

Microbiology

RESIDUAL EFFECTS OF DIFFERENT STRAINS OF *RHIZOBIUM JAPONICUM*†

by M. C. KABI*, *Nodule Research Laboratory, Botany Department,
Burdwan University, Burdwan-713101*

Residual effect of eight strains of *Rhizobium japonicum* was determined in four varieties of soybean, both in virgin soil and in plots where soybean was grown and inoculated in the preceding years. Experiments were conducted in three different locations in West Bengal.

In general, the local strains of West Bengal expressed maximum residual effect in nodulating soybean varieties whereas the exotic strains showed least effect. Above conclusions were drawn after comparing the data of the experimental plots where the soybeans have been grown in previous years to that of freshly inoculated virgin soil.

Enrichment of soil with specific *Rhizobium* produced a short durational effect in boosting up the nodule numbers of the test hosts which did not persist in the next crop years. The frequency of nodules containing unidentified rhizobial strains also appeared in a decreasing order during the successive years of experimentation.

INTRODUCTION

Introduction of a crop like soybean to any location needs artificial soil inoculation with suitable strains of *Rhizobium* for achieving the desired yield. It is necessary to know beforehand to what extent the inoculating strains are adaptable to their new ecological environment. Johnson, Means and Weber (1965) have indicated that the residual effect of an inoculum at a given place may be considered as one of the important factors contributing to the success of the inoculum in the following years at the same place. Abel and Erdman (1964) obtained a correlation between effectiveness of inocula with the growth characteristics of "Lee" soybean where the degree of effectiveness of a strain in a particular crop season may not remain unchanged in the next crop-years. They have also found that inoculation by single strain was not better than when strains were given in mixtures.

MATERIALS AND METHODS

Residual effects of eight testing strains were determined in four varieties of soybean (Baramali, K-30, Lee and Kent). All the test strains of *Rhizobium* (i.e., BU.S1-BU.S8) are serologically different from each other (Bhaduri *et al.* 1970). Serological characterisation of the rhizobial strains has been made following the method of

*Present address: Nodule Research Laboratory, Bidhan Chandra Krishi Viswa Vidyalaya, Research Building No. 2, Mohanpur 741246.

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Kleczkowski and Thornton (1944). Strain numbers BU.S1–BU.S4 have been isolated from different locations of West Bengal; strain numbers BU.S5–BU.S7 have been obtained through the courtesy of the USDA and the strain BU.S8 was obtained from the J.N.K.V., Jabalpur.

Paired experiments were conducted both in (a) virgin soil, in the sense that it does not contain any soybean *Rhizobium*, and side by side in (b) plots where soybean was grown with the mixed inoculum of the same eight testing strains in equal proportions in the previous year. The latter plots were kept uninoculated and the former one was freshly inoculated with the mixed culture of eight test-strains given in equal proportions. Treatment (a) may be considered as the inoculated check to the treatment (b). There were also uninoculated control rows of each variety of the host plants along with the treatment (a). Experiments were conducted in replications at three locations, viz., Burdwan, Berhampur and Kalimpong. Random sampling of ten plants per treatment per replication were made at the onset of flowering and the nodules were harvested from these plants and the sero-diagnosis of these nodule-isolates were made following the usual schedule as mentioned earlier.

RESULTS AND DISCUSSION

Data in Table I–III indicate that Baramali variety got relatively more nodules by the strains BU.S1, BU.S2, BU.S4 and BU.S7 from the one year old inoculated field when compared to the freshly inoculated one. Strain numbers BU.S1, BU.S2, BU.S3 and BU.S4 behaved similarly to the variety K-30. Only in Berhampur, in the field (b), strain numbers BU.S4 and BU.S8 produced more nodules on both of the newly introduced varieties—Lee and Kent; which in Kalimpong was produced by BU.S5 and BU.S3 respectively. It may be mentioned that in Kalimpong field, BU.S3 has shown an increased residual effect over its normal nodulating ability on Lee, but on Kent it decreased significantly. In the field (b) at Kalimpong, frequency of unknown sero-types decreased when compared to that of the freshly inoculated one. In Berhampur, BU.S4 produced significantly higher numbers of nodules on all the test-hosts grown in the field (b) than those from the field (a). In the field (b) of Berhampur, BU.S8 caused more nodules than the fresh inoculation by the same strain. An opposite trend has been noted on these varieties by BU.S3. At Burdwan, the previous year's inoculated field preferred the native isolates of the mixed inoculant to produce more nodules on Baramali and K-30 varieties which is significantly higher when compared to field (a). All the exotic strains showed a decreased residual response in nodulation to Baramali and K-30, the already introduced varieties. Lee and Kent, the newly introduced germ-plasms, did not produce any significant change in nodulation when grown at (a) and (b) fields.

