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RHIZOBIUM-INDUCED CHLOROSIS IN SOYBEAN
I. EFFECT OF POPULATION DENSITY OF THE INOCULANTS

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Increased nodulation due to increasing doses of the inoculants did not bear simple proportion; different varieties responded differently in this regard. The average size of the nodules in a particular variety remains unaffected in spite of increasing the population density of the inoculants. The number of nodules is positively correlated to the total nodular fresh weight, the former has shown an inverse relationship with the size of the nodules. Inter-varietal differences exist in respect of nodulation as well as response to chlorosis. Increased doses of inoculants induced increasing degrees of chlorosis. Expression of chlorosis is positively correlated more with the total nodular fresh weight than with the total number of nodules produced. Further, unit nodular contribution to the degree of chlorosis varies in different varieties.

INTRODUCTION

Rhizobium-induced chlorosis in soybean was first reported by Erdman *et al.* (1956) who observed that certain strains of *Rhizobium japonicum* were responsible for inducing chlorosis in soybean. They also pointed out that such chlorosis showed no marked effect on the growth, nodulation or the yield of soybean. Expression of such chlorosis was reported to vary considerably in different varieties of soybean (Erdman *et al.*, 1957; Johnson & Means, 1960; Bhaduri *et al.*, 1968). It has also been established that the source of stimulation for induction of chlorosis are the nodules which harbour the specific chlorosis-inducing strains of *Rhizobium* (Johnson & Clark, 1958; Johnson *et al.*, 1959). Subsequently, Owens (1961) and Owens and Wright (1965a) were able to isolate a phytotoxic amino compound from the nodules of the chlorotic soybeans which, only in a minute amount, was capable of inducing chlorosis to the soybean seedlings. Production of this toxin in pure cultures in a number of strains of *R. japonicum* was also reported by Owens and Wright (1965b).

Interaction of different host genotypes (soybean) and the chlorosis-inducing strain of *Rhizobium japonicum* with regard to nodulation and in the expression of chlorosis, has been examined in the present study.

MATERIALS AND METHODS

Soybean varieties—Following twelve soybean cultivars were selected :

Sl. No.	Variety	Seed colour	Maturity	Source
1.	Baramali	Yellowish white	Late	State Oilseed Berhampore Res. Stn.
2.	B.M.J.	Black	Medium	IARI, New Delhi
3.	EC 1774	Black	Medium	-do-
4.	EC 2542	Black	Medium	-do-
5.	IC 196	Black	Medium	State Oilseed Berhampore
6.	IC 202	Black	Medium	Res. Stn. -do-
7.	IC 216	Brown	Early	IARI, New Delhi
8.	IC 219	Light yellow	Medium	State Oilseed Berhampore
9.	K 16	Black	Early	Res. Stn.
10.	K 30	Black	Early	-do-
11.	Nepal	Brown	Medium	-do-
12.	Soymax	Yellow	Early	-do-

The Rhizobium strain—Experiment was conducted with a chlorosis-inducing marker strain of *R. japonicum*, S_6 (strain No. 76, obtained through the courtesy of the United States Dept. of Agriculture). The strain showed its best growth in yeast-mannitol-agar medium and could nodulate effectively all the twelve soybean varieties mentioned above. Single cell isolation was made from this strain following the standard schedule. This was kept as stock cultures after confirming its purity by comparing it against the parent culture both in respect of serological and cultural characteristics. The stock culture was maintained carefully throughout the course of the present investigation. Purity of the strain was checked regularly.

Field experiment—Experiment was conducted at the Burdwan University Farm where legumes were not grown for the last 4-5 years. Absence of soybean-rhizobia in these fields was confirmed by analysing soil samples collected at random from the experimental plots. Twelve soybean varieties inoculated with six different doses of inoculants were placed in a randomised block design with three replications and the sub-plot size was 1.5 m × 1.5 m. Uninoculated checks were maintained as the controls. Seeds were sown in rows with an uniform spacing (25 cm between rows and 10 cm between plants). These were then covered immediately with the inoculum at the rate of 10 g for every 25 seeds. Afterwards, a very light irrigation was applied. All inoculants were prepared by mixing broth cultures with a sterile charcoal base appropriately, so as to get inoculants of six different population densities (indicated in Tables I–IV). Inoculant samples were tested separately following the method given by Date and Vincent (1962) to ensure the desired viable populations per g of the inoculant.

Ten plants were taken from each of the treatment after 45 days of growth and data on nodulation and degree of chlorosis were recorded for each plant. The degree of chlorosis in each plant was calculated by measuring the total area of leaves: total area of chlorosis and represented in percentages.

