Gupta *et al.* 1989) might be responsible for the reduction in photosynthesis observed in the present study. Garg *et al.* (2001) have also observed significant differences in rate of net photosynthesis under increasing water stress in some moth bean genotypes. The present study indicates that CZM-99 among the early and CAZRI-moth-1 among the late genotypes showed more reduction due to water stress in photosynthesis than other genotypes. Water stress also reduced the chlorophyll concentration in different genotypes but reduction was more pronounced in late flowering genotypes, particularly at the flowering stage (Table 2). Among all the genotypes the decrease in chlorophyll content was least in RMO-40 in early flowering group and Maru moth in late flowering group at both the growth stages.

Water stress significantly decreased the content of starch in all the genotypes but the early flowering genotypes displayed comparatively less reduction than late genotypes (Table 3). The detrimental effect, however, was less at flowering stage with respect to changes in starch content. The decrease in starch level was associated with a significant increase in the level of reducing sugars.

Table 2. Effect of moisture stress at vegetative and flowering stages on net photosynthetic rate and total chlorophyll content of moth bean genotypes.

Genotypes	Net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)						Total chlorophyll (mg g^{-1} dry wt.)					
	Vegetative			Flowering			Vegetative			Flowering		
	C	D	GxD	C	D	GxD	C	D	GxD	C	D	GxD
RMO-40	9.09	4.12	GxD	7.96	2.73	GxD	6.69	6.02	GxD	5.34	4.38	GxD
RMO-257	7.62	3.63	GxD	8.81	2.90	GxD	6.45	5.42	GxD	4.87	3.79	GxD
CZM-99	9.69	3.33	GxD	8.06	2.66	GxD	7.24	5.58	GxD	5.18	3.88	GxD
CAZRI moth-1	8.96	2.69	GxD	11.33	2.33	GxD	8.23	6.32	GxD	6.43	3.02	GxD
Maru moth	10.23	3.50	GxD	8.86	2.79	GxD	7.79	6.91	GxD	7.79	6.04	GxD
Jawala	11.25	4.83	GxD	10.53	3.81	GxD	9.26	7.59	GxD	7.86	4.31	GxD
Mean	9.47	3.68	GxD	9.26	2.87	GxD	7.61	6.31	GxD	6.25	4.24	GxD
LSD(0.05)	G	D	GxD	G	D	GxD	G	D	GxD	G	D	GxD
	0.97	0.56	1.37	0.46	0.27	0.66	0.44	0.25	0.62	0.37	0.21	0.52

C- Control plants; D- Stressed plants
G- Genotype; NS- Non- significant

Table 3. Effect of moisture stress at vegetative and flowering stages on contents of starch and reducing sugars of moth bean genotypes.

Genotypes	Starch (mg g ⁻¹ dry wt.)				Reducing sugars (mg g ⁻¹ dry wt.)							
	Vegetative		Flowering		Vegetative			Flowering				
	C	D	C	D	C	D	GxD	C	D	GxD		
RMO-40	213.3	110.6	117.8	114.8	12.48	16.19		23.43	19.81			
RMO-257	200.7	77.0	195.0	123.9	15.62	23.62		20.10	14.75			
CZM-99	225.1	79.5	199.4	129.1	16.96	29.36		23.35	12.19			
CAZRI moth-1	217.6	62.2	188.1	99.4	13.17	32.99		15.76	20.55			
Maru moth	170.1	64.8	199.4	113.1	10.16	27.31		14.82	20.20			
Jawala	175.8	82.8	160.5	98.4	11.34	21.99		16.10	20.81			
Mean	200.2	79.5	185.1	113.3	13.29	25.24		18.93	18.06			
LSD(0.05)	G	D	GxD	G	D	GxD	G	D	GxD	G	D	GxD
	9.8	5.7	13.9	11.7	6.8	16.6	1.27	0.74	1.80	1.29	0.75	1.83

C- Control plants, D – Stressed plants, G - Genotypes

Garg *et al.* (2001) also reported similar finding in moth bean genotypes under various intensities of water stress at the pre-flowering stage. At the vegetative stage reducing sugars increased by 29 to 73 % in early flowering genotypes while increase was 94 to 150 % in case of late flowering genotypes, indicating large disturbances in carbohydrate metabolism in late as compared to early flowering genotypes. Water stress at flowering stage, however, reduced the net pool of reducing sugars in early flowering genotypes, indicating adverse effect on synthesis *per se* besides an accumulation due to hydrolysis of starch. Genotypic differences in carbohydrate metabolism under water stress at different growth stages have also been reported in clusterbean (Kuhad and Sheoran 1986, Garg *et al.* 1998).

