

A FURTHER EXPERIMENT ON SOYA INOCULATION IN CEYLON

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IN a previous paper, the writers (Park and Fernando, 1937) recorded the results of preliminary tests of the nitrogen-fixing efficiency of strains of *Rhizobium japonicum* Kirch. A yellow-seeded variety of soybean, *Glycine max* (Linn.) Merr., inoculated with these strains, was grown in pots in sand and watered with a culture solution containing the various essential elements other than nitrogen. The results of the experiment showed that, under these conditions, seed inoculation resulted in increase in vigour in the plants and also that the plants reacted in different degree to the strains of *Rhizobium* used. In the present communication are presented the results of a field trial of these same strains.

DESIGN OF THE EXPERIMENT

Five inoculation treatments, *viz.*, inoculations with four strains of *R. japonicum* and an uninoculated control, were combined with two varieties of soybean. Three of the strains of *Rhizobium*, *viz.*, strains Nos. 73, 36 and 30 received through the courtesy of Dr. H. J. Toxopeus, Agricultural Experiment Station, Buitenzorg, had been isolated from plants grown in soils of different types in Middle, Eastern and Western Java, respectively. The fourth strain, which is referred to in this paper as the Rothamsted strain, was originally strain No. 9 of the Wisconsin Experiment Station, Wisconsin, U. S. A.; this strain was tested at the Rothamsted Experiment Station and proved so efficient under the conditions prevailing in England that it is to-day marketed commercially in England.

The names and the history of the two varieties of soybean have not been determined. They will be referred to here as varieties L and S. The variety S is identical with the one used in the preliminary pot experiments (Park and Fernando, 1937). It was originally imported from Poona. The variety L approximates to the well-known Mammoth Yellow variety. Summary

descriptions of the two varieties are given in Table 1. The varieties L and S appear to fall into groups 2 and 4 respectively in the classification of Kaltenbach and Legros (1936):—

TABLE 1

Variety of Soybean	Colour of Flowers	Colour of Pods	Average growth Period	Colour of Seeds	Colour of Hilum	Shape of Seeds	Dimensions of Seeds in Mms.		
							Length	Breadth	Diameter
L	..white	..grey-brown	..3 months	..bright yellow	..yellow	..rounded	8.5 ± 0.29	7.8 ± 0.27	6.3 ± 0.39
S	..purple	..grey-brown	..4 months	..dull yellow	..brown	..flattened ovoid	7.2 ± 0.31	5.6 ± 0.27	4.0 ± 0.19

The ten treatments were replicated in four randomized blocks. The experimental area, which was adjacent to the Plant Pathological Laboratory, Peradeniya, received dressings of lime on February 19 and March 5, these dressings being at the rate of 10 cwt. per acre. The soil was a heavy loam of fair nitrogen content and slightly acid in reaction (pH value = 6.16). The detailed analysis of the soil was as follows:

				Per Cent.
Gravel	2.41
Coarse sand	24.42
Fine sand	26.93
Silt	4.10
Clay	39.65
Moisture	2.73
Loss by solution	2.17
				100.00
				Per Cent.
Loss on ignition	8.33
Nitrogen	0.119

The presence of three citrus plants in the experimental area appeared to contribute considerably to the heterogeneity in soil fertility.

The area was divided up into plots measuring 4 feet by 8 feet with paths 2 feet wide between plots. Adjacent blocks were separated by paths 4 feet wide.

METHODS

Seed-disinfection.—It was considered desirable to free the seed testas of contaminating rhizobia prior to inoculation. In preliminary tests, surface-sterilization by wet heat, *viz.*, immersion in water at 65°C for 5 minutes, resulted in a 70 per cent. reduction in germination. Exposure to 0.1 per cent. mercuric chloride for 3 minutes followed by washing in several changes of tap water, had no depressant effect on germination. The experimental seed was disinfected by this method; the last traces of mercuric chloride were removed by soaking the seed in milk and subsequently washing it in tap water. The control plots were sown with some of this disinfected seed.

Seed-inoculation.—Fresh cultures of the strains of *R. japonicum* were prepared on Carroll's asparagus extract-mannitol agar (Carroll, 1934). The bacterial slime was rubbed off the agar slants and dispersed in a 0.1 per cent. solution of calcium diacid phosphate in skim milk. The disinfected seed was inoculated by immersion in the various bacterial suspensions, allowed to dry in the shade and sown on the same day.