RESULTS

Soybean varieties responded differently to various doses of inoculants in respect of the number of nodules produced per plant (Table I). Maximum number of nodules were produced by IC 216 and EC 1774, and lowest by K-16. IC 219, K-30 and Nepal showed almost equal response, while the remaining six varieties differed significantly. In general, the number of nodules increased under increasing doses of the inoculant; the number, however, falling at the highest dose.

The differential response of different varieties to different doses of inoculants were also evident when mean nodular fresh weight was taken into consideration (Table II). The maximum and the minimum nodular fresh weights were observed in cases of EC 1774 and Baramali respectively. Varieties IC 202 and Nepal produced almost an equal amount of nodular fresh weight but the rest of the varieties remained distinct because they differed significantly from each other in this respect. Further, the nodular fresh weight increased with the application of increasing doses of inoculants although there was a fall at the fourth dose. But when the varieties were compared between themselves, a fluctuating response similar to nodule number was also reflected in their nodular fresh weight.

The size of the nodules, as reflected in their mean values, was found to be different in different varieties (Table III). The largest nodules were observed in case of K-16 whereas Baramali and IC 216 produced very small nodules. The nodules were almost of equal size in B.M.J., EC 1774, K-30 and Nepal. In rest of the varieties, the nodules were of varying sizes and the differences were found to be statistically significant. Correlation between the nodule size and the population density of the inoculants was not found linear.

It appears from Table IV that different degrees of chlorosis were exhibited by different varieties of soybean. Chlorosis was maximum in Soymax and minimum in K-16. An almost equal amount of chlorosis was noted in case of EC 2542, IC 202 and IC 219 whereas the rest of the varieties differed from each other significantly in regard to the degree of chlorosis.

In general, the degree of expression of chlorosis increased with increasing doses of the inoculants (Table V). It declined at doses IV and V and again increased at dose VI. Chlorosis in Baramali, IC 196 and K-30 showed an increasing tendency with increasing doses of inoculants. Varieties EC 1774, IC 216, Nepal and Soymax produced increasing chlorosis till they reached their peaks, but then they declined with further increasing of the doses. EC 2542, B.M.J., IC 202, IC 219 and K-16, however, responded erratically.

It appears from Table VI that nodule number is correlated with nodular fresh weight in almost all the varieties (except K-16, Nepal and Soymax). A significant negative correlation between nodule number and per nodule fresh weight

TABLE I

Mean number of nodules in twelve varieties of soybean inoculated with different doses of Rhizobium japonicum *(S_6)*

Varieties	Doses of inoculants*						Average
	I (6.28)	II (6.98)	III (7.28)	IV (7.98)	V (8.28)	VI (8.98)	
Baramali	12.8	15.7	16.0	20.2	23.3	26.4	19.07
B. M. J.	17.2	14.3	11.0	12.8	21.0	13.3	14.93
EC 1774	22.4	27.6	30.0	35.2	33.3	35.5	30.67
EC 2542	14.0	11.9	15.7	13.3	10.6	12.8	13.05
IC 196	7.3	9.0	10.8	12.5	13.6	14.0	11.20
IC 202	9.8	11.5	13.3	12.3	13.0	12.7	12.08
IC 216	28.0	32.5	37.3	34.6	30.9	27.2	31.75
IC 219	20.1	16.6	19.5	17.0	25.8	29.5	21.42
K-16	9.0	8.4	9.6	7.0	10.2	8.5	8.78
K-30	17.2	17.5	19.8	23.2	25.6	27.0	21.72
Nepal	18.4	21.3	25.5	23.0	21.8	20.6	21.77
Soymax	13.3	15.3	16.6	14.1	12.7	9.2	13.53
Average	15.79	16.80	18.75	18.77	20.15	19.73	

C. D. at 5% level (Treatments) =2.47
C. D. at 5% level (Variety) =0.41
C. D. at 5% level (Dose) =0.21

*Log number of cell counts/g of culture indicated within parentheses for each dose.

