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Rhizobium-Induced Chlorosis in Soybean. II.* Effect of Ageing

M C KABI and P N BHADURI, FNA
Nodule Research Laboratory, Bidhan Chandra Krishi Viswa Vidyalaya, Mohanpur 741246, West Bengal

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Nodulation in soybean increased with the increasing age of the host plant to a peak at flowering time and then it declined. The size of the nodules also increased with increasing age of the host. The rate of nodulation with increasing age varied considerably between different varieties. The host varieties differed from each other in respect of the time taken for initiation of chlorosis in them. Expression of chlorosis increased with age of the host. It attained its peak and then started declining till it was fully recovered. Reappearance of chlorosis did not occur afterwards.

Key Words: Chlorosis, Nodulation, *Rhizobium*

Introduction

Chlorosis in soybean induced by specific strains of *Rhizobium* (Erdman et al. 1956) occurs on the upper leaves before initiation of flowering. Such chlorosis is subsequently recovered almost spontaneously during later stages of plant growth. Nodules being the exact source of stimulation in inducing chlorosis (Johnson & Clark 1958) and as its expression is positively correlated with nodulation (Kabi & Bhaduri 1979), it is reasonable to expect that increased nodular activity with advancement of age of the host would be reflected on the expression of the amount of chlorosis. It is generally agreed that the nodulation is increased with the advancement of age of plant, reaching its peak after which it starts declining (Pate 1958a, b; Sen & Bhaduri 1971, Gibson 1974).

The nodulation and expression of chlorosis at different ages of growth in different host germplasms of soybean are discussed.

Materials and Methods

The soybean varieties and *Rhizobium* strain used and other management and cultural

*This is part II in the series. Part I is....Effect of population density of the inoculants; *Proc. Indian natn. Sci. Acad.* B45, pp. 163-174 (1979)

practices followed in these experiments were the same as described previously by the authors (Kabi & Bhaduri 1979). The population density of the inoculum used was maintained strictly at 10^6 cells per g of charcoal-based inoculants. Seeds in all the treatments were sown simultaneously but samplings of plants of different treatments were collected at different ages of plant growth, viz., 4, 6, 8, 10 and 12 weeks. Observations on nodulation and chlorosis were recorded at these ages following usual methods.

Results

In general, the number of nodules increased with ageing of the plants, reaching its peak around the 8th week and then started declining (table 1). Further, the rate of nodulation at different weeks varied considerably in different varieties. While EC 1774, EC 2542 and IC 216 produced maximum number of nodules during 6th week, IC 196 and K-16 produced the same amount during the 10th week. However, in rest of the varieties, the number of nodules was maximum during the 8th week.

The nodular fresh weight of different varieties varied at different weeks (table 2)—the nature of variation with increasing ages of the plants was similiar to that observed in the number of nodules.

The size of the nodules increased with increasing age of the plant also (table 3). However, the rate of increase in nodule size was different in different varieties.

The appearance of chlorosis commenced from the 4th week in IC 216, K-30 and Soymax while in rest of the varieties it started from the 6th week (table 4) indicating that different varieties responded differently in respect of appearance of chlorosis at different ages of the plants. However, in all the varieties, the nature of expression of chlorosis was that at first it increased and then after reaching to its peak it declined with the advancement of age.

Statistical analysis of the data (table 5) revealed that both the varieties as well as ages contributed significantly in inducing variation in nodulation and also in the expression of chlorosis. Except for the nodule size, variety × age interaction also played an important role in inducing variability.

Discussion

Progressive increase in nodulation with age of the host plant was evident till peak nodulation was attained. Afterwards, nodulation started declining, probably due to progressive scenescence of the older nodules (tables 1 and 2). This is in agreement with the earlier findings of Sen and Bhaduri (1971). According to Pate (1958a, b), nodular activity in *Pisum arvense* and *Vicia sativa* rapidly declines following initiation of flowering. But with *Centrosema pubescens* (Bowen 1959), *Desmodium unicatum*, *Phaseolus atropurpureus* (Whiteman & Lulham 1970), *D. intortum* and *D. sandwicense* (Whiteman 1970), it was difficult to establish direct relationship between flowering and nodule scenescence. In soybean, it is known that the varieties belonging to earlier maturity groups showed peak nodular activity during flowering, followed by a rapid decline at the end of flowering (Gibson 1974). Vincent (1974) has pointed out that the longivity of nodules usually reflects the growth habits of the hosts. Nodules of the herbaceous legumes are relatively fragile and function for a relatively shorter period during the plant's active vegetative growth. Removal of plant tops, unfavourable moisture conditions, lack of photosynthesis and attack by nematodes, fungi or insects can lead to their disintegration or shedding. 'Nodule necrosis' begins in the oldest (basal) area of the