Sowing.—The seeds were dibbled at the rate of two to three seeds per hole on May 28. Each plot had 24 holes in six rows of four; the holes were spaced one foot within and $1\frac{1}{2}$ feet between rows. By May 31, most of the seeds had germinated and the cotyledons had developed chlorophyll. Supplying vacancies was begun on June 7; this was done by removing plants from holes containing more than one seedling. No nodules were observed on any of the plants examined on this date. The cotyledons on plants of the variety L had, by this time, yellowed and been shed. Cotyledon-fall did not occur in variety S till very much later. The plants were finally thinned down to one per hole during the period June 12–17.

RESULTS

Nodulation was first observed in the varieties L and S on June 12 and 16, respectively. Flowers appeared in the variety L on June 22. The variety S did not flower till July 4, and even then the flowering exhibited considerable lack of uniformity. On July 12, when all the plants of the variety L and some plants of the variety S had set pod, there were still numerous plants of the latter variety in the vegetative stage. At this time differences between inoculated and control plots in the variety S were becoming evident; there were no corresponding obvious differences in the plots containing the variety L.

On July 25, records were made of the heights of individual plants of the variety S. Flowers had set in nearly all the plants of this variety. Plants in control plots exhibited a marked chlorosis while the inoculated plants were deep green. The height data and their analysis of variance are presented in Tables 2 and 3 respectively. Detailed records of the heights of the plants of the variety L were not made as the differences were obviously not significant.

The harvesting of the varieties L and S was commenced on August 26 and September 29 respectively and was continued until it was completed. The harvesting of variety S was spread over about two weeks. Pods, especially of the variety S, exhibited a tendency to shatter. Table 4 records the average yields per plot for the various treatments.

The chemical analysis of samples of seed from plants of the variety S subjected to the various treatments is given in Table 5.

TABLE 2

Heights of Plants of Variety S in Cms.

Strain of Bacterium	73	36	30	R	Uninoculated Control	Total
Block I	895	878	828	719	561	3,881
II	799	687	744	728	639	3,597
III	794	846	861	660	658	3,819
IV	877	527	704	646	538	3,292
Total	3,365	2,938	3,137	2,753	2,396	Grand Total: 14,589
Mean	841.25	734.5	784.25	688.25	599.0	General Mean: 729.45

TABLE 3

Analysis of Variance of Heights of Plants in Variety S

	D.F.	S.S.	Variance	F	Onepercent. point
Strains	4	136,969.7	34,242.4	5.46	5.41
Blocks	3	42,572.95	14,190.98		
Error	12	75,168.3	6,264.0		
Total	19	254,710.95			
Standard error		79.15			
Coefficient of variability		10.8 per cent.			

MEANS OF TREATMENTS IN CENTIMETRES

Strain 73	Strain 36	Strain 30	Rothamsted Strain	Control	General mean	Significant Difference
841.25	734.5	784.25	688.25	599.0	729.45	122.0

TABLE 4

Strain of <i>R. japonicum</i>	Mean Yield of Seed per Plot	
	Variety L	Variety S
73	122.6 gm.	358.1 gm.
36	90.9	348.6
30	122.8	344.2
Rothamsted	79.9	235.9
(Uninoculated control)	92.4	169.3

TABLE 5

Chemical Analysis of Samples of Soybean Seed (Variety S) from Experimental Plots

	Seed from Plants inoculated with Strains				Seed from Uninoculated Controls
	73	36	30	Rothamsted	
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Moisture	11.38	11.46	11.76	12.25	11.73
Nitrogen	5.96	6.29	6.19	5.92	5.56
Nitrogen on moisture-free basis	6.72	7.10	7.01	6.75	6.30

DISEASES AND PESTS

In the early stages, damage by hares seriously menaced the successful continuation of the experiment but this was checked by enclosing the area with wire mesh. *Lamprosema indicata* F. caused considerable leaf injury and consequent reduction in the photosynthetic surface. The unthrifty appearance of certain

plants was traced to root-damage by the common bean fly *Agromyza phaseoli* Coq. No major fungus diseases occurred.

METEOROLOGICAL DATA

The records of daily precipitation at Peradeniya during the experimental period are given in Table 6. Days of no rain are omitted.

TABLE 6.

