

SHORT COMMUNICATION

AZOSPIRILLUM APPLICATION ON RECOVERY OF DROUGHT STRESSED RAGI (*ELEUSINE CORACANA* GAERTN)

L. LOUIS JESUDAS, R. KARUNAKARAN AND K. SANTHAGURU

Centre for Research and P.G. Department of Botany,
Thiagarajar College, Madurai—625 009.

Received on 31 March, 1992

Influence of *Azospirillum* inoculation on recovery of drought stressed *Eleusine coracana* was studied. Drought stress caused a sharp decline in total nitrogen content, soluble proteins, proline content and *in vivo* nitrate reductase activity in leaves. On rewatering, the recovery from stress was much faster in *Azospirillum* inoculated plants as compared to the uninoculated ones. It appears that *Azospirillum* inoculation alleviates the stress effect in ragi.

Drought stress caused an increase in nitrate and total nitrogen content (Mattas and Pauli, 1965) and total soluble proteins (Stutte and Todd, 1968). Accumulation of proline has also been found in water stressed plants (Savitskaya, 1967). Nitrate

Table I. Effect of drought stress on total nitrogen, soluble proteins, proline

Parameters	Uninoculated control plants					Uninoculated stressed	
	Plant age (days)					Plant age	
	38	45	52	55	60	38	45
Total nitrogen (mg/g dry leaf)	38.25 ±0.27	29.00 ±0.26	27.00 ±0.25	28.00 ±0.3	29.00 ±0.2	22.80 ±0.28	27.50 ±0.3
Soluble proteins (mg/g fr. leaf)	3.25 ±0.25	3.50 ±0.27	7.50 ±0.3	6.07 ±0.25	10.50 ±0.5	4.00 ±0.3	2.50 ±0.2
Proline (mg/g fr. leaf)	1.50 ±0.25	2.50 ±0.4	10.00 ±0.75	2.50 ±0.4	2.50 ±0.5	1.50 ±0.25	6.25 ±0.3
<i>In vivo</i> nitrate reductase activity (μ moles NO_2^- formed/h/g fr. leaf)	0.88 ±0.03	0.63 ±0.02	0.06 ±0.01	0.53 ±0.05	1.04 ±0.02	1.03 ±0.05	0.81 ±0.03

± Standard deviation.

reductase is considered as the rate limiting enzyme in nitrogen assimilation. Water stress is known to decrease the activity of nitrate reductase in plants (Bardzik *et al.*, 1971). Application of nitrogen has been reported to reduce the adverse effect of drought on dry matter and grain yield of pearl millet (Lahiri, 1980). *Azospirillum*, a Gram negative bacterium associates with C₃ and C₄ grasses and fixes atmospheric nitrogen. Inoculation with *Azospirillum* increased the yield and total nitrogen content in cereal crops (Kapulnik *et al.*, 1981). The present study reports the influence of *Azospirillum* inoculation on recovery of drought stressed *Eleusine coracana*.

Eleusine coracana (Co-11) seeds were treated with *Azospirillum lipoferum* (commercial inoculum obtained from Agricultural College and Research Institute, Madurai) and sown in earthen pots (10" diameter and 12" height) filled with red soil. Five days after sowing, the seedlings were thinned to 10 plant per pot. Tap water was used to water the plants. All the experimental plants were maintained in a green house under conditions of broad day light. Drought stress was given by withholding water supply for a specific period of plant growth (*viz.* 45th day to 52nd day).

Dry weight was determined by drying the plant materials in an oven at 90°C till the weight became constant. Total nitrogen content (Umbreit *et al.*, 1972), proline content (Bates *et al.*, 1973) and soluble proteins (Lowry *et al.*, 1951) were measured colorimetrically. For *in vivo* nitrate reductase assay 1 mm leaf sections

and *in vivo* nitrate reductase activity in leaves of *E. coracana*

drought plants			<i>Azospirillum</i> inoculated drought stressed plants				
(days)			Plant age (days)				
52	55	60	38	45	52	55	60
25.00 ±0.25	27.00 ±0.28	29.00 ±0.3	27.00 ±0.27	28.50 ±0.29	26.00 ±0.31	30.00 ±0.4	32.00 ±0.35
12.00 ±0.6	10.00 ±0.7	12.50 ±0.75	2.50 ±0.2	12.00 ±0.5	4.00 ±0.3	11.00 ±0.5	13.50 ±0.65
65.00 ±0.5	5.00 ±0.28	5.00 ±0.28	10.00 ±0.7	1.50 ±0.3	40.00 ±0.75	1.50 ±0.3	1.50 ±0.3
0.13 ±0.05	0.75 ±0.05	0.98 ±0.05	1.15 ±0.06	0.70 ±0.04	0.10 ±0.03	1.13 ±0.05	1.43 ±0.06

were introduced into test tubes containing 5 ml of assay mixture. The assay mixture was prepared by mixing 0.5 ml of n-propanol, 1.0 ml of 5% Triton X-100, 40.0 ml of buffered KNO_3 solution (0.68 g of KH_2PO_4 and 0.505 g of KNO_3 were dissolved in 34 ml of water and the pH was adjusted 7.5 with 2 N KOH; it was made upto 40.0 ml with distilled water) and 8.5 ml of distilled water. The assay was carried

