VARIETAL SPECIFICITY FOR RHIZOBIAL SEROTYPES IN RELATION TO NODULATION AND CROP YIELD

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The nodulation pattern and yield with native and introduced rhizobia (as distinguished serologically) were examined in different genotypes of bengal-gram (*Cicer arietinum* L.), pea (*Pisum sativum* L.) soybean (*Glycine max* L. Merr.), green-gram (*Phaseolus aureus* Roxb.) and some of the radiation induced mutants of green-gram in M_3 generation, under natural green house conditions. It was found that under similar soil and environmental conditions with similar doses of inoculum, the number of nodules produced and the efficiency of an inoculating strain varied with the genotype of the host species. Both nodulation and response to yield were found to be quantitative characters. Possibility of improving these characters through mutation breeding is discussed. The time of flowering and maturity of the host were not related with the efficiency.

The nodule bacteroids showed homology for somatic antigens with cultured rhizobia but differed in internal protein antigen bands.

INTRODUCTION

The major controlling factors which determine the efficiency of an inoculating strain of root nodule bacteria are reported to be the host genotype (Hamatova 1963; Saubert and Scheffler 1967) and the ability of the strain to dominate over the native rhizobia (Damirgi *et al.* 1967; Brockwell and Dudman 1968). Serological methods had been applied for determining the nodulation pattern of an inoculating strain (Thornton and Kleczkowska 1950; Means and Johnson 1968; Brockwell and Dudman 1968; Skrdleta 1969). In most of these studies, isolates either from a single early nodule of randomly selected plant or from nodules sampled randomly, were tested. No attempt had been made to determine the relation, if any, between the nodulation by inoculating serotype and yield. The present investigation was therefore, carried out to find out (i) competition between native and introduced rhizobia to nodulate the host, (ii) the effect of host genotype on the relative efficiency of the inoculating strain, and (iii) effect of mutation breeding on nodulation.

MATERIALS AND METHODS

Crops

Following genotypes of various crops were used in the present studies :

Bengal-gram (*Cicer arietinum* L.) : NP53, G24, P320, P3637, C235.

Pea (*Pisum sativum* L.) : P6115, P353-23, P41B, Br12, T163, P172M, B450, P4793.

Soybean (*Glycine max* L. Merr.) : Bragg, Clark 63.

Green-gram (*Phaseolus aureus* Roxb.) : Baisakhi, Hybrid 45.

Green-gram mutants (M3) : Dry seeds of Baisakhi and hybrid 45 were irradiated with 30 Kr and 70 Kr gamma rays (from Co^{60}). Plants raised from treated seeds were considered as Ml plants. Mutants based on yield, seed colour, leaf shape and stem habit were selected in M2 generation and single plant harvests were taken for symbiotic studies in M3 generation. These mutants were (i) Mutants of Baisakhi : Dull green seed colour (70 Kr), shining dark green seed colour (70 Kr), light brown seed colour (30 Kr), mosaic seed colour (30 Kr), dullest green seed colour (30 Kr), and (ii) mutants of hybrid 45 : Trailing type stem (70 Kr) and early mutant maturing with Baisakhi (30 Kr).

Rhizobium cultures

(i) G2 from *Cicer arietinum* L., (ii) P2 from *Pisum sativum* L., (iii) SB16 from *Glycine max* L. Merr., and (iv) M1 and M7 from *Phaseolus aureus* Roxb.

Soil

Unsterile surface soil was used in clay pots (8 kg capacity) with basal dressing of superphosphate (2g per pot) having 16 per cent. P_2O_5.

Antigen

Whole cell antigen was prepared by growing rhizobia on yeast extract mannitol agar slopes (Allen 1957). Harvested cells were used for subsequent injections and antigen-antibody reactions.

Antisera

Rabbit antisera were prepared (Vincent 1970) for each strain.

Pot culture experiment

All crop genotypes were raised in pots under unsterile conditions. Seeds soaked overnight in water were inoculated with broth cultures of respective rhizobia. Soaked but uninoculated seeds were sown as control for comparing the nodule serotypes as well as yield with inoculated series. After germination three seedlings were kept per pot in all crops.

Nodulation

The plants were uprooted at flowering stage of the crop grown in pots, by gentle washing off the soil with tap water. All nodules from both inoculated as well as uninoculated plants were detached, washed with distilled water, surface sterilized and kept immersed in saline in freezer (0°C) for agar diffusion analysis.

Agar diffusion

Agar diffusion was carried out in petri—plates using 0.75 per cent agar as gelling medium containing 0.025 per cent sodium azide (Vincent 1970). Four sets of wells

in hexagonal arrays around a central well were prepared in each plate. Each individual nodule was crushed separately in serum viales (1 × 7.5 cm) in two drops of saline, steamed for 30 min over a water bath and poured into individual wells. One well in each set was filled with reference antigen for comparing precipitin bands and the central well was filled with the corresponding antiserum. Nodule homology was determined by looking for the identity of preciptin bands (Vincent 1970). The number of nodules showing homology with the inoculant antigen in uninoculated treatments were deducted from those showing identity in inoculated plants to calculate the homologous nodules formed exclusively by the inoculating rhizobia.