Table 1 *Mean number of nodules at different ages in twelve varieties of soybean inoculated with* Rhizobium japonicum (S_6)

Varieties	Age of plants (in weeks)					Average
	4th	6th	8th	10th	12th	
Baramali	10.3	14.5	22.0	13.3	9.4	13.9
B.M.J.	12.5	16.6	25.8	19.7	12.6	17.4
EC 1774	15.3	26.0	23.7	14.5	10.2	17.9
EC 2542	9.1	13.3	13.0	11.5	8.5	11.1
IC 196	6.5	8.5	7.3	8.9	5.2	7.3
IC 202	8.6	11.3	15.8	12.4	9.3	11.5
IC 216	23.7	28.9	27.5	21.5	19.7	24.3
IC 219	16.6	20.5	23.3	18.5	12.4	18.3
K-16	5.5	8.2	10.6	11.0	7.2	8.5
K-30	12.0	17.6	26.5	16.6	9.3	16.4
Nepal	15.1	20.0	26.8	18.5	10.9	18.3
Soymax	12.3	14.0	19.7	13.6	9.5	13.8
Average	12.3	16.6	20.2	15.0	10.4	

C.D. at 5% level (Treatments)= 2.57
C.D. at 5% level (Variety) = 0.52
C.D. at 5% level (Age) = 0.21

Table 2 *Mean nodular fresh weight (in mg) at different ages in twelve varieties of soybean inoculated with* Rhizobium japonicum (S_6)

Varieties	Age of plants (in weeks)					Average
	4th	6th	8th	10th	12th	
Baramali	27.3	46.4	70.5	64.5	54.5	52.6
B.M.J.	112.2	154.3	286.4	225.5	155.3	186.7
EC 1774	116.3	281.3	239.3	186.6	132.3	191.2
EC 2542	143.4	264.3	247.5	243.1	184.2	216.5
IC 196	155.3	236.5	230.1	292.2	168.4	216.5
IC 202	113.6	207.6	273.5	245.4	184.6	204.5
IC 216	107.4	138.4	121.4	125.5	160.4	120.0
IC 219	165.2	231.6	262.2	223.3	163.3	209.1
K-16	108.3	210.2	265.5	264.5	208.6	211.4
K-30	78.4	147.4	220.6	161.2	116.4	144.8
Nepal	140.1	232.2	296.4	217.6	148.5	207.0
Soymax	136.5	185.4	264.5	190.6	151.4	185.7
Average	117.0	194.6	231.5	203.3	147.9	

C.D. at 5% level (Treatments) = 11.01
C.D. at 5% level (Variety) = 2.20
C.D. at 5% level (Age) = 0.92

Table 3 *Mean nodule size (per-nodule fresh weight in mg) at different ages in twelve varieties of soybean inoculated with* Rhizobium japonicum (S_6)

Varieties	Age of plants (in weeks)					Average
	4th	6th	8th	10th	12th	
Baramali	2.82	3.19	3.29	4.82	6.10	4.05
B.M.J.	9.27	9.43	11.24	11.66	12.54	10.83
EC 1774	7.89	10.81	10.17	12.91	13.12	10.98
EC 2542	15.84	20.25	19.30	21.30	22.50	19.84
IC 196	24.72	28.47	32.25	33.61	33.80	30.57
IC 202	13.53	18.57	17.46	19.99	20.52	18.03
IC 216	4.55	4.80	4.49	5.88	5.62	5.07
IC 219	10.09	11.68	11.51	12.27	13.38	11.79
K-16	21.29	25.70	25.52	26.59	29.04	25.63
K-30	6.86	8.75	8.37	9.87	15.52	9.27
Nepal	9.42	13.64	11.27	12.79	13.64	12.15
Soymax	11.36	13.63	14.00	14.58	16.18	13.95
Average	11.47	14.08	14.07	15.53	16.58	

C.D. at 5% level (Treatments) = 3.40
C.D. at 5% level (Variety) = 0.68
C.D. at 5% level (Age) = 0.28

Table 4 *Percent chlorosis at different ages in twelve varieties of soybean inoculated with* Rhizobium japonicum (S_6)

