Table 1 *L-Asparaginase production by mutant strains of E. carotovora*

Mutant strain	Phenotypic characters	PCV* (%)	Enzyme activity (IU/ml)
389	MSG^+ Ala^+ Sm^R	3	0.2
189	Asn^+ Gln^+ Ala^+ Sm^S	4	0.08
213	MSG^+ Tr^+ Met^+ Sm^R	3	0.38
56	Asn^+ Tr^+ Met^+ Sm^S	5	0.0
106	Asn^+ Met^+ Tr^+ Sm^S	3	0.0
122	MSG^+ Gln^+ Sm^R	5	1.5

*Packed cell volume.

Table 2 *L-Asparaginase production by recombinants of E. carotovera obtained by genetic recombination*

Recombinant	Mutant strains in cross	PCV (%)	Enzyme activity (IU/ml)
R1	389 × 213	3.0	1.58
R2	189 × 56	3.0	1.43
R3	106 × 213	2.5	3.13
R4	106 × 122	2.5	3.13
R5	122 × 213	2.5	5.93
R6	389 × 122	2.0	6.10
R7	389 × 106	3.0	2.10
R8	56 × 109	3.0	1.30

strains are seen as poor producers. These strains, when hybridized, yielded, among others, the recombinants R5 and R6, which were at least four times more active than the original strains (table 2). It was further observed that the mutant strains requiring monosodium glutamate specifically and showing streptomycin resistance produced these high yielding recombinants. Hence Sm^R was considered a suitable selective marker. The prototrophic recombinants were also found to be induced when L-asparagine, at 0.1–0.3% was added to the culture medium; there was a 3.5-fold increase in enzyme activity. The parental strains were never capable of showing such induction.

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EFFECT OF *RHIZOBIUM* IN ASSOCIATION WITH GRANULAR INSECTICIDES ON NODULATION AND YIELD IN SOYBEAN

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INSECTICIDES containing mercury, copper or zinc, when used for seed protection, were found to be extremely toxic to *Rhizobium* sp.[1–4] Oblisami *et al.*[5], found in *in vitro* tests that endrin, carbofuran and disulfoton inhibited the growth of *Rhizobium* from red gram (*Cajanus cajan*). Seed inoculation with *Rhizobium* is advocated for achieving higher grain production in soybean. We therefore studied the effect of pesticides on nodulation *vis-a-vis* yield of soybean to evolve an effective combination of pesticide and rhizobial culture.

The experiment was conducted in randomized block design at the Research Farm of IARI, New Delhi. Soybean seeds, var. Harosoy-63, were coated with effective culture of *Rhizobium japonicum* (IARI strain, obtained from the Division of Microbiology, IARI) using 10% sucrose solution before sowing. The inoculated seeds were air-dried. Granular insecticides phorate 10, quinalphos 5, mephosfolan 5 and carbofuran 3 were drilled in soil furrows before sowing. All the insecticides except phorate were applied at 3 g/m row and phorate was applied at 1.5 g/m row. There were six treatments, each replicated four times. The size of each plot was 3.0 × 2.25 m. Row-to-row and plant-to-plant distances were 37.5 and 5 cm respectively.

The crop was given two light irrigations before uprooting, one at the beginning of flowering and the other at early pod formation stage, in addition to normal rainfall received during the crop season. Ten plants were uprooted from each plot each time.

Table 1 *Effect of Rhizobium inoculation on nodulation, height of plants and grain yield of soybean with and without insecticides*