It may be summarised from the above observations that the frequency of nodules produced by the residual ability of the native isolates of the previous year's inoculation was significantly more over that of the freshly inoculated one. But, on the contrary, the exotic strains introduced in field (b) one year back failed to restore their normal nodulating ability specially with regard to the already introduced soybean varieties.

Preliminary experiments on the residual effects conducted earlier and discussed above required further clarification for which a more critical set of experiments was

TABLE

Residual effects (percentage of sero-recovery) of the test strains of *Rhizobium japonicum* (BU.S1-

Host varieties	Percentage of sero-recovery of the test							
	BU.S1		BU.S2		BU.S3		BU.S4	
	a	b	a	b	a	b	a	b
Baramali	11.9	10.0	17.0	11.2	9.8	10.8	7.5	24.6***
K-30	9.3	11.0	14.0	11.8	15.3	10.0	8.3	24.2***
Lee	9.7	5.5	7.7	4.5	12.6	5.0*	15.5	30.0***
Kent	9.3	5.0	14.0	14.5	15.3	3.0**	8.3	26.5***

Note: Plot 'a' indicates virgin soil, whereas 'b' indicates the soil where soybean was grown during tested by χ^2 test. Sn indicates unidentified sero-types of *Rhizobium*
 ***P = .001; **P = .01; *P = .05

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Residual effects (percentage of sero-recovery) of the test strains of *Rhizobium japonicum* (BU.S1-

Host varieties	Percentage of sero-recovery of the test							
	BU.S1		BU.S2		BU.S3		BU.S4	
	a	b	a	b	a	b	a	b
Baramali	7.5	7.5	7.6	8.5	7.7	8.0	6.6	10.0
K-30	5.0	6.0	6.6	6.6	7.6	10.2	13.0	20.0
Lee	10.7	4.0	6.3	3.4	8.0	0.0***	8.2	6.6
Kent	7.9	6.0	6.0	6.0	0.0	2.0***	6.5	5.4

Note: Plot 'a' indicates virgin soil whereas 'b' indicates the soil where soybean was grown during tested by χ^2 test. Sn indicates unidentified sero-types of *Rhizobium*
 ***P = .001; **P = .01; *P = .05

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	BU.S1		BU.S2		BU.S3		BU.S4	
	a	b	a	b	a	b	a	b
Baramali	8.0	31.3***	8.0	31.4***	7.8	5.1	6.6	11.6
K-30	8.6	22.6***	8.6	20.0***	8.8	20.0***	17.0	18.0
Lee	11.0	15.0	9.0	12.5	14.3	2.5**	18.0	11.0
Kent	12.0	15.0	17.0	12.5	10.0	5.0	8.0	10.0

Note: Plot 'a' indicates virgin soil whereas 'b' indicates the soil where soybean was grown during the tested by χ^2 test. Sn indicates unidentified sero-types of *Rhizobium*
 ***P = .001; **P = .01; *P = .05

I

BU.S8) against four varieties of soybean (Baramali, K-30, Lee and Kent) at the Berhampore Farm

strains both in 'a' and 'b' plots

BU.S5		BU.S6		BU.S7		BU.S8		Sn	
a	b	a	b	a	b	a	b	a	b
17.3	10.0	12.3	18.0	10.0	6.6	9.0	8.0	5.2	2.8
13.0	7.6	17.0	17.6	9.0	6.0	9.0	8.0	5.1	3.8
15.5	15.0	8.0	6.5	6.5	6.0	6.0	16.5***	18.5	11.0
13.0	10.5	17.0	8.0*	9.0	7.5	9.0	15.0*	5.1	10.0*

the previous year with inoculum. The differences between 'a' and 'b', for each treatment, have been recovered from the nodules.

