HOMEOSTATIC CONTROL IN NODULATION

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Seeds of two varieties of soybean were treated with different doses of X-rays. X_1 seedlings and distinct variants of X_3 population were inoculated with four serologically distinct strains of *Rhizobium japonicum* both individually and mixed in equal proportion.

In general, some proportionality was found between the induced variability in plant characters (excluding nodulation) and the X-ray doses employed. The induced variation in nodule number and total nodule volume could be represented by a normal unimodal curve plotted against the increasing doses of X-rays showing that there exists an autonomous control which tends to conserve the normal limit of nodular character even when subjected to X-irradiation.

Changes induced by X-rays in the host genotype did not produce any significant change in the number of respective nodule sites of individual competing strains in a mixed inoculum. The existence of an autonomous control in nodulation is thus further supported.

INTRODUCTION

Considerable amount of work has been done in the past in inducing mutations in soybean (Humphrey 1951, 1954, 1959; Zacharias 1956; Papa *et al.* 1961; Rawlings *et al.* 1958; Dunleavy 1959). It has been well established that application of X-rays and thermal neutrons to soybean seeds increase the estimated genetic variability in quantitative characters. In the present paper a brief account of experiments has been presented that were performed to understand basic aspects of the effect of X-irradiation to soybean and the consequent changes induced in the host-*Rhizobium* relationship. Previous investigations done on this line by William and Lynch (1954), Caldwell (1966) and Visona *et al.* (1964) have not led to any definite conclusion.

MATERIALS AND METHODS

Seeds of two varieties of soybean, Soymax and K-30 (recommended by the State Agricultural Department for grain and fodder types respectively) were treated with different doses of X-rays (30, 50, 60, 70, 80 and 90 KR). Plants were raised from the irradiated seeds kept in sterilised bottles containing sterilised sand medium, supplemented with Hogland's nitrogen-free nutrient solution. Seedlings thus raised in the bottles were inoculated with a definite population of serologically distinct strains of *Rhizobium japonicum* (BU/S4, BU/S5, BU/S6 and BU/S8) both individually as well as with a mixture of four strains given in equal proportion. Nodules were harvested from these plants during the onset of flowering and nodule number, total nodule volume and per nodule volume were determined in each case. Isolates from single nodules were grown on YWA-mannitol medium and each isolate was

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serologically tested. Variations in the general characters of X_1 plants, including the percentage of germination, leaf-type, plant height, branch number, nature of pubescence, pod colour, pollen and seed sterility, seed weight and seed size were recorded. X_2 plants were raised from the selfed seeds of the selected X_1 plants. From the seeds of the X_2 population X_3 populations were raised and inoculated in the same manner as was done with the X_1 seeds. From the harvested nodules of the X_3 progenies, isolations were made on YWA-mannitol slants and their serological reactions were determined.

Results and Discussion

In general, some amount of proportionality was found between the induced variability in plant characters and the X-ray doses employed. However, changes in the number and volume of nodules did not show any linearity with the increasing doses of X-rays. The induced variation in nodule number and volume could be represented by a normal unimodal curve plotted against the increasing doses of X-rays (Table I). Fig. 1 and 2 show the normal distribution of nodule number and nodule volume both in the treated and control population. It suggests that either nodulation is not at all affected by the X-ray doses in contrast to other plant characters or there exists an autonomous control which tends to conserve the normal limit of the nodular characters even when subjected to such an adverse external influence of X-irradiation. The former assumption is not tenable because of the following observations :

The statistically computed and pooled data presented in the table show that nodule number, total nodule volume and per nodule volume responded differently to different doses of X-rays. The four serologically distinct testing strains of *Rhizobium* also responded differentially to the nodular characters. The genotypic

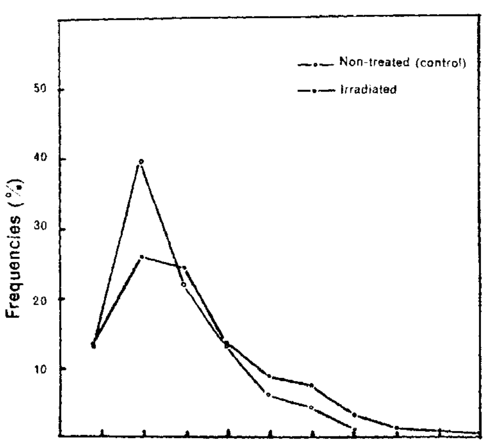

Fig. 1. Frequency distribution of nodule number in irradiated and control population of Soybean.