Date 1937.	Rainfall in.	Date 1937.	Rainfall in.
May 1	0.02	July 17	0.41
4	0.26	19	2.80
5	0.10	20	0.21
6	0.58	21	0.29
12	0.02	22	0.79
17	0.13	23	0.03
18	0.81	24	0.06
19	0.19	26	0.01
20	0.07	27	0.03
23	0.35	28	0.44
27	2.30	August 4	0.60
28	0.10	5	1.63
29	0.26	7	2.77
31	0.47	9	0.02
June 3	0.12	10	0.02
4	0.29	23	0.11
5	0.06	27	0.38
7	0.64	28	0.57
8	0.01	30	1.77
10	0.01	September 1	1.18
11	0.19	2	0.09
12	0.50	3	0.05
14	0.21	4	0.14
18	0.02	17	0.06
19	0.07	25	0.09
21	0.37	27	0.04
26	0.65	28	0.11
28	0.26	29	0.39
30	0.17	October 1	1.82
July 1	0.19	2	0.98
2	0.73	4	0.90
3	0.07	6	0.61
5	0.44	8	0.8
6	0.42	15	0.42
7	1.05	16	0.06
8	0.01		
9	0.15		
10	0.58		
12	0.63		
13	0.39		
16	0.16		

DISCUSSION

In the analysis of variance of height totals in the variety S (Table 2), the value of F (Snedecor, 1934) for treatment exceeds

the 1 per cent. point and is accordingly indicative of a significant effect. The means for the various inoculations are all superior to the uninoculated control and, except in the instance of the Rothamsted strain, this superiority is significant. Strain 73 is significantly better than the Rothamsted strain. The bacterial strains have taken up the same order of efficiency as in the preliminary pot experiment (Park and Fernando, 1937), except that the relative positions of strains 30 and 36 have been reversed.

The correlation coefficient provides an estimate of the strength of the relationship between plant height and fresh weight. A value of $r = +0.9$ was obtained; the value was highly significant ($P < 0.01$). Plant height may be considered a satisfactory index of vegetative vigour.

The records of mean yields of seed per plot given in Table 3 exhibit no obvious response of the variety L to inoculation. The failure of this variety to respond to inoculation may at least, in part, be explained by the poor nodulation of these plants; the percentage of nodulated plants in this variety at harvest time was only 24.1 per cent. as against 76.5 per cent. for the variety S. It must be remembered, however, that these figures are underestimates: soon after flowering the nodules disintegrate rapidly, and nodule vestiges may not be evident on plants uprooted after harvest. The early maturing of the variety L may also have contributed to the failure to benefit by inoculation.

The yield figures for the variety S were subjected to an analysis of variance; the value of F for treatment did not reach the 5 per cent. point. The failure to demonstrate significance was apparently due to the extremely large experimental error which amounted to 52.8 per cent. of the general mean. The records in Table 4 of chemical analyses of samples of seed obtained from plants of the variety S subjected to various treatments show that inoculation has in every instance resulted in an increase in percentage nitrogen.

The dissimilarity in nodulation between the varieties L and S is not without parallel in other species of legumes. Duggar (1935) demonstrated differences in the extent of nodulation between peanut varieties; in field experiments in Alabama, the 3-year averages for Spanish and runner peanuts were found to be 11.0 and 126.7 nodules per plant respectively.

The present experiment yields no precise information regarding the effect of seed-disinfection on nodulation. It may be mentioned, however, that seeds of the variety L, on account of their tendency to sloughing off of the testas if soaked too long, did not receive as thorough a washing subsequent to immersion in mercuric chloride, as seeds of the variety S; residual mercuric

chloride may have contributed to the unsatisfactory nodulation of the former variety. Duggar (1935), working with peanuts, observed that seed-disinfection had usually a depressant effect on nodulation.

SUMMARY

1. The above communication contains a description of a small field experiment on the effect of seed inoculation of soya. Two varieties of soya were used : a large-seeded variety akin to the Mammoth Yellow variety and a small, yellow-seeded variety originating from Poona. These seeds were surface sterilized by immersion in 0·1 per cent. mercuric chloride solution and were subjected to four treatments, *viz.*, inoculation with four strains of *Rhizobium japonicum* Kirch., and there was a fifth uninoculated control.

2. The large-seeded variety did not respond to inoculation.

3. The small-seeded variety reacted markedly to inoculation. Mathematically significant differences were obtained in growth, as measured by plant height. The experimental error was unfortunately so high that the differences in yield were not mathematically significant.

4. Chemical analyses of the seeds of the small-seeded variety harvested from the different plots showed distinct differences in nitrogen content as a result of seed inoculation.

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