Annals of Arid Zone 28 (1&2) : 143-147, 1989

GROWTH OF FRUIT PLANTS AS INFLUENCED BY NITROGEN FIXING BACTERIA

A.V. RAO AND H.C. DASS*

Central Arid Zone Research Institute Jodhpur-342 001, India

ABSTRACT

Inoculation of jujube (*Ziziphus mauritiana*) and pomegranate (*Punica granatum*) plants with nitrogen fixing bacteria enhanced the height and dry weight of plants as compared to the uninoculated control plants. There was a strain-variety interaction among the strains. A marked increase in the root biomass of these fruit plants upon inoculation with nitrogen fixing bacteria was also observed. Though there was no increase in the per cent N in the plants wiih inoculation, the total N-uptake was more with the inoculated plants. The rhizosphere of inoculated plants carried higher population of *Azospirillum brasilense* as compared to the uninoculated control plants.

INTRODUCTION

Azospirillum, an associative symbiotic nitrogen fixing bacterium, is known to occur in the rhizosphere of many monocots and dicots but the studies on interaction between this bacterium and the host plants are restricted to cereals and millets (Von Bulow and Dobereiner, 1975; Venkateswarlu and Rao, 1983). Very little information is available on the association of N_2-fixing bacteria with horticultural plants. Nair and Subba Rao (1977) observed the stimulation of N_2- fixing bacteria in the rhizosphere of coconut plants in mixed farming. Subba Rao (1983) reported the association of nitrogen fixing bacteria such as *Azotobacter*, *Azospirillum* and *Beijerinckia* within the rhizosphere of many plantation and orchard plants including jujube (*Ziziphus mauritiana*) and pomegranate (*Punica granatum*). The present investigation was undertaken to study the effects of nitrogen fixing bacteria on the growth of jujube and pomegranate plants in pot culture.

MATERIAL AND METHODS

Pure cultures of the strains of S_{14}, S_{51} and S_{54} of *Azospirillum brasilense* and *Azotobactor chraococcum* were maintained in the Microbiology section of the Central Arid Zone Research Institute, Jodhpur on the slants of N-free malate medium supplemented with 250 ppm ammonium sulphate and Jenson's N-free medium respectively. Pure cell suspension of 4-day old culture was used as inoculum. The cells were centrifuged, washed twice and suspended in 0.15 M phosphate buffer of pH 7.0.

* Director, National Research Centre on Citrus, Nagpur - 440016

Six-month old budded plants of jujube (*Ziziphus mauritiana*) cv 'Seb' and 'Gola' and rooted cuttings of pomegranate (*Punica granatum*) cv 'Jalore Seedless' were each transplanted in 12-kg earthern pots (containing a mixture of sand, clay and FYM in equal proportions of each) in the month of Feb 1985. The soil was sandy loam, having 81% sand, 7% silt and 12% clay and contained 0.162% organic carbon, 0.021% total nitrogen 17 kg ha^{-1} of available phosphorus and 190 kg ha^{-1} of available potassium. After establishment in the pots, the plants were inoculated with 100 ml/pot of pure cell suspension ($5x10^1$ CFU/ml). The control plants were supplied with 100 ml of autoclaved cell suspension. For each treatment, 10 replicates were maintained. At the end of 6 months, the rhizosphere soil samples were collected and analysed for *Azospirillum* population by MPN method employing N-free semi-solid malate medium (Dobereiner and Day, 1976). The plants were uprooted and the observations on the height and dry weights of shoot and root were recorded. Nitrogen content in plant samples was determined after Jackson (1967). The data were statistically analysed for analysis of variance.

RESULTS AND DISCUSSION

Following inoculation with nitrogen fixing bacteria, the height of the jujube plants increased significantly (Table 1), more in cv 'Seb' (7.7 to 20.3%) than in cv 'Gola' (3.7 to 13.5%). Increase in the heights of pomegranate plants was not significant. But Kapulnik et al,, (1981) with wheat, sorgham and *Panicum*, and Venkateswarlu and Rao (1983) with pearl millet reported the enhancement in the growth of these plants upon inoculation with the strains of *Azospirillum brasilense*.

Table 1. Effect of nitrogen fixing bacteria on the height (cm) of fruit plants

Treatments	Jujube		Pomegranate
	cv 'Seb'	cv 'Gola'	
Control	73.9	86.5	66.6
Azospirillum brasilense			
S_{14}	83.9 (20.3)	98.2 (13.5)	70.3 (5.5)
S_{51}	88.1 (19.2)	94.3 (9.3)	72.7 (9.1)
S_{54}	87.3 (18.1)	89.7 (3.7)	68.7 (3.1)
A. chroacoccum	79.6 (7.7)	90.5 (4.5)	67.6 (1.5)
LSD (P = 0.05	47.3	52 4	ns

Figures in parentheses are per cent increases over control.

The strain S_{14} was superior for the varieties of jujube and S_{51} was superior for pomegranate. The dry weights of shoots and roots of inoculated plants were also higher than of uninoculated plants (Table 2). The increase in the growth of all the seedlings with *Azotobacter chroococcum* was marginal.