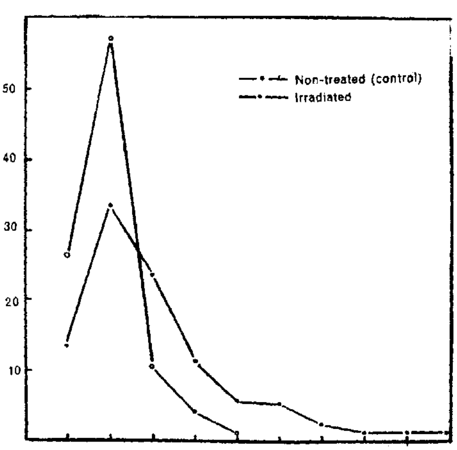

Fig. 2. Frequency distribution of nodule volume in irradiated and control population of soybean.

TABLE 1

Mean values and standard errors of the nodule number, total nodular volume and per-nodule volume of the X-ray treated soybean (vars. K-30 and Soymax)

Variety	Doses of X-rays	Nodule number $\pm S_{\bar{x}}$	Total nodular volume $\pm S_{\bar{x}}$	Per-nodule volume $\pm S_{\bar{x}}$
K-30	0 KR	18.7 ± 2.17	0.147 ± 0.017	0.0079 ± 0.00017
	30 KR	31.7 ± 2.72	0.277 ± 0.032	0.0090 ± 0.00049
	50 KR	23.9 ± 2.23	0.277 ± 0.038	0.0127 ± 0.00220
	60 KR	32.1 ± 3.09	0.450 ± 0.061	0.0146 ± 0.00310
	70 KR	24.6 ± 3.13	0.260 ± 0.034	0.0104 ± 0.00044
	80 KR	16.9 ± 1.77	0.214 ± 0.007	0.0112 ± 0.00220
	90 KR	14.2 ± 1.82	0.117 ± 0.011	0.0096 ± 0.00012
Soymax	0 KR	20.9 ± 0.86	0.146 ± 0.011	0.0082 ± 0.00040
	30 KR	24.3 ± 2.48	0.216 ± 0.022	0.0094 ± 0.00062
	50 KR	24.9 ± 1.79	0.221 ± 0.025	0.0088 ± 0.00152
	60 KR	25.9 ± 2.36	0.283 ± 0.029	0.0128 ± 0.00322
	70 KR	24.6 ± 3.01	0.198 ± 0.020	0.0092 ± 0.00062
	80 KR	12.1 ± 1.28	0.106 ± 0.011	0.0095 ± 0.00062
	90 KR	11.4 ± 1.28	0.106 ± 0.009	0.0101 ± 0.00125

difference between the soybean varieties had direct effect on the number of nodules only and not on the total nodule volume and per nodule volume. These observations partly corroborated the findings madee arlier by Nutman (1967) in subterranean clover. He found that nodule number and the whole nodule volume were dependent on bacterial strains. With a few exceptions host and strain difference tended to be independent. The average nodule length was dependent on bacterial strains and often regulated by the host genome. The whole nodule volume was strongly influenced by the bacterial strains, but the relative volumes of the different tissues of the nodules were under the dual control. Different doses of X-rays and different strains of *Rhizobium* when considered together, could exert considerable influence on all the three aforesaid nodular characters, but on the other hand these characters remained unaffected from the combined influence of the different doses of X-rays and host varieties. The host genome, with different combinations of the inoculating strains could produce significant change in the nodule number and per nodule volume only. When three factors, i.e., host genome, doses of X-rays and inoculating strains were considered simultaneously they did not show any significant difference on the nodular characters. The situation became more complex when interactions of all the factors were taken into consideration for understanding the phenomenon of nodulation. The present data are inadequte to provide precise information in understanding the relative role of the individual factors in this complex interacting system.

TABLE II(a)

Competition between the strains of Rhizobium *in mixture of four at equal proportions against X-ray treated soybean (var. K-30) (Differences between observed and expected frequencies tested by χ^2-test)*

Doses of X-rays		Frequency of sero-recovery					
		BU/S_4	BU/S_5	BU/S_6	BU/S_8	Total	S_n
	O =	52.00	52.00	28.00	16.00	148.00	4.00
0 KR	E =	37.00	37.00	37.00	37.00		
	χ^2 = 26.25***						
	O =	48.00	30.00	27.00	24.00	129.00	15.00
30 KR	E =	32.25	32.25	32.25	32.25		
	χ^2 = 10.80*						
	O =	48.00	42.00	24.00	12.00	126.00	18.00
50 KR	E =	31.50	31.50	31.50	31.50		
	χ^2 = 25.99***						
	O =	45.00	35.00	25.00	10.00	115.00	35.00
60 KR	E =	28.75	28.75	28.75	28.75		
	χ^2 = 23.26***						
	O =	42.00	36.00	30.00	18.00	126.00	36.00
70 KR	E =	31.50	31.50	31.50	31.50		
	χ^2 = 10.00*						
	O =	35.00	45.00	25.00	10.00	115.00	30.00
80 KR	E =	28.75	28.75	28.75	28.75		
	χ^2 = 23.36***						
	O =	30.00	36.00	24.00	18.00	108.00	42.00
90 KR	E =	27.00	27.00	27.00	27.00		
	χ^2 = 6.66(NS)						

O = Observed frequency; E = Expected frequency (i.e., 1:1:1:1); * = Significant at 5% level; *** = Significant at 0.1% level; NS = Not significant; df=3.