II

BU.S8) against four varieties of soybean (Baramali, K-30, Lee and Kent) at the Kalimpong Farm

strains both in 'a' and 'b' plots

BU.S5		BU.S6		BU.S7		BU.S8		Sn	
a	b	a	b	a	b	a	b	a	b
22.5	24.0	17.0	18.0	9.8	16.0*	15.7	6.4*	5.6	1.6
14.0	20.0	9.8	14.0	11.2	17.0	5.6	5.0	27.2	1.2***
19.0	40.0***	13.5	14.0	14.9	22.0	8.3	6.0	11.1	4.0*
22.0	30.0	10.0	16.0	11.3	16.0	18.0	12.0	18.3	6.6**

the previous year with inoculum. The differences between 'a' and 'b', for each treatment, have been recovered from the nodules.

III

BU.S8) against four varieties of soybean (Baramali, K-30, Lee and Kent) at the Burdwan Farm

strains both in 'a' and 'b' plots

BU.S5		BU.S6		BU.S7		BU.S8		Sn	
a	b	a	b	a	b	a	b	a	b
24.0	5.0***	18.0	6.6**	10.0	2.5*	16.0	2.5***	1.6	4.0
19.0	7.4**	12.8	8.0	14.6	2.0***	6.0	1.0*	4.6	1.0
18.0	19.0	9.0	13.7	8.0	11.6	7.0	11.0	5.7	3.7
18.0	17.5	12.6	15.0	10.2	12.5	10.0	10.5	2.2	2.0

previous year with inoculum. The differences between 'a' and 'b', for each treatment, have been recovered from the nodules.

TABLE IV

Residual effects (percentage of sero-recovery) of the test strains of Rhizobium japonicum (BU.S1-BU.S8) during three consecutive years (1967, 1968 and 1969) against four varieties of Soybean (Baramali, K-30, Lee and Kent) at the Burdwan Farm. Inoculum was applied during the year, 1967 (The data of 1968 and 1969 have been tested against that of 1967 by χ^2 test)

Soybean varieties	Year	Percentage of sero-recovery of <i>Rhizobium japonicum</i> strains (inoculated during the year, 1967)								
		BU.S1	BU.S2	BU.S3	BU.S4	BU.S5	BU.S6	BU.S7	BU.S8	Sn
Baramali	1967	8.0	8.0	7.8	6.6	24.0	18.0	10.0	16.0	1.6
	1968	32.0***	32.0***	5.0	7.0	6.0***	8.0*	5.0	5.0**	0.0***
	1969	40.0***	40.0***	3.5	6.0	3.5***	4.0***	1.5***	1.5**	0.0***
K-30	1967	8.6	8.6	8.8	17.0	19.0	12.8	14.6	6.0	4.6
	1968	22.0***	22.0***	18.0**	18.0	7.4**	7.0	2.0***	1.0*	2.6
	1969	23.0***	22.8***	21.2***	21.0	6.0**	4.0*	0.0***	0.0***	2.0
Lee	1967	11.0	9.0	14.3	18.0	18.0	9.0	8.0	7.0	5.7
	1968	15.0	13.0	3.0**	11.6	18.0	14.4	11.0	11.0	3.0
	1969	16.0	16.0*	0.0***	12.0	14.0	15.0*	12.0	12.0	3.0
Kent	1967	14.0	13.0	10.0	9.2	18.0	13.6	10.2	10.0	2.0
	1968	15.0	13.5	5.5	10.0	17.0	16.0	12.0	10.0	1.0
	1969	16.0	14.0	0.0***	12.0	16.0	16.0	13.0	11.0	2.0

