

BRIEF COMMUNICATION

Influence of water stress on water relations, photosynthetic parameters and nitrogen metabolism of moth bean genotypes

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Abstract

Effects of water stress at pre-flowering stage were studied in three genotypes (RMO-40, Maru moth and CZM-32 E) of moth bean [*Vigna aconitifolia* (Jacq.) Marechal]. Increasing water stress progressively decreased plant water potential, leaf area, net photosynthetic rate, starch and soluble protein contents and nitrate reductase activity while contents of reducing sugars, total soluble sugar, free amino acids and free proline progressively increased. Significant genotypic differences were observed and genotype CZM-32-E displayed a better drought tolerance than other genotypes.

Additional key words: nitrate reductase, proline, relative water content, starch, *Vigna aconitifolia*.

Moth bean is one of the important arid legumes, known for its tolerance to drought and high atmospheric temperature in arid and semi-arid regions (Kharb *et al.* 1987, Kumar *et al.* 1998). In the sandy soils where moisture retention is poor, the development of stress is rapid. Soil moisture deficits adversely influence the metabolism, growth and yield of many plants. In this regard, Vyas *et al.* (1996) reported that increasing water stress at the critical pre-flowering stage progressively and significantly decreased the activities of nitrate reductase, glutamine synthetase and glutamate synthase, and soluble protein content in moth bean plants. Furthermore, in several arid zone crops genotypic differences in response to water stress exist (Garg *et al.* 1981, 1998, Kuhad and Sheoran 1986). Though moth bean crop is known to be adapted to dry land conditions information is meagre on the physiology of this crop (Vyas *et al.* 1996, Kumar *et al.* 1998). Therefore, an attempt was made to investigate the influence of water stress on water relations and biochemical changes in selected genotypes of moth bean.

The present investigation was conducted in the net

house with three elite genotypes of moth bean [*Vigna aconitifolia* (Jacq.) Marechal] Maru moth, RMO-40 and CZM-32E. 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). Soil moisture in the pots was maintained close to field capacity until 40 d after sowing (*i.e.* pre-flowering stage). Plants in the control were maintained at soil moisture close to field capacity throughout their growing period. Irrigation was withheld for 3, 6 and 9 d, respectively, in separate sets of pots and was simultaneously terminated by rewatering in all the treatments, on the 50th day after sowing.

Just prior to the termination of water stress on the 50th day, plant water potential was measured using pressure chamber (*M/s PMS Instrument Company*, Corvallis, USA) and leaf relative water content (RWC) by standard procedure (Slatyer and McIlroy 1961). At the same time rates of net photosynthesis were measured in two fully expanded upper most leaves of intact plants in the field using *LICOR-6200* (Lincoln, USA) portable photosynthetic system which also recorded leaf stomatal

Received 3 May 2000, accepted 27 October 2000.

Abbreviations: g_s - stomatal conductance; d - days; NRA - nitrate reductase activity; P_N - net photosynthetic rate; RWC - relative water content.

Acknowledgements: Thanks are due to the Director CAZRI for providing facilities. Technical help rendered by Mr. M.R. Naval & P.K. Bhardwaj is duly acknowledged. This study was funded by USDA Grant no. IN-AES-680 (FG-IN-720).

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Table 1. Influence of water stress on plant water potential (Ψ_w plant) and relative water content (RWC) of moth bean genotypes (D - drought, G - genotype, NS - non-significant).

| Water stress [d] | Ψ_w [-MPa] RMO-40 | Maru moth | CZM-32E | RWC [%] RMO-40 | Maru moth | CZM-32E |
|------------------|---------------------------|-----------|--------------|-------------------|-----------|--------------|
| 0 | 0.60 | 0.70 | 0.70 | 83.6 | 87.5 | 91.9 |
| 3 | 0.90 | 0.95 | 0.90 | 81.8 | 80.7 | 87.6 |
| 6 | 1.25 | 1.20 | 1.10 | 73.1 | 75.7 | 81.5 |
| 9 | 1.50 | 1.35 | 1.30 | 70.3 | 72.1 | 76.9 |
| LSD at | D | G | D \times G | D | G | D \times G |
| $P = 0.05$ | 0.06 | 0.05 | NS | 2.9 | 2.5 | NS |
| $P = 0.01$ | 0.08 | 0.07 | NS | 3.9 | 3.6 | NS |