The inter-strain competition in nodulation of two host varieties changed considerably after X-irradiation. Although, X-irradiation does exert its influence on the host in determining its differential preferentiality towards the infecting strains of *Rhizobium*, the order of relative competing ability of the inoculating strains in the inter-strain competition remained almost unaffected (Tables IIa and IIb). Strain Nos. BU.S4 and BU.S5, as in the control material, were found always superior to strain Nos. BU.S6 and BU.S8 when inoculated to X_1 progenies. Even in the

TABLE II(b)

Competition between the strains of Rhizobium *in mixture of four at equal proportions against X-ray treated soybean (var. Soymax). (Differences between observed and expected frequencies tested by χ^2-test)*

Doses of X-rays		Frequency of sero-recovery					
		BU/S_4	BU/S_5	BU/S_6	BU/S_8	Total	S_n
	O =	48.00	44.00	28.00	12.00	132.00	8.00
0 KR	E =	33.00	33.00	33.00	33.00		
	χ^2 = 24.58***						
	O =	48.00	48.00	20.00	12.00	128.00	20.00
30 KR	E =	32.00	32.00	32.00	32.00		
	χ^2 = 23.62***						
	O =	45.00	45.00	20.00	15.00	125.00	20.00
50 KR	E =	31.25	31.25	31.25	31.25		
	χ^2 = 24.60***						
	O =	52.00	40.00	16.00	12.00	120.00	32.00
60 KR	E =	30.00	30.00	30.00	30.00		
	χ^2 = 36.90***						
	O =	55.00	55-00	25.00	15.00	150.00	20.00
70 KR	E =	37.50	37.50	37.50	37.50		
	χ^2 = 34.29***						
	O =	60.00	50.00	10.00	20.00	140.00	25.00
80 KR	E =	35.00	35.00	35.00	35.00		
	χ^2 = 48.54***						
	O =	36.00	42.00	18.00	18.00	114.00	36.00
90 KR	E =	28.50	28.50	28.50	28.50		
	χ^2 = 16.08**						

O = Observed frequency; E = Expected frequency (i.e., 1:1:1:1); ** = Significant at 1% level; *** = Significant at 0.1% level; NS = Not significant; df = 3.

X_3 progenies this relationship remained more or less unchanged (Table V). Changes in the host genotypes induced by X-rays did not produce any significant change in the number of respective nodule sites of individual competing strains for a mixed inoculum. Unknown serotypes which normally appear during nodule isolation started to appear more frequently with the increasing doses of X-rays. Appearance of induced phenotypic variations also showed some proportionality with the frequency of occurence of unknown serotypes. Observations recorded above in regard to the influence of X-irradiation on inter-strain competition suggest that an autonomous control in nodulation exists in the symbiotic system.

TABLE III

Analysis of variances of the nodule number, total nodular volume and per-nodule volume among the X_1 population of soybean

Sources of variations	Mean sum of squares		
	Nodule number	Total nodular volume	Per-nodule volume
1. Replication	228.46	0.008	0.0001225
2. Treatments :			
(i) Variety	817.78*	0.234	0.0003500
(ii) Dose of X-ray	2058.81**	0.425**	0.0005150**
(iii) Inocula (strain)	510.03**	0.135**	0.0005850**
(iv) Var. × X-ray	145.34	0.022	0.0001650
(v) Var. × Strain	599.53**	0.018	0.0003500*
(vi) X-ray × Strain	236.72*	0.054**	0.0003195**
(vii) Var. × Strain × X-ray	118.86	0.019	0.0002662**
3. Error	156.49	0.015	0.0001247

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

TABLE IV

Mean values and standard errors of the nodule number, total nodular volume and per-nodule volume of the mutations of soybean (vars. K-30 and Soymax) raised in the X_3 generation