TABLE II

Mean nodular fresh weight (in mg) in twelve varieties of soybean inoculated with different doses of Rhizobium japonicum (S_6)

Varieties	Doses of inoculants*						Average
	I (6.28)	II (6.98)	III (7.28)	IV (7.98)	V (8.28)	VI (8.98)	
Baramali	40.2	53.3	55.2	76.1	79.4	121.2	70.9
B. M. J.	140.2	141.0	116.2	128.1	192.2	145.0	143.8
EC 1774	238.2	265.1	281.3	332.2	315.5	347.3	296.6
EC 2542	282.6	260.3	345.4	259.1	195.3	256.2	266.5
IC 196	208.3	246.3	246.3	272.4	281.6	297.3	258.7
IC 202	158.4	189.4	232.2	227.3	239.3	216.4	210.5
IC 216	105.3	124.4	149.5	131.1	113.6	108.4	122.1
IC 219	226.4	207.4	218.4	196.4	236.4	256.3	223.6
K-16	230.6	224.4	224.5	203.3	268.7	228.4	233.3
K-30	157.3	168.4	186.4	214.2	243.3	259.5	204.9
Nepal	176.3	216.5	248.4	219.3	209.2	188.5	209.7
Soymax	182.3	196.4	207.4	187.2	172.1	167.4	185.5
Average	178.8	191.1	210.9	203.9	212.2	216.0	

C. D. at 5% level (Treatments) = 14.34
C. D. at 5% level (Variety) = 2.39
C. D. at 5% level (Dose) = 1.20

*Log number of cell counts/g of culture indicated within the parentheses for each dose.

TABLE III

Mean size of the nodules (i.e. 'per-nodule fresh weight' in mg) in twelve varieties of soybean inoculated with different doses of Rhizobium japonicum (S_6)

Varieties	Doses of inoculants*						Average
	I (6.28)	(II) (6.98)	(III) (7.28)	(IV) (7.98)	(V) (8.28)	(VI) (8.98)	
Baramali	3.26	3.52	3.52	3.97	3.48	4.77	3.75
B. M J.	8.29	9.96	10.68	10.29	9.32	11.29	9.97
EC 1774	10.82	9.68	9.40	9.47	9.55	9.83	9.79
EC 2542	20.45	22.35	22.26	19.72	18.69	20.08	20.59
IC 196	28.78	30.30	23.29	22.32	20.84	21.47	24.50
IC 202	19.49	16.86	17.68	18.73	18.58	17.18	18.09
IC 216	3.89	3.82	4.02	3.81	3.75	4.18	3.91
IC 219	11.49	12.74	11.44	11.66	9.18	8.81	10.89
K-16	26.47	27.32	25.67	30.09	27.24	26.88	27.28
K-30	9.36	9.71	9.54	9.29	9.84	9.63	9.56
Nepal	9.68	10.28	9.99	9.60	9.69	9.32	9.76
Soymax	13.97	13·05	12.86	13.34	13.77	18.43	14.24
Average	13.83	14.13	13.36	13.52	12.83	13.49	

C. D. at 5% level (Treatments) =2.79
C. D. at 5% level (Variety) =0.47
C. D. at 5% level (Dose) =0.23

*Log number of cell counts/g of cultures indicated within the parentheses for each dose.

TABLE IV

Mean per cent chlorosis in twelve varieties of soybean inoculated with different doses of Rhizobium japonicum (S_6)

Varieties	Doses of inoculants* I (6.28)	II (6.98)	III (7.28)	IV (7.98)	V (8.28)	VI (8.98)	Average
Baramali	16.1	28.4	27.6	32.1	45.7	83.4	38.9
B. M. J.	33.4	29.7	25.1	91.3	50.0	39.1	44.8
EC 1774	33.4	40.4	41.7	69.1	63.5	60.1	51.3
EC 2542	37.0	33.4	71.5	30.0	29.5	46.7	41. 4
IC 196	18.4	22.2	25.7	35.7	48.8	54.6	34.2
IC 202	41.2	46.8	45.6	36.7	38.1	39.0	41.2
IC 216	20.1	28.7	60.3	40.0	36.9	27.6	35.6
IC 219	41.3	39.0	46.7	36.7	38.1	45.6	41.2
K-16	39.0	23.9	36.0	28.1	37.5	34.6	33.2
K-30	25.3	21.1	43.5	37.6	41.2	70.1	39.8
Nepal	43.0	45.6	80.1	40.6	38.1	36.7	47.4
Soymax	33.4	50.1	85.7	60.4	48.1	45.6	53.9
Average	31.8	34.1	49.1	44.9	43.0	48.6	

C. D. at 5% level (Treatments) =4.32
C. D. at 5% level (Variety) =0.72
C. D. at 5% level (Dose) =0.36

*Log number of cell counts per g of cultures indicated within the parentheses for each dose.