Table 2. Influence of water stress on net photosynthetic rate, leaf stomatal conductance and leaf area of moth bean genotypes (D - drought, G - genotype, NS - non-significant).

| Water stress [d] | Net photosynthetic rate [$\mu\text{mol m}^{-2} \text{s}^{-1}$] | | | Leaf stomatal conductance [$\text{mol m}^{-2} \text{s}^{-1}$] | | | Leaf area [$\text{cm}^2 \text{plant}^{-1}$] | | |
|------------------|---|-----------|--------------|--|-----------|--------------|--|-----------|--------------|
| | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E |
| 0 | 8.00 | 8.42 | 11.78 | 0.270 | 0.200 | 0.210 | 423.3 | 432.2 | 412.9 |
| 3 | 4.15 | 3.51 | 9.35 | 0.180 | 0.160 | 0.200 | 296.3 | 298.1 | 323.9 |
| 6 | 2.71 | 2.59 | 5.20 | 0.060 | 0.050 | 0.070 | 208.2 | 207.1 | 251.9 |
| 9 | 1.46 | 1.72 | 4.21 | 0.040 | 0.040 | 0.030 | 126.7 | 116.9 | 163.6 |
| LSD at | D | G | D \times G | D | G | D \times G | D | G | D \times G |
| $P = 0.05$ | 0.42 | 0.36 | 0.72 | 0.016 | 0.014 | 0.027 | 22.5 | 19.5 | NS |
| $P = 0.01$ | 0.56 | 0.49 | 0.98 | 0.021 | 0.018 | 0.037 | 30.3 | NS | NS |

Table 3. Influence of water stress on contents of starch, reducing sugars and total soluble sugars [$\text{mg g}^{-1}(\text{d.m.})$] in leaves of moth bean genotypes (D - drought, G - genotype, NS - non-significant).

| Water stress [d] | Starch | | | Reducing sugars | | | Total soluble sugars | | |
|------------------|--------|-----------|--------------|-----------------|-----------|--------------|----------------------|-----------|--------------|
| | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E |
| 0 | 131.5 | 126.8 | 145.9 | 20.61 | 19.51 | 19.79 | 73.2 | 68.3 | 79.0 |
| 3 | 77.1 | 120.0 | 127.9 | 24.52 | 24.88 | 23.15 | 73.6 | 72.5 | 83.3 |
| 6 | 60.3 | 72.6 | 76.7 | 26.69 | 26.60 | 30.17 | 84.7 | 90.0 | 94.5 |
| 9 | 55.0 | 57.4 | 70.9 | 29.16 | 28.01 | 34.34 | 85.3 | 93.1 | 95.8 |
| LSD at | D | G | D \times G | D | G | D \times G | D | G | D \times G |
| $P = 0.05$ | 3.8 | 3.3 | 6.6 | 0.92 | 0.80 | 1.60 | 3.3 | 2.9 | NS |
| $P = 0.01$ | 5.1 | 4.4 | 8.8 | 1.24 | 1.08 | 2.15 | 4.5 | 3.9 | NS |

Table 4. Influence of water stress on nitrate reductase activity [NRA [$\mu\text{g}(\text{NO}_2) \text{g}^{-1}(\text{d.m.}) \text{s}^{-1}$] and contents of soluble protein, free amino acids and free proline [$\text{mg g}^{-1}(\text{d.m.})$] in leaves of moth bean genotypes (D - drought; G - genotype).