Water stress at both the growth stages significantly decreased the nitrate reductase activity (Fig.1) and concentration of soluble protein in all the genotypes (Table 4). Under water stress nitrate reductase activity (NRA) was comparable in all the genotypes. However, in late flowering genotypes, which have higher NRA under control condition, the magnitude of decrease was more in stressed plants indicating their higher sensitivity to water stress. Per cent reduction was more at flowering than at the vegetative stage. Among the late flowering genotypes, Jawala reflected least decrease in NRA and soluble protein content. RMO-257 among early flowering genotypes showed less adverse effect except with respect

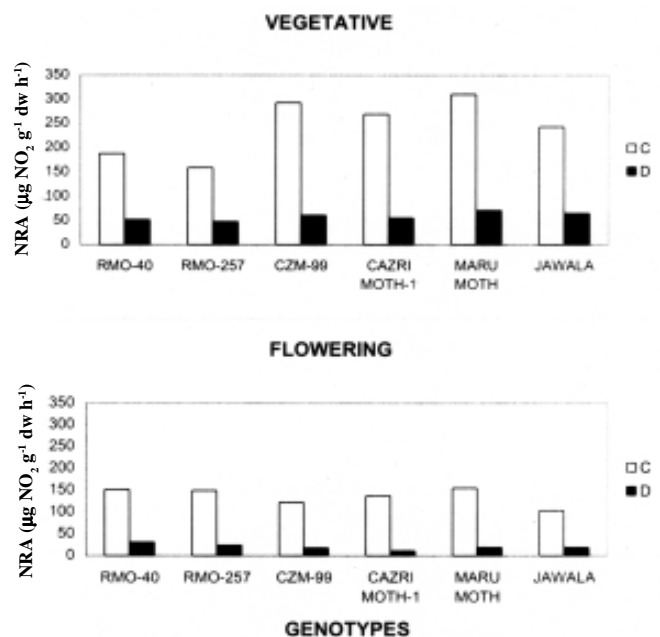


Fig. 1. Nitrate reductase activity (NRA) in leaves of control (C) and stressed (D) plants of different moth bean genotypes at vegetative and flowering stages, LSD ($P = 0.05$) values for genotypes, stress and their interaction are 14, 8 and 20 for the vegetative stage respectively. Corresponding values at flowering stage are 11, 6 and 15.

to NRA at flowering stage, where it registered 3.6 % more reduction than RMO-40. In previous studies with moth bean increasing water stress progressively decreased NRA and after 10 days its activity decreased by 88.2 % (Vyas *et al.* 1996). Significant genotypic differences in

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Table 4. Effect of moisture stress at vegetative and flowering stages on contents of soluble protein and free amino acids in leaves of moth bean genotypes.

Genotypes	Soluble Protein (mg g ⁻¹ dw)						Free amino acids (mg g ⁻¹ dw)					
	Vegetative			Flowering			Vegetative			Flowering		
	C	D	GxD	C	D	GxD	C	D	GxD	C	D	GxD
RMO-40	50.2	32.8		66.8	45.3		10.52	12.54		5.94	6.93	
RMO-257	46.1	30.2		72.7	49.5		10.62	12.98		5.96	6.67	
CZM-99	56.2	29.8		75.2	38.8		10.73	14.71		4.49	5.48	
CAZRI moth-1	60.3	27.7		69.4	23.7		12.97	19.28		6.22	10.02	
Maru moth	48.8	32.1		90.8	48.6		13.04	16.16		6.90	10.92	
Jawala	49.4	34.5		73.6	40.6		13.45	16.51		7.92	10.26	
Mean	51.8	31.2		74.8	41.1		11.89	15.36		6.24	8.36	
LSD (0.05)	G	D	GxD	G	D	GxD	G	D	GxD	G	D	GxD
	12.7	7.0	NS	4.9	2.8	7.0	0.88	0.51	1.24	0.58	0.33	0.82

C- Control plants, D – Stressed plants

G - Genotypes

NRA under water stress in moth bean (Garg *et al.* 2001) and clusterbean (Garg *et al.* 1998) have also been reported.