TABLE V

Analysis of variances of nodular characters and per cent chlorosis in soybean varieties induced by Rhizobium japonicum (S_6)

Sources of variation	d. f.	Mean sum of squares: Number of nodules	Nodular fresh weight	Nodule size	Per cent chlorosis
Replication	2	5.56	89.59	0.03	16.39
Treatment	71	186.00**	13843.79**	165.52**	754.55**
Variety	11	996.64**	73877.25**	1027.41**	752.29**
Inoc. dose	5	103.75**	7468.20**	6.29	1946.75**
Interaction	55	31.34**	2416.68**	7.61	646.62**
Error	142	7.13	240.97	9.13	21.82

**Significant at 1% level.

(i.e. nodule size) was obtained in case of B.M.J., EC 1774, IC 196, IC 219, K-16, Nepal and Soymax. However, no significant relationship was found to exist between nodular fresh weight and per nodule fresh weight except in Baramali, IC 216 and Nepal. Although nodule number was apparently correlated with percentage of chlorosis, this relationship was statistically significant only in case of Baramali, EC 1774, EC 2542, IC 196, IC 216 and K-30. But positive correlation between nodular fresh weight and chlorosis percentage was found significant in all the cases except in B.M.J. and IC 202.

TABLE VI

Correlation coefficients between nodular characters and per cent chlorosis in twelve varieties of soybean

Varieties	Nodule No. and Nodule wt.	Nodule No. and Nodule size	Nodule wt. and Nodule size	Nodule No. and Chlorosis (%)	Nodule wt. and Chlorosis(%)
Baramali	0.689**	−0.103	0.633**	0.704**	0.964**
B. M. J.	0.762**	—0.671**	—0.051	0.048	0.157
EC 1774	0.838**	—0.729**	—0.179	0.795**	0.927**
EC 2542	0.686**	—0.360	0 425	0.535*	0.821**
IC 196	0.779**	—0.873**	—0.456	0.776**	0.910**
IC 202	0.585*	—0.445	0.456	0.025	0.067
IC 216	0.549*	—0.450	0.475*	0.635**	0.845**
IC 219	0.638**	—0.861**	—0.205	0.119	0.674**
K-16	0.384	—0.820**	0.158	0.289	0.676**
K-30	0.737**	—0.458	0.242	0.647**	0.842**
Nepal	0.391	—0.610**	0.478*	0.372	0.771**
Soymax	0.411	—0.879**	—0.001	0.427	0.683**
Pooled	0.023	—0.706**	0.530**	0.283**	0.326**

** Significant at 1% level.
* Significant at 5% level.

Effects of population density of the inoculants on nodular characters, as well as on chlorosis percentage, in twelve varieties of soybean were studied and are represented in terms of regression coefficients (Table VII). Population density of the inoculants was found to have contributed significantly towards the number of nodules, nodular fresh weight and percentage of chlorosis in respect of four varieties only (viz. Baramali, EC 1774, IC 196 and K-30). Regression coefficients of number of nodules on population density of the inoculant in B.M.J. and IC 216; coefficients of nodular fresh weight on population in EC 2542 and Soymax; and of chlorosis percentage on population density in EC 2542, IC 202 and Nepal, were not only insignificant but indicated a negative trend.

TABLE VII

Regression coefficients of nodular characters and per cent chlorosis on population density of the inoculant in twelve varieties of soybean

Varieties	Number of nodules	Nodular fresh weight	Per cent chlorosis
Baramali	5.2257**	29.8948**	21.8715*
B.M.J.	—0.0591	9.2677	9.2957
EC 1774	4.8966**	41.7400**	12.8493*
EC 2542	0.6981	—22.8052	—0.8360
IC 196	2.6646**	32.1168**	14.6957**
IC 202	0.9652	23.0000	—2.4772
IC 216	—0.6466	1.9575	1.7620
IC 219	3.7542	10.8672	0.3624
K-16	0.0867	2.3548	0.0289
K-30	4.1519**	41.4318**	15.0793**
Nepal	0.5110	0.3257	—5.7390
Soymax	1.7080	—9.1525	1.3314
Pooled	1.5886	12.9530	5.6875**

** Significant at 1% level.
* Significant at 5% level.

DISCUSSION

Nodulation : Inter-varietal differences in respect of average number of nodules as well as total nodular fresh weight per plant may be produced by a specific strain of *Rhizobium*. Data presented in tables I and II confirm the above relationship; such differences being associated with different genomic constitution of the host-germplasm. Similar results were reported earlier by a number of investigators (Nutman, 1967; Arutjunjan, 1964; Dobereiner *et al.*, 1966). However, variation in respect of number of nodules has been found induced by different rhizobial strains as well (Chen & Thornton, 1940). So, it may be justified to suggest that it is the complex genomic interaction between both the symbiotic partners that actually determines the average number of nodules and so also the total nodular fresh weight. Similarly, the average size of nodules has also been found to vary from one variety to the other (Table III) which is in conformity with the earlier findings of Nutman (1959; 1967); But, this character is also influenced by the host-rhizobium interaction indirectly, because the size of the nodules was often found inversely correlated with the average number of nodules produced (Nutman, 1959; Sen & Bhaduri, 1971; Banerjee, 1974; Chandra, 1974), although such relationship does not hold good in a few cases (Nutman, 1949; Bowen & Kennedy, 1961). Further evidence in support of the former contention as against the latter is the association of large and small nodules with the sparse and abundantly nodulating habits respectively (Nutman, 1952).