| Water stress [d] | NRA | | | Soluble protein | | | Free amino acids | | | Free proline | | |
|------------------|--------|-----------|--------------|-----------------|-----------|--------------|------------------|-----------|--------------|--------------|-----------|--------------|
| | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E | RMO-40 | Maru moth | CZM-32E |
| 0 | 0.076 | 0.106 | 0.083 | 30.75 | 41.90 | 33.70 | 8.12 | 11.90 | 9.50 | 0.77 | 0.38 | 0.84 |
| 3 | 0.070 | 0.096 | 0.066 | 28.50 | 42.70 | 34.70 | 9.00 | 13.00 | 10.10 | 1.16 | 0.71 | 1.02 |
| 6 | 0.055 | 0.078 | 0.038 | 26.65 | 33.20 | 27.71 | 14.00 | 13.50 | 11.30 | 1.80 | 1.71 | 1.38 |
| 9 | 0.047 | 0.021 | 0.030 | 26.50 | 31.20 | 30.80 | 13.10 | 13.85 | 14.20 | 1.93 | 1.82 | 1.48 |
| LSD at | D | G | D \times G | D | G | D \times G | D | G | D \times G | D | G | D \times G |
| $P = 0.05$ | 0.002 | 0.002 | 0.004 | 1.35 | 1.17 | 2.34 | 0.60 | 0.52 | 1.03 | 0.14 | 0.12 | 0.23 |
| $P = 0.01$ | 0.003 | 0.003 | 0.006 | 3.90 | 3.60 | 3.15 | 0.80 | 0.70 | 1.39 | 0.18 | 0.16 | 0.32 |

conductance. Leaf area was also measured in all the treatments using *LICOR-3000* leaf area meter. All the measurements were made in 4 replicates under each treatment. Two upper most fully expanded leaves from eight plants from each treatment were taken for the estimation of reducing sugars (Nelson 1944), soluble protein (Lowry *et al.* 1951), free amino acids (Yemm and Cocking 1955), free proline (Bates *et al.* 1973), starch, and total soluble sugar (Yemm and Willis 1954) contents as well as nitrate reductase activity (Jaworski 1971). The significance of the data was adjudged through analyses of variance adopting factorial design.

Increase in the duration of water deprivation progressively decreased both plant water potential and RWC in all the genotypes (Table 1). Genotype CZM-32E maintained relatively higher plant water potential and RWC as compared with other genotypes. A rapid decline in plant water potential and leaf water content with increasing stress has also been reported earlier by Vyas *et al.* (1996) in moth bean as well as in other crops (Vyas *et al.* 1985, Kathju *et al.* 1990).

Decrease in plant water status resulted in significant and progressive decline in net photosynthetic rate (P_N). This was associated with a simultaneous decrease in leaf area and leaf stomatal conductance (g_s) (Table 2). There was about 81.7 and 79.6 % decrease in P_N after 9 d of water deprivation in genotypes RMO-40 and Maru moth, respectively. In contrast genotype CZM-32E showed only 64.3 % reduction in P_N . Similarly, reduction in leaf area was significantly less in this genotype. P_N is known to be adversely affected by water deficit in a number of crops. Genotypic differences in response to P_N to water stress have also been reported by Kuhad and Sheoran (1986). Decreased photosynthesis seems to be mainly due to decreased g_s and decreased leaf area. On an average leaf area decreased by 27.6, 47.4 and 67.9 % after 3, 6 and 9 d of water stress, respectively.

Increased water stress progressively decreased the

contents of starch in all the genotypes but the effects were more pronounced in cv. RMO-40. The decrease in starch content was generally associated with a progressive increase in the contents of reducing sugars and total soluble sugars (Table 3). However, differences between 6 and 9 d of stress were not significant. Genotype CZM-32E displayed higher contents of starch and total soluble sugars. Genotypic differences have also been reported in clusterbean (Kuhad and Sheoran 1986, Garg *et al.* 1998).

Increasing water stress significantly reduced the nitrate reductase activity (NRA) and soluble protein content while the contents of free amino-acids and free proline progressively increased (Table 4). On an average NRA decreased by 13.0, 35.4 and 63.5 % after 3, 6 and 9 d of water deprivation, respectively. However, soluble protein content decreased only at 6 and 9 d of water stress. Free amino acids increased by 8.7 to 39.4 % while free proline increased from 45.5 to 163.6 %.

In previous experiments with moth bean, increasing water stress progressively decreased NRA and after 10 d stress its activity decreased by 88.2 % (Vyas *et al.* 1996). An increase in the concentration of proline is reported as an index of drought resistance (Singh *et al.* 1972). However, reports are available both in favour (Aspinall and Paleg 1981) and against (Stewart and Hanson 1980) this hypothesis. Proline accumulation may also vary widely depending on the species. In case of moth bean its accumulation has been relatively small as compared to other dry land crops (Garg *et al.* 1981, 1998) but significant genotypic differences may exist as found in the present study. The overall increase in free amino acids with associated decrease in soluble protein content indicate that stress had interfered with protein synthesis or induced proteolysis (Naylor 1972) or both. The lesser magnitude of changes in cv. CZM-32E indicate higher drought tolerance of this genotype as compared with genotypes RMO-40 and Maru moth.