Yield

The yield of grain both in uninoculated and inoculated series, was determined (g) and statistically analysed at 5 per cent level of significance. The percent increase or decrease in yield was calculated in inoculated series over control.

Results and Discussion

The number of nodules produced by native and inoculating rhizobia, the per cent increase or decrease in inoculating serotype nodules and yield in inoculated plants are depicted in Tables I and II. The soil contained least number of naturalized rhizobia for soybean (12.10 and 12.80 nodules per pot) and Bengal-gram (16.16 to 25.83 nodules per pot). Pea contained large number (40.33 to 90.33 nodules per pot) and maximum number of nodules ranging from 35.66 to 117.16 per pot was observed in green-gram. The mutant genotypes of green-gram showed greater variation in the nodulation behaviour with native rhizobia as compared to the established genotypes.

Perusal of the data given in Tables I and II revealed the existence of different type of response patterns to inoculation so far as the effect of nodulation and yield were concerned :

(*a*) Both nodulation and yield increased significantly due to inoculation, e.g. Bengal-gram varieties P320, G24, P3637 and C235; pea varieties : Br12, T163, and P4793; soybean varieties : Bragg and Clark; green-gram varieties Baisakhi, shining dark green seed colour, dullest green seed colour (with both M1 and M7), dull green seed colour, large bold seeded, hybrid 45, early maturing mutant (only with M1). For these genotypes the inoculating serotype could be considered efficient.

(*b*) Number of nodules increased significantly but not yield, e.g. Bengal-gram NP 53; mutants of green-gram : light-brown seed colour, mosaic seed colour (with both M1 and M7), dull green seed colour, large bold seeded, early maturing mutant and the parent hybrid 45 (with M7). This showed that the genetic factors governing nodulation and efficiency are independent to each other. Similar reports have been made in case of clover (Nutman 1967).

(*c*) Significant decrease in the number of nodules e.g. trailing type stem mutant of hybrid 45 inoculated with M1 (Tabe II). The inoculating serotype was not compatible for this host as revealed by serological studies. However, the mutant profusely nodulated with native rhizobia indicating the possibility of developing mutants for increased nodular tissues.

TABLE I

Effect of inoculation on nodulation and yield of different crop genotypes

(figures indicate average o five replicates)

Crop genotype	Number of nodules per pot		Inoculant serotype in nodules of the inoculated plants (%)	Increase in yield over control (%)
	Uninoculated	Inoculated		
Bengal-gram				
P 320	16.16	81.83*	84.12*	60.05*
G 24	25.83	174.50*	91.21*	65.23*
NP 53	24.16	208.50*	91.60*	33.33
P 3637	24.50	181.66*	89.90*	85.76*
C 235	22.66	254.16*	94.42*	90.81*
Pea				
P 6115	59.50	97.16	42.71	12.66
P 353-23	47.16	64.83	39.04	3.85
P 41B	40.33	64.66	39.18	—3.46
Br 12	74.00	136.66*	55.85*	30.19*
T 163	90.33	176.66*	53.01*	34.57*
P 172M	57.16	61.33	14.69	—13.10
B 450	54.16	52.83	—5.43	—0.67
P 4793	79.16	152.00*	57.57*	47.51*
Soybean				
Bragg	12.80	129.66*	96.52*	145.93*
Clark 63	12.10	118.16*	94.21*	169.75*

*Values significantly different from the control at 5% level of significance.
— indicates decreased values.

Nutman (1949, 1954) reported that the resistance to nodule formation and the efficiency were monogenic characters. In the present studies none of the 25 genotypes tested showed complete resistance to nodulation. Secondly, the responding genotypes showed a graded variation in the number of nodules produced by an inoculating serotype and increase in the yield. These findings shows that both these characters are quantitative.

The variation in the number of nodule tissues produced by different genotypes with a single serotype (Tables I and II) and by a single genotype with two different serotypes e.g. Baisakhi with M1 and M7 (Table II) showed that the total number of nodules produced are under the genetic control of the host and its expression depends on strain compatibility.

TABLE II

Effect of inoculation on nodulation and yield of different genotypes of green-gram (figures indicate average of five replicates)

Crop genotype	Number of nodules per pot			Inoculant serotype in nodules of the inoculated plants (%)		Increase in yield over control (%)	
	Uninoculated	Inoculated					
		with M1	with M7	M1	M7	M1	M7
Baisakhi	93.99	225.98*	224.38*	85.17*	69.52*	64.94*	53.33*
Dull green seed colour (70 Kr)	108.16	184.33*	164.33*	45.84*	38.78*	35.01*	8.45
Shining dark green seed colour (70 Kr)	84.83	196.16*	207.33*	65.11*	66.02*	55.61*	41.41*
Large bold seeded (70 Kr)	55.35	183.33*	162.82*	91.91*	90.48*	34.51*	12.96
Light brown seed colour (30 Kr)	84.66	152.88*	155.00*	59.85*	53.72*	24.45	13.23
Mosaic seed colour (30 Kr)	35.66	118.50*	103.50*	78.05*	66.66*	20.42	6.41
Dullest green seed colour (30 Kr)	42.88	175.33*	138.00*	83.64*	76.00*	46.49*	28.41*
Hybrid 45	76.16	153.00*	123.66*	52.72*	44.20*	46.92*	—1.21
Trailing type stem (70 Kr)	117.16	92.33*	97.50	—3.61	2.73	—11.80	—17.86
Early maturing mutant (30 Kr)	51.16	145.83*	108.56*	71.54*	67.86*	59.70*	4.62

* Values significantly different from the control at 5% level of significance.
— indicates decreased values.