The present findings show that by increasing the doses of inoculum, there was an increase in nodule number and total fresh weight of the nodules although their magnitudes varied from one variety to the other. In some varieties, however, this relationship was not so distinct as in others (Tables I and II). This finding has also been confirmed from the regression analysis (Table VII). Population density of the inoculant contributed significantly towards the number of nodules and nodular fresh weight in respect of four varieties only; in the remaining varieties, its contribution was insignificant. The regression coefficients of number of nodules on population density of the inoculant in B.M.J. and IC 216 and that of nodular fresh weight on population in EC 2542 and Soymax were not only insignificant but indicated a negative trend. Hence, nodulating behaviour of different host-germplasms against increasing doses of inoculants differed considerably. Relationship between nodulation and population density of rhizobia in rhizospheres has been described differently by various workers. Perkins (1925), Thornton (1929) and Bhaduri (1951) found that nodulation was related to the size of the population of the nodule bacteria in the rhizosphere, but not in simple proportion. According to Purchase and Nutman (1957), nodule number increases asymptotically to a maximum with increase in bacterial density; and their data were best fitted to either of the two forms of a compound 'Mitscherlich Equation'. Lim (1963) indicated that the amount of infection made by inocula of varying sizes is distributed following Poisson's Law of Distribution. Quispel (1954a, b) found that the number of nodules produced on *Alnus glutinosa* did not increase in simple proportion to inoculum size. On the basis of the findings obtained during the present investigation as well as from the available literatures cited above, it would appear that no generalized statement in this regard would be tenable because of the differential response of different hosts to specific rhizobium strains. However, from the data obtained during the present investigation, it may be pointed out that the average size of the nodules for a particular variety remained constant and was independant of the doses of the inoculants used (Table III). Nutman (1959) has also reported similar results.

Chlorosis : Inter-varietal difference in respect of response to chlorosis (Table IV) is corroborative to the earlier reports made by Erdman *et al.* (1957), Johnson and Means (1960) and Bhaduri *et al.* (1968). This is probably due to the differential host-rhizobial interactions that have resulted to differential expression of chlorosis as suggested earlier by Johnson and Means (1960). The overall picture shows that increased doses of inoculants induced increasing degree of chlorosis which might be attributed to the increased nodulation. Besides, the regression analysis revealed that population density of inoculants contributed significantly towards expression of chlorosis as well as nodulation in four varieties only. When different host varieties were compared with each other, they did not show any linear relationship in this regard. So, the whole phenomenon becomes more complex and as such it would be more reasonable to suggest that the resultant of two interacting components ultimately governs the net expression of chlorosis. These are :

(i) Nodulating efficiency of the chlorosis inducing rhizobial strain to a particular host genotype; and

(ii) Per nodular contribution or contribution of per unit mass of nodular tissue to the degree of chlorosis expressed in that particular host.

Relationship between nodulation and chlorosis : From further analysis of the data (Table VI) it may be noted that the relationship between different nodular characters (viz. nodule number, nodular fresh weight and nodule size) remained almost similar as suggested earlier by different workers (Nutman, 1959; Sen & Bhaduri, 1971; Banerjee, 1974; Chandra, 1974). The interesting finding that has emerged out of the present investigation is the differential nature and degree of relationships between different nodular characters as experienced by different host varieties. This may be accounted as due to their differential susceptibilities as well as due to different kinds of interactions to a specific strain of *Rhizobium.*

Expression of chlorosis has been found to be positively correlated with nodulation (i.e. either nodule number or nodular fresh weight). However, this relationship was more prevalent only in case of nodular fresh weight. This may be explained in the light of the findings reported earlier by Chen and Thornton (1940) who found a strong correlation between fresh weight of nodules and the volume of the bacterial tissue; and it is the bacterial tissue which is supposed to be responsible, directly or indirectly, for the induction of chlorosis (Johnson & Clark, 1958; Johnson *et al.*, 1959; Owens, 1961).

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