GROWTH AND YIELD OF SOYBEAN AS AFFECTED BY INOCULATION WITH STRAINS OF *Rhizobium japonicum*

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ABSTRACT

Three soybean varieties (Clark-63, UPLB Sy-2, and TK-5) were inoculated with strains of Rhizobium japonicum **(TAL 102, TAL 103, TAL 377, and Allen 527) using broth culture. The inoculated plants grew taller and formed more nodules than the uninoculated ones but no significant differences on the flowering and maturity of the three soybean varieties were observed. Significant differences in grain yield were noted among varieties, among inoculants, and their interactions. Variety UPLB Sy-2 inoculated with Allen 527 had the highest yield (1.13 t/ha) followed by TK-5 with TAL 102 (0.91 t/ha). Clark-63 treated with TAL 377 gave the lowest grain yield (0.64 t/ha). Among the uninoculated varieties, Clark-63 was the lowest yielder (0.54 t/ha). The results indicate that a specific variety requires a specific strain of** R. japonicum.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merill) is a leguminous plant which fixes nitrogen from the atmosphere through symbiosis with nitrogen-fixing bacteria like *Rhizobium japonicum.* Soybean was observed to yield better when inoculated than when applied with inorganic nitrogenous fertilizer (Calendacion, 1975; PCARR, 1976) and produced high dry matter yield than the uninoculated ones (Kang, 1974). Even with other legumes such as mungbean, inoculation of seeds resulted in significant increase in grain yield (Bunoan and Protacio, 1973). Ishawaran (1974) added that the protein content of nodulated legume has improved by inoculation compared to the uninoculated ones. Seeds of leguminous crops are inoculated before planting to augment the lower population of the microorganisms in the soil. It is hypothesized that an increased population of the right bacterial strain in the soil

will increase nitrogen-fixation from the atmosphere and supply nitrogenous compounds to the plant, thus increasing the yield of the crop.

Success in obtaining high nitrogen-fixation through inoculation depends on several factors such as (1) effectiveness and efficiency of *R. japonicum* strains present in the soil in relation to the varieties and environment, (2) number of rhizobium cells in the inoculum in relation to the native population of *R. japonicum,* (3) technique of inoculation and seeding to provide higher survival rate and multiplication of *rhizobia* around the roots, and (4) the environmental factors such as soil and climate which affect the survival of the introduced rhizobia in the soil (NifTAL, 1976). When conditions are favorable, the organisms become active in the nitrogen-fixation activity which helps in the physiology and nutrition of the plant.

This study presents the effect of inoculating different strains of *R. japonicum* on the growth and yield of three soybean varieties and the *Rhizobium*-variety interaction.

MATERIALS AND METHODS

Field Layout and Experimental Design. — An area of 465 sq m was plowed and harrowed alternately for two times and then leveled using an animal-drawn implement. Furrows were cut 50 cm apart. The experimental design used was split-plot arranged in randomized complete block with 4 replications. The treatments were designated as follows:

Soybean varieties - (mainplot)

V_1 — Clark-63
V_2 — UPLB Sy-2
V_3 — TK-5

Rhizobial strains as inoculants - (subplot)

T_0 — control
T_1 — Tal 102
T_2 