Treatment	Mean number of nodules*		Mean weight of nodules (g)*				Mean height of plants* (cm)		Grain yield	
			Fresh		Dry				Per plot (g) mean	Per ha. (Q)
	30 DAS	48 DAS	30 DAS	48 DAS	30 DAS	48 DAS	30 DAS	48 DAS		
Control	0	0.25	0	0	0	0	227.50	288.25	646.06	9.57
Rhizobium alone	186.50	280.25	1.0965	2.63	0.30	0.94	242.00	395.25	771.42	11.42
Rhizobium + phorate	196.25	319.50	1.3475	3.54	0.35	1.233	243.25	370.75	1137.85	16.85
Rhizobium + quinalphos	201.50	322.00	1.5420	3.39	0.41	1.197	257.25	404.50	983.56	14.57
Rhizobium + mephosfolan	183.25	303.25	1.4586	2.86	0.34	1.021	243.50	375.50	858.21	12.71
Rhizobium + carbofuran	184.75	293.75	1.4757	3.43	0.39	1.330	242.50	385.25	858.21	12.71
SE of mean	19.73	21.34	0.1272	0.24	0.036	0.108	4.94	27.48	96.61	
C.D. at 0.05	59.45	64.31	0.3840	0.72	0.108	0.326	14.88	N.S.	291.15	
C.V. (%)									22.7	

DAS, Days after sowing. *Mean of 10 plants.

Nodules were removed carefully from each plant and cleaned and counted. Fresh weight of the nodules (ten plants in each treatment) was determined on an electrical microbalance. Afterwards, the nodules were dried at 80°C for three days before recording their dry weight. The height of each plant was measured from soil level to the tip of the stem. At harvest, grain yield per plot was recorded and analysed. The yield per plot was converted into yield per hectare.

The data are shown in table 1. In the uninoculated plants there was virtually no nodulation in pre-flowering or early pod formation stage of the crop. There were no significant differences in nodule number between treatments with *Rhizobium* alone and with *Rhizobium* in association with insecticides. The maximum number of nodules was recorded with *Rhizobium* in association with quinalphos. The results agree with those reported by Mundade *et al.*[6] who observed increased nodulation in peanut (*Arachis hypogea*) treated with *Rhizobium* association with carbofuran.

A significant increase in nodule fresh weight was recorded with *Rhizobium* + quinalphos at the pre-flowering stage and with *Rhizobium* + phorate at early pod formation stage.

Rhizobium in association with insecticides had the beneficial effect of increasing plant height at pre-flowering stage. A significant increase in height was however, recorded only when *Rhizobium* was in association with phorate, quinalphos or mephosfolan insecticides. At early pod formation stage, no significant difference in height was observed.

All these findings indicate the effectiveness of the combination of *Rhizobium* culture and insecticides.

Rhizobium inoculated seeds recorded an increase of 19.4% yield over uninoculated seeds but significantly higher yield (43.20%) was recorded when *Rhizobium*-inoculated seeds were sown in association with phorate. The reason for this may be significantly greater number of nodules as well as higher nodule fresh and dry weight at early pod formation stage. These observations reveal that seeds inoculated with *Rhizobium* culture can be safely sown in association with these granular insecticides, preferably with phorate-10.

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AZOTOBACTER CHROOCOCCUM IN ROOT, STEM AND LEAF TISSUES OF CELLS OF *TRITICUM AESTIVUM* L. AND *TRITICUM DURUM* L.

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NITROGEN fixation by free-living bacteria associated with plants had received a good deal of attention[1]. It is known that efficiency of nitrogen fixation is more common in symbiotic associations of bacteria and plants but Parker[2] suggested that it should not be confined to root nodules and that other existing associations for nitrogen fixation must not be ignored. In this context the intracellular detection of *Azotobacter chroococcum*, a free-living nitrogen fixer, in root, stem and leaf tissues of *Triticum aestivum* L. and *Triticum durum* L. and its probable role in the nitrogen economy of wheat are presented.

Wheat genotypes of RAJ-1555 and Kiran of *T. durum* L., and APAU-1577 and DWR-16 of *T. aestivum* L. were obtained from Wheat Breeder, AICRP on Wheat, Dharwad. They were surface-sterilized following the method of Bhide and Purandare[3] and grown aseptically on Trelease and Trelease agar media[4] in 2 l conical flasks. After 10 days of growth, transverse sections of roots, stem and leaves were observed under a phase contrast microscope.

At higher magnifications parenchymatous cells of the root cortex (figure 1a), the parenchymatous cells of the leaf sheath, especially those near the peripheral region of the stem (figure 1b), and the