Time of flowering and maturity of the host did not seem to be related with host strain compatibility or efficiency for e.g. Hybrid 45 took twice as much time to mature as Baisakhi but both nodulated effectively with M1 *Rhizobium*. For a host to obtain material benefit by inoculation with an efficient and compatible strain more than 50 per cent nodules should be of inoculating serotype under competition with native rhizobia (Tables I and II).

Effect of host genotype on the bacteroid antigens

In agar diffusion the somatic antigen bands (Vincent and Humphrey 1970), developed with bacteroid suspension from nodules, were identical with the injecting antigen bands, but the fast diffusable internal antigen bands gave anomalous results with nodular suspension of all the genotypes studied (Fig. 1). These bands were formed by heat labile antigens (Means and Johnson 1968), being inactivated by steaming for 30 min. These results indicate that the internal antigens of *Rhizobium* cells

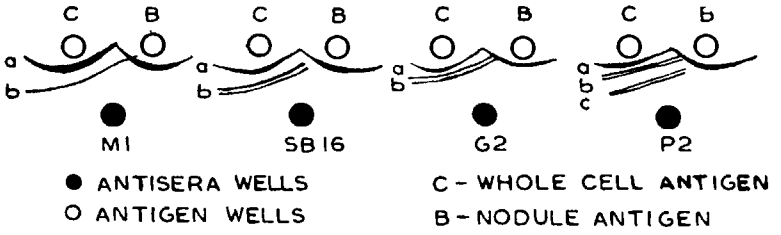

FIG. 1. Type of precipitin bands formed against respective antiserum with whole cell and nodule antigens. M1, *Phaseolus* rhizobium, SB16, *Rhizobium japonicum*; G2, *Cicer* rhizobium, P_2, *R. leguminosarum*; a, Somatic antigen bands; b and c, Internal antigen bands (heat labile)

might undergo certain modifications during bacteriod formation. Nodule suspensions from efficient as well as inefficient nodules gave identical antigen-antibody reactions.

ACKNOWLEDGEMENTS

Thanks are due to Dr. N. S. Subba Rao, Head, Division of Microbiology, for providing necessary facilities and encouragement.

REFERENCES

Allen, O. N. (1957). Experiments in Soil Bacteriology, Burgess Publishing Co., Minnesota, U.S.A.

Brockwell, J., and Dudman, W. F. (1968). Ecological studies of root nodule bacteria introduced into field environments. II initial competition between seed inocula in the nodulation of *Trifolium subterraneum* seedlings. *Aust. J. agric. Res.*, **19**, 749–757.

Damirgi, S. M., Frederick, L. R., and Anderson, I. C. (1967). Serogroups of *Rhizobium japonicum* in soybean nodules as affected by soil type. *Agron. J.*, **59**, 10–12.

Hamatova, E. (1963). The specificity of *Rhizobium japonicum* towards various soya varieties. *Rost. Vyroba* **14**, 728–731.

Means, U. M., and Johnson, H. W. (1968). Thermostability of antigens associated with serotypes of *Rhizobium japonicum*. *Appl. Microbiol.*, **16**, 203–206.

Nutman, P. S. (1949). Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. *Heredity*, **3**, 263–291.

———(1954). Symbiotic effectiveness in nodulated red clover (ii) A major gene for ineffectiveness in the host. *Heredity*, **8**, 47–60.

———(1967). Varietal differences in nodulation of subterranean clover. *Aust. J. agric. Res.*, **18**, 381–425.

Saubert, S., and Scheffler, J. G. (1967). Strain variation and host specificity of *Rhizobium*. III Host specificity of *Rhizobium* on African clover. *S. Afr. J. agric. Sci.*, **10**, 357–364.

Skrdleta, V. (1969). Application of gel immuno-precipitation in agar gel for the serological typing of soybean root nodules. *Folia Microbiol.*, **14**, 32–35.

Thornton, H. G., and Kleczkowska, J. (1950). Use of antisera to identify nodules produced by inoculation of legumes in the field. *Nature, Lond.*, **166**, 1118–1119.

Vincent, J. M. (1970). A manual for the Practical Study of Root-Nodule Bacteria. Handbook. No. 15, IBP Black well Scientific Publication, Oxford & Edinburgh.

Vincent, J. M., and Humphrey, B. A. (1970). Taxonomically significant group antigens in rhizobia. *J. gen. Microbiol.*, **63**, 379–382.