***P = .001; **P = .01; *P = .05

TABLE V

Percentage sero-recovery of the test strains of *Rhizobium japonicum* (BU.S1-BU.S8) from the fields inoculated during the year 1967 with the mixture of eight test strains in equal proportions. The soil was enriched with specific strains (The differences between VI and RE; and between RE and En, for each strain, were tested by χ^2 test)

Host variety	Experiment	Percentage of sero-recovery of the testing strains																			
		BU.S1	BU.S2	BU.S3	BU.S4	BU.S5	BU.S6	BU.S7	BU.S8	Sn											
Baramali	VI (1969)	8.0	8.0	7.0	6.8	24.2	18.0	10.0	16.0	2.0											
	Re (1968)	32.0***	34.0***	5.0	10.0	5.8***	7.0**	2.2*	3.0**	1.0											
	En-S3	30.0	32.0	7.5	10.5	6.0	7.8	2.0	3.0	1.2											
	En-S5	28.0	28.0	5.0	10.0	21.0***	4.0	2.0	1.0	1.0											
	En-S6	29.0	30.0	6.0	9.0	5.0	16.0***	1.2	2.0	1.8											
	En-S7	30.0	30.0	6.0	9.0	6.0	7.0	8.0	2.0	2.0											
	En-S8	28.0	30.0	6.0	9.0	5.0	7.0	3.0	11.0***	1.0											
			9.0	9.0	9.0	17.0	19.0	13.0	15.0	6.0	3.0										
K-30	RE (1968)	20.0***	20.0***	19.0***	18.0	7.4**	8.0	3.0**	2.0	2.6											
	En-S5	18.0	20.0	16.0	14.0	20.0***	8.0	2.0	1.0	1.0											
	En-S6	20.0	20.0	20.0	15.0	7.0	12.0	2.0	2.0	2.0											
	En-S7	18.0	18.0	18.0	14.0	7.0	8.0	14.0***	2.0	1.0											
	En-S8	22.0	20.0	18.0	14.0	5.0	7.0	2.0	12.0***	0.0***											
Lee	VI (1969)	10.0	9.0	15.0	18.0	18.0	9.0	8.0	7.0	6.0											
	RE (1968)	15.0	14.0	2.5**	10.5	19.0	13.0	12.0	12.0	2.0											
	En-S3	15.0	13.0	10.0***	8.0	16.0	13.0	12.0	11.0	2.0											
Kent	VI (1969)	16.0	13.0	10.0	8.0	18.0	12.0	11.0	10.0	2.0											
	RE (1968)	15.0	12.0	5.5	10.0	17.5	15.0	12.0	10.0	2.0											
	En-S3	16.0	10.0	7.0	10.5	16.0	16.5	13.0	10.0	1.0											

VI Inoculated in virgin soil; RE = Residual effect; En = Enriched with specific strain.

***P = .001; **P = .01; *P = .05

laid out at the State Seed Multiplication Farm, Burdwan. From this second set of experiments (Table IV) it has been observed that the strains BU.S1 and BU.S2 produced increasing number of nodules in Baramali and K-30 varieties of soybean for three consecutive years whereas strain number BU.S5 produced nodules at a decreasing rate in those above mentioned varieties, in successive years. The rate of decrease in nodule numbers was almost proportional to the rate of increase shown by BU.S1 and BU.S2. Strain numbers BU.S5, BU.S6, BU.S7 and BU.S8 produced nodules, also in a decreasing order, in K-30 variety of soybean.

Experiments performed in three consecutive years (1967, 1968 and 1969) at the Burdwan field (Table V) have indicated that local strains produced nodules at an increased rate whereas the exotic strains lost their nodulating abilities in the same soil during this period. The effective population of the residue strains of the abovefield that was losing their potency of nodulation were then enriched separately with individual strains. Enrichment of soil by reinoculating with specific *Rhizobium* produced and immediate effect in boosting the nodule numbers of the test-hosts but did not persist in the next crop year. It appears from the experiments that most of the enriching strains of *Rhizobium japonicum*, responded well to the respective hosts. Some of the strains, viz., BU.S3, BU.S6 and BU.S7 failed to give the expected result to Baramali, K-30 and Kent varieties of soybean in the enrichment experiment.

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