Varieties	egA of plants (in weeks)					Average
	4th	6th	8th	10th	12th	
Baramali	0.00	27.27	33.43	24.98	14.28	19.99
B.M.J.	0.00	31.50	50.25	46.66	12.19	28.12
EC 1774	0.00	33.33	28.57	13.33	8.33	16.71
EC 2542	0.00	15.38	40.04	29.43	11.13	19.20
IC 196	0.00	25.02	25.74	14.31	2.54	13.52
IC 202	0.00	40 06	26.66	8.63	3.23	15.72
IC 216	14.28	29.09	25.05	16.73	16.64	20.36
IC 219	0.00	36.69	41.26	26.70	7.18	22.37
K-16	3.00	35.05	38.54	28.61	13.36	23.71
K-30	5.55	26.62	33.35	16.25	5.86	17.53
Nepal	0.00	36.65	43.31	28.59	13.32	24.37
Soymax	50.00	76.64	90.29	20.09	10.95	49.59
Average	6.07	34.44	39.71	22.86	9.92	

C.D. at 5% level (Treatments) = 3.74
C.D. at 5% level (Variety) = 0.76
C.D. at 5% level (Age) = 0.32

Table 5 *Analysis of variances of nodular characters and percent chlorosis in soybean varieties induced by* Rhizobium japonicum (S_6) *at different doses*

Sources of variation	d.f.	Mean sum of squares			
		Number of nodules	Nodular fresh weight	Nodule size	Percent chlorosis
Replication	2	1.81	13.84	2.87	1.95
Treatment	59	114.80**	13997.69**	186.05**	1015.67**
Variety	11	348.56**	36941.95**	937.05**	1332.70**
Age	4	523.80**	75614.74**	133.16*	7803.78**
Interaction	44	19.18**	2660 08**	3.11	319.32
Error	118	7.76	142.11	13.54	16.77

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

bacteroid zone. Autolysis leads to the disintegration of the host cells and collapse of their walls but the remains of the bacteroids disappear even more rapidly. Residual intercellular rhizobia therefore are likely to multiply in the intercellular space and invade the middle lamellae of the host cell walls. In the present investigation, nodulation peaks of different varieties varied due to the variation of their peak flowering times. So, it may be concluded that nodulation is maximum during peak flowering time of the plants' growth. During actual reproductive phase of the plants, i.e., at the time of pod formation, nodulation starts declining.

It has also been noted that the rate of nodulation at different weeks varied considerably among different varieties which might be chiefly attributed to the genomic differences of the host varieties. Because Nutman (1948, 1953) has pointed out that the rate of nodulation is considerably influenced by the host genome.

Although it is well known that the number of nodules bears an inverse relationship with average size of the nodules (Nutman 1959, Sen & Bhaduri 1971, Banerjee 1974, Chandra 1974, Kabi & Bhaduri 1979), the latter was also found to be increased with increasing age of the plants. This is due to the continued activity of the meristematic tissue of the nodules and till the bacteroid tissue becomes fully developed and mature. Increase in the average size of the nodules with increase in age (table 3) has also been reported earlier (Pate 1958a, b). But here again, the rate of increase in the average size of the nodules was found to vary from variety to variety which might be due to the intrinsic differences between the host-genotypes. The inverse relationship between the number and size of the nodules holds good at any point of the age of the plant.

Different varieties of soybean responded differently towards the appearance of chlorosis at different ages (table 4). This is probably due to the variation in the time required for nodule initiation in different varieties which ultimately influence the expression of chlorosis. There may exist a threshold condition for induction of chlorosis. This appears to vary from one host-genome to the other. Unimodal nature of distribution of the degree of chlorosis with increasing ages (table 4) might have resulted from the same nature of relationship obtained for nodulation

at different ages (table 1). Therefore, it is clear that chlorosis appears only after the initiation of the nodular activity and recovery of chlorosis would occur as soon as nodular activity ceases. Johnson and Clark (1958) have indicated that chlorosis develops only after the formation of root nodules. That nodules are the only source of stimulation for induction of chlorosis has been confirmed by Johnson et al. (1959) and Owens and Wright (1965). But after the full recovery of chlorosis the plants still possessed fewer nodules but these were either not sufficient enough to induce chlorosis to the fully mature plant or after a certain period of growth the plant itself does not permit to induce chlorosis any further or certain changes in the metabolic state of the plant that have occurred during the reproductive phase of growth, normal development of the chlorophyll does not get interrupted. Whatever may be the reason, in none of the treatments, reappearance of chlorosis occurred after the full recovery of chlorosis.

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