Table II. Summary of "Two way ANOVA"

Source	df	SS	MS	F-ratio	Remarks
(a) <i>Total nitrogen</i>					
Treatment	14	504.10	36.01	24.00	**
(i) Age	4	85.91	21.48	14.32	**
(ii) Stress	2	116.22	58.11	38.78	**
(iii) Age \times Stress	8	301.96	37.75	25.19	**
(iv) Error	30	44.96	1.50		
Total	44	549.06			
(b) <i>Soluble proteins</i>					
Treatment	14	715.19	51.08	4.92	*
(i) Age	4	405.53	101.38	9.76	*
(ii) Stress	2	51.29	25.64	2.47	@
(iii) Age \times Stress	8	258.37	32.30	3.11	@
(iv) Error	30	311.53	10.38		
Total	44	1036.72			
(c) <i>Proline</i>					
Treatment	14	13608.85	972.06	920.98	**
(i) Age	4	8834.17	3434.46	3166.18	**
(ii) Stress	2	1225.49	612.74	564.88	**
(iii) Age \times Stress	8	3549.19	443.65	408.99	**
(iv) Error	30	32.54	1.08		
Total	44	13641.39			
(d) <i>in vivo nitrate reductase</i>					
Treatment	14	6.88	0.491	8.72	*
(i) Age	4	5.97	1.49	26.50	**
(ii) Stress	2	0.57	0.29	5.07	*
(iii) Age \times Stress	8	0.34	0.04	0.76	@
(iv) Error	30	1.69	0.056		
Total	44	8.58			

** indicates highly significant.

* indicates significant.

@ indicates not significant.

out at 30°C for 1 or 2 h in dark and the nitrite released into the medium was estimated by naphthylethylene diamine/sulphanilamide couple.

The results presented in table I showed changes in total nitrogen content, soluble proteins, proline content and *in vivo* nitrate reductase activity in leaves of ragi upon drought stress.

The results of "Two way ANOVA" tests (Table-II) showed that the age of the plant and the stress had significant influence over the various parameters such as total nitrogen content, soluble proteins, proline content and *in vivo* nitrate reductase activity. The interaction between the age of the plant and the stress was found to be significant for total nitrogen and proline, but not for soluble proteins and *in vivo* nitrate reductase activity.

The decrease in total nitrogen content of ragi during stress is similar to the finding obtained for *Sorghum* (Rego *et al.*, 1986). However, *Azospirillum* inoculation induced quick recovery as compared to the uninoculated plants. The accumulation of proline during drought stress is in accordance with results reported in barley (Savitskaya (1967). As has been shown (Barnett and Naylor, 1966), proline accumulation under stress could act as storage compound for carbon and nitrogen. This view is substantiated by the occurrence of high quantities of soluble proteins during the period of recovery from water stress.

The decrease in nitrate reductase activity during drought stress and subsequent recovery after rewatering is in accordance with the results of Bardzik *et al.* (1971). This increased activity during recovery period appears to be the result of greater mobilization of storage compound as energy source.

In conclusion, it may be suggested that *Azospirillum* inoculation can be exploited to alleviate the stress effects in millets like ragi.

Authors are grateful to Prof. P. Vallinayagam, Head of the Department of Botany, Thiagarajar College, Madurai for providing facilities.

REFERENCES

- Bardzik, J.M., Marsh, H.V. Jr. and Haris, J.R. (1971). Effects of water stress on the activities of three enzymes in maize seedlings. *Plant Physiol.*, 47 : 828-831.
- Barnett, N.M. and Naylor, A.W. (1966). Amino acid and protein metabolism in Bermuda grass during water stress. *Plant Physiol.*, 41 : 1222-1230.
- Bates, L.S., Waldran, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil*, 39 : 205-207.
- Kapulnik, Y., Sarig, S., Nur, I., Okon, Y., Kigel, J. and Hanis, Y. (1981). Yield increase in summer cereal crops in Israeli fields inoculated with *Azospirillum*. *Exp. Agric.*, 17 : 170-187.

- Lahiri, A.N. (1980). Interaction of water stress and mineral nutrition on growth and yield. In : *Adaptation of plants to water and higher temperature stress*. ed. by N.C. Turner and P.J. Kramer, New York : John Wiley and Sons, pp. 341-352.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol Chem.*, 193 : 265-275.
- Mattas, R.E. and Pauli, A.W. (1965). Nitrate reduction and N fractions in young corn plants during heat and moisture stress. *Crop Sci.*, 5 : 181-184.
- Rego, T.J., Grundon, N.J., Asher, C.J. and Edwards, D.G. (1986). Effects of water stress on nitrogen nutrition of grain *Sorghum*. *Aust. J. Plant Physiol.*, 13 : 499-508.
- Savitskaya, N.N. (1967). Problem of accumulation of free proline in barley plants under conditions of soil water deficiency. *Fiziol. Rast.* 14 : 737-739.
- Stutte, C.A. and Todd, G.W. (1968). Ribonucleotide compositional changes in wheat leaves caused by water stress. *Crop Sci.* 8 : 319-321.
- Umbreit, W.W., Burris, R.H. and Stauffer, J.F. (1972). Method for nitrogen. In : *Manometric and Bio-chemical techniques* (5th ed.). Burgess Publishing Company, Minnesota, pp. 259-260.