Decrease in soluble protein was associated with an increase in free amino acids content indicating stress induced increase in proteolytic activity (Naylor 1982, Levitt 1980). As in case of reducing sugars, the net pool of free amino acids was low at flowering stage under moisture deficit condition in both the groups of genotypes. Early variety RMO-40 experienced least changes in free

amino acids among all the genotypes indicating its higher tolerance to drought condition. Garg *et al.* (2001) have also indicated that higher drought tolerance of moth bean genotypes (CZM-32E and RMO-40) was related to less metabolic disturbances under increasing water stress.

Water stress significantly reduced the seed yield of all the genotypes irrespective of the stage at which drought was imposed. However, drought at flowering stage was more detrimental to seed yield than at vegetative stage (Table 5). The magnitude of yield reduction due to

Table 5. Effect of moisture stress at vegetative and flowering stages on seed yield and shoot dry matter of moth bean genotypes.

Genotype	Control	Seed yield (g plant ⁻¹)			Mean	Shoot Dry Matter (g plant ⁻¹)			Mean
		Stress		Mean		Stress		Mean	
		Vegetative	Flowering			Vegetative	Flowering		
RMO-40	2.25	1.70	1.38	1.78	8.15	4.03	4.90	5.69	
RMO-257	2.06	1.47	1.27	1.60	5.82	3.82	4.11	4.58	
CZM-99	2.49	1.47	1.25	1.74	9.32	4.54	5.48	6.45	
CAZRI-Moth-1	3.76	1.79	1.53	2.36	14.62	5.29	7.20	9.04	
Maru Moth	2.94	1.60	1.41	1.98	11.76	6.37	6.78	8.30	
Jawala	3.94	2.14	1.87	2.65	17.37	7.25	9.85	11.49	
Mean	2.91	1.70	1.45	-	11.17	5.22	6.39	-	
LSD (0.05)	G	D	GxD		G	D	GxD		
	0.33	0.23	0.57		0.83	0.59	1.44		

water stress was variable in different genotypes and growth stages. The highest reduction in seed yield was observed in CAZRI moth-1 at both vegetative (52.4%) and flowering stages (59.3%), while RMO-40 recorded the lowest reduction (26.7 and 40.9%, respectively). In general the reduction in seed yield was consistently more in late than early flowering genotypes at both the growth stages. It is well known that several short duration genotypes of legumes show higher and more stable yields than long duration genotypes under low rainfall condition (McBlain and Hume 1980, Hall and Patel 1985, Rose *et al.* 1992, Garg 2002). Although this phenomena has generally been related to drought escape mechanism of early genotypes (Levitt 1980), but the present study indicates that they may also possess higher drought tolerance potential. Data on dry matter production (DMP) indicate that though biomass accumulation was markedly higher in late than early genotypes but water stress induced decrease in dry matter was relatively more in the late flowering genotypes (Table 5). Furthermore, the reduction in DMP was significantly more due to drought at the vegetative than at flowering stage in all the genotypes. Similar observations have been reported earlier in cluster bean genotypes (Garg *et al.* 1998), wherein vegetative stage was more sensitive for DMP, while for seed yield it was flowering stage.

The study revealed that in general late flowering genotypes were more susceptible to drought than early flowering genotypes. However, Maru moth, a late flowering genotype, reflected more drought tolerance than early flowering CZM-99. Additionally, CZM-99 and CAZRI-Moth-1 were more susceptible to drought in their respective groups, at either stages of growth. These genotypes also invariably reflected less favourable plant water status and more metabolic derangements in terms of photosynthetic rate, nitrate reductase activity, chlorophyll loss, starch hydrolysis, proteolysis and associated accumulation of reducing sugars and free amino acids. Furthermore, moth bean genotypes were more sensitive to water stress at flowering as compared to stress at vegetative stage.

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REFERENCES

- Arnon, A.E. (1949). Copper enzymes in isolated chlorophyll. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1-15.
- Garg, B.K. (2002). Physiological aspects of abiotic stress tolerance in arid legumes. Theme paper presented at, "National Symposium on Arid Legumes for Food, Nutrition Security and Promotion of Trade", CCS, HAU, Hisar, 15-16 May, 2002. pp. 144-149.
- Garg, B.K. and Burman, U. (2002). Moth bean physiology. In: D. Kumar and N.B. Singh (Eds.), *Moth Bean in India* pp. 87-102. Scientific Publishers, Jodhpur.
- Garg, B.K., Vyas, S.P., Kathju, S. and Lahiri, A.N. (1998). Influence of water deficit stress at various growth stages on some enzymes of nitrogen metabolism and yield in clusterbean genotypes. *Indian J. Plant Physiol.* **3**: 214-218.
- Garg, B.K., Kathju, S. and Burman, U. (2001). Influence of water stress on water relations, photosynthetic parameters and nitrogen metabolism of moth bean genotypes. *Biol. Plant.* **44**: 289-292.
- Hall, A.E. and Patel, P.N. (1985). Breeding for resistance to drought and heat. In : S.R. Singh, and R.O. Rachie (Eds.), *Cowpea Research, Production and Utilization*. pp. 137-151. Wiley, U. K.
- Jaworski, E. (1971). Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **43**: 1274-1279.
- Kharb, R.P.S., Singh, V.P. and Tomer, Y.S. (1987). Moth bean [*Vigna aconitifolia* (Jacq.) Marechal]- A review. *Forage Res.* **13**: 113-132.
- Kuhad, M.S. and Sheoran, I.S. (1986). Physiological and biochemical changes in clusterbean (*Cyamopsis tetragonoloba* L.) genotypes under water stress. *Indian J. Plant Physiol.* **29**: 46-52.
- Kumar, D. and Singh, N.B. (2002). *Moth bean in India*. Scientific Publishers, Jodhpur.
- Levitt, J. (1980). *Responses of Plants to Environmental Stresses*. Vol. II. Academic Press, New York.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193**: 262-275.
- Indian J. Plant Physiol.*, Vol. 9, No. 1, (N.S.) pp. 29-35 (Jan.-Mar., 2004)

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- McBlain, B.A. and Hume, D.J. (1980). Physiological studies of higher yield in new early maturing soybean cultivars. *Can. J. Plant Sci.* **60**:1315-1326.
- Naylor, A.W. (1972). Water deficits and nitrogen metabolism. In: T.T. Kozlowski (Ed.). *Water Deficits and Plant Growth Vol. III* pp. 241-254. Academic Press, New York.
- Nelson, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. *J. Biol. Chem.* **153**: 375-380.
- Rose, I.A., Mewhirter, K.S. and Spurway, R.A. (1992). Identification of drought tolerance in early maturing indeterminate soybeans (*Glycine max.* L. Merr.) *Aust. J. Agric. Res.* **43**: 645-657.
- Sen Gupta, A., Berkowitz, G.A. and Pier, P.A. (1989). Maintenance of photosynthesis at low leaf water potentials in wheat. *Plant Physiol.* **89**: 1358-1365.
- Slatyer, R.D. and McIlroy, E.C. (1961). *Practical Microclimatology with Special Reference to the Water Factor in Soil Plant Atmospheric Relationships.* UNESCO, Paris .
- Vyas, S.P., Kathju, S., Garg, B.K. and Lahiri, A.N. (1996). Activities of nitrate reductase and ammonia assimilating enzymes of moth bean under water stress. *Sci Cult.* **62**: 213-214.
- Yemm, E.W. and Cocking, E.C. (1955). The determination of amino acids with ninhydrin. *Analyst* **80**: 209-230.
- Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **57**: 508-514.