References

- Aspinall, D., Paleg, L.G.: Proline accumulation: Physiological aspects. - In: Paleg, L.G., Aspinall, D. (ed.): *The Physiology and Biochemistry of Drought Resistance in Plants*. Pp. 205-241. Academic Press, New York 1981.
- Bates, L.S., Waldren, R.P., Teare, L.D.: Rapid determination of proline for water stress studies. - *Plant Soil* **39**: 205-208, 1973.
- Garg, B.K., Kathju, S., Lahiri, A.N., Vyas, S.P.: Drought resistance in pearl millet. - *Biol. Plant.* **23**: 182-185, 1981.
- Garg, B.K., Vyas, S.P., Kathju, S., Lahiri, A.N.: 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, 1998.
- Kathju, S., Vyas, S.P., Garg, B.K., Lahiri, A.N.: Fertility induced improvements in performance and metabolism of wheat under different intensities of water stress. - In: Sinha, S.K., Sane, P.V., Bhargava, S.C., Aggarwal, P.K. (ed.): *International Congress of Plant Physiology*. Pp. 854-858. Society for Plant Physiology and Biochemistry and Water Technology Centre, IARI, New Delhi 1990.
- Kharb, R.P.S., Singh, V.P., Tomer, Y.S.: Moth bean (*Vigna aconitifolia* (Jacq.) Marechal). A review. - *Forage Res.* **13**: 113-132, 1987.
- Kuhad, M.S., Sheoran, L.S.: Physiological and biochemical changes in clusterbean (*Cyamopsis tetragonoloba* L.) genotypes under water stress. - *Indian J. Plant Physiol.* **29**: 46-52, 1986.
- Kumar, D., Henry, A., Manga, V.K.: Improvement of arable crops. - In: Faroda, A.S., Singh, M. (ed): *Fifty Years of Arid Zone Research in India*. Pp. 253-272. CAZRI, Jodhpur 1998.

- Jaworski, E.: Nitrate reductase assay in intact plant tissue. - Biochem. biophys. Res. Commun. **43**: 1274-1279, 1971.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with Folin-phenol reagent. - J. biol. Chem. **193**: 245-275, 1951.
- Naylor, A.W.: Water deficits and nitrogen metabolism. - In: Kozlowski, T.T. (ed.): Water Deficits and Plant Growth. Vol. III. Pp. 241-254. Academic Press, New York 1972.
- Nelson, N.: A photometric adaptation of the Somogyi method for determination of glucose - J. biol. Chem. **153**: 375-380, 1944.
- Singh, T.N., Paleg, L.G., Aspinall, D.: Proline accumulation and varietal adaptation to drought in barley, a potential metabolic measure of drought resistance. - Nature new Biol. **236**: 188-190, 1972.
- Slatyer, R.O., McIlroy, J.C.: Practical Microclimatology with Special Reference to the Water Factor in Soil Plant Atmosphere Relationships. - UNESCO, Paris 1961.
- Stewart, C.R., Hanson, A.D.: Proline accumulation as a metabolic response to water stress. - In: Turner, N.C., Kramer, P.J. (ed.): Adaptation of Plants to Water and High Temperature Stress. Pp. 173-189. Wiley Interscience, New York 1980.
- Vyas, S.P., Kathju, S., Garg, B.K., Lahiri, A.N.: Performance and metabolic alterations in *Sesamum indicum*. L. under different intensities of water stress. - Ann. Bot. **56**: 323-331, 1985.
- Vyas, S.P., Kathju, S., Garg, B.K., Lahiri, A.N.: Activities of nitrate reductase and ammonia assimilation enzymes of moth bean under water stress. - Sci. Cult. **62**: 213-214, 1996.
- Yemm, E.W., Cocking, E.C.: The determination of amino acids with ninhydrin. - Analyst **80**: 209-230, 1955.
- Yemm, E.W., Willis, A.J.: The estimation of carbohydrates in pearl millet extracts by anthrone. - Biochem. J. **57**: 508-514, 1954.