Mutants	Nodule number ± Sx	Total nodular volume ±Sx	Per-nodule volume ±Sx
1. K-30 (Control)	25.3 ± 5.92	0.120 ± 0.014	0.00554 ± 0.00057
2. K-30/30/34	17.0 ± 2.03	0.081 ± 0.007	0.00507 ± 0.00044
3. K-30/50/5	31.8 ± 4.84	0.138 ± 0.010	0.00503 ± 0.00054
4. K-30/80/3	25.2 ± 1.10	0.101 ± 0.014	0.00451 ± 0.00037
5. K-30/80/10	24.8 ± 1.57	0.105 ± 0.004	0.00427 ± 0.00022
6. Soymax (Control)	9.8 ± 2.25	0.068 ± 0.014	0.00759 ± 0.00109
7. Soy/30/1	22.5 ± 4.00	0.123 ± 0.020	0.00513 ± 0.00066
8. Soy/30/9	10.1 ± 1.43	0.067 ± 0.008	0.00606 ± 0.00092
9. Soy/30/13	18.5 ± 3.02	0.087 ± 0.010	0.00556 ± 0.00093
10. Soy/30/14	16.6 ± 3.29	0.070 ± 0.012	0.00468 ± 0.00044
11. Soy/30/18	24.6 ± 5.60	0.128 ± 0.034	0.00522 ± 0.00040
12. Soy/50/9	21.5 ± 6.60	0.091 ± 0.018	0.00532 ± 0.00084
13. Soy/70/9/2	10.8 ± 1.32	0.060 ± 0.019	0.00705 ± 0.00032
14. Soy/90/2	13.0 ± 2.76	0.065 ± 0.010	0.00567 ± 0.00069

TABLE V

Competition between the strains of Rhizobium *in a mixture of four at equal proportions against the mutations raised through X-irradiation of soybean (vars. K-30 and Soymax) (Variations of the observed frequency of sero-recovery of the nodules of the mutants from that of their respective parents tested by χ^2-test*

Mutants			Frequency of sero-recovery (%)			
			BU/S_4	BU/S_5	BU/S_6	BU/S_8
	O =		18.18	31.82	22.73	27.27
1. K-30/30/34	E =		42.31	38.46	7.69	11.54
	χ^2 =	65.75***				
	O =		52.38	35.72	7.14	4.76
2. K-30/50/5	E =		42.31	38.46	7.69	11.54
	χ^2 =	6.60(NS)				
	O =		30.31	18.18	27.27	24.24
3. K-30/80/3	E =		42.31	38.46	7.69	11.54
	χ^2 =	77.91***				
	O =		30.91	36.36	20.00	12.73
4. K-30/80/10	E =		42.31	38.46	7.69	11.54
	χ^2 =	23.00***				
	O =		56.52	34.78	4.35	4.35
5. Soy/30/1	E =		40.90	31.84	13.63	13.63
	χ^2 =	18.85***				
	O =		53.85	30.76	3.85	11.54
6. Soy/30/9	E =		40.90	31.84	13.63	13.63
	χ^2 =	11.47**				
	O =		35.29	47.06	11.77	5.88
7. Soy/30/13	E =		40.90	31.84	13.63	13.63
	χ^2 =	12.68**				
	O =		47.62	38.10	4.76	9.52
8. Soy/30/14	E =		40.90	31.84	13.63	13.63
	χ^2 =	8.73*				
	O =		31.82	40.91	18.18	9.09
9. Soy/30/18	E =		40.90	31.84	13.63	13.63
	χ^2 =	7.55(NS)				
	O =		26.31	47.37	15.79	10.53
10. Soy/50/9	E =		40.90	31.84	13.63	13.63
	χ^2 =	13.81**				
	O =		35.30	47.06	11.76	5.88
11. Soy/70/9/2	E =		40.90	31.84	13.63	13.63
	χ^2 =	12.68**				
	O =		34.15	41.46	14.63	9.76
12. Soy/90/2	E =		40.90	31.84	13.63	13.63
	χ^2 =	5.17 (NS)				

O = Observed frequency of the mutants; E = Expected frequency, i.e., observed frequency of the parent varieties; *** = Significant at 0.1% level; ** = Significant at 1% level; * = Significant at 5% level; NS = Not significant; df=3.

Conclusions

It would appear that a leguminous nodule is not only an incidental appendage of the root or a mere structural manifestation of the host-rhizobial interaction. It has, on the contrary, a distinct biological identity characterised by a dynamic, self-regulatory system that maintains the internal environment within a limit. In other words, it may be stated that there is an internal homeostatic control in nodulation. Homeostatic effect in regulating the variability of agronomic charaters of soybean, grown under different environmental conditions has been previously reported by Byth and Weber (1968) and Auckland (1967). But homeostasis in nodulation is of unique a kind because it is regulated by two interacting organisms of absolutely different natures.

Acknowledgements

The author expresses his deep sense of gratitude to Prof. P. N. Bhaduri, Head of the Department of Botany, Burdwan University, for his direct supervision and guidance in this work. Thanks are also due to Sri K. K. Lahiri, for his helpful discussion during the course of this investigation. The financial assistance provided by the Indian Council of Agricultural Research in carrying out this research work is gratefully acknowledged.

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