Table 2. Influence of nitrogen fixing bacteria on shoot and root growth dry matter (g/plant) and N-uptake (mg/plant) in different cultivars of jujube and pomegranate

Treatment	Jajube cv 'Seb'			Jajube cv 'Gola'			Pomegranate cv Jalore seedless		
	Shoot	Root	N-uptake	Shoot	Root	N-uptake	Shoot	Root	N-uptake
Control	68 1	27.4	926	51.9	18 8	858	34 7	22.5	496
S_{14}	84.5	32.2	1149	62 6	25.6	1036	39.0	23 5	558
S_{51}	74.6	29.9	1014	49.4	18.4	817	34.2	22.6	490
S_{54}	76.7	29·3	1043	53.7	22.8	889	40.7	26.5	582
Azotoboacter	72.3	28.1	983	52.7	18.5	872	39.2	23.7	561
LSD (P=0 05)	9.6	4.3	—	5.9	NS	—	4.1	NS	—

The enhancement in root growth following inoculation with *Azospirillum* is believed to be due to the production of growth regulators, besides nitrogen fixation (Tien et al., 1979; Venkateswarlu and Rao, 1983). The marked increase in root biomass particularly suggested the role of growth hormones. All the strains of *A. brasilense* were found to synthesize indoles as well as gibberellins (Venkateswarlu and Rao, 1983). while Tien et al. (1979) had shown the production of cytokinins by *A. brasilense*. The stimulation varied with the strains and there was a strain-variety interaction among orchard plants also. In case of ber S_{14} was found to be better while with pomegranate S_{54} proved to be more efficient as compared to be other strains.

The better performance of *Azospirillum* strains over *Azotobacter* might be due to the fact that *Azospirillum*, besides multiplication in the rhizosphere, infect the root hairs and establish within the cortex (Okon et al., 1976; Magalhaes et al., 1979; Rao and Venkateswarlu, 1985).

The date on *Azospirillum* population in the rhizosphere soil samples indicated that there was a better population build-up in the rhizosphere of inoculated plants as compared to the uninoculated plants (Table 3). The maximum incease in the population was observed in cv 'Seb' variety of jujube followed by 'Gola' and pomegranate.

Table 3. Average population (± SD) of *Azespirillum brasilense* in the soils

Soil	Population (X 10^4/g soil)	
	Control	Inoculated
Non-rhizosphere soil	1.56 ± 026	—
Rhizosphere soil:		
Jujube cv 'Seb'	2.94 ± 0.14	12.00 ± 1.22
Jujube cv 'Gola'	2 08 ± 0.18	7.96 ± 0.85
Pomegranate	1.96 ± 0 18	5.15 ± 0.56

It is concluded that the enhanced growth of seedlings of ber and pomegranate upon inoculation might be due to the cumulative effect of nitrogen fixtion and production of growth regulators. The extent to which each of these various processes contributes to increased growth of inoculated fruit plants remains to be assessed.

ACKNOWLEDGEMENT

The authors thank Mr. H.S. Panwar and Mr. S.C. Vyas for their technical help during this study.

REFERENCES

Dobereiner, J. and Day, J.M. 1976. Associative symbiosis in tropical grasses. Characterization of micro-organisms and nitrogen fixing sites. pp. 538. *In* Newton, W.E. and Nymans, C.J. (ed.). Proceedings of First International Symposium on N_2 fixation. Washington State University Press, Pullman.

Jackson, M.L. 1967. Soil chemical analysis. Prentice Hall of India Private Limited, New Delhi.

Magalhaes, L.M.S., Patriquin, D.G. and Dobereiner, J. 1979. Infection of field grown maize *Azospirillum* spp. Review of Brazilian Biology. 39 : 587-596.

Nair, S.K. and Subba Rao, N.S. 1977. Distribution and activity of phosphate solubilising micro-organisms in the rhizosphere of coconut and cocao under mixed cropping. Journal of Plantation Crops. 5 : 67-73.

Okon, Y., Albrecht, S.L. and Burris, R.H. 1977. Methods for growing *Spirillum lipoferum* for counting it in pure culture and in association with plants. Applied and Environmental Microbiology, 33 : 85-88.

Rao, A.V. and Venkateswarlu, S. 1985. Most probable number of *Azospirillum* associated with the roots of inoculated pearl miilet. Plant and Soil. 88 : 153-158.

Subba Rao, N.S. 1983. Nitrogen fixing bacteria associated with plantation and orchard plants. Canadian Journal of Microbiology 29 : 863-866.

Tien, J.M., Gaskins, M.H. and Hubbel, D.H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of pearl millet [*Pennisetum americanum* (L.)]. Applied and Environmental Microbiology. 37 : 1016-1024.

Venkateswarlu, B. and Rao, A.V. 1983. Response of pearl millet to inoculation with different strains of *Azospirillum brasilense*. Plant and Soil. 74 : 379-386.

Von Bulow, J.F.M. and Dobereiner, J. 1975. Potential for nitrogen fixation in genotypes in Brazil. Proceedings of the National Academy of Sciences, U.S.A. 72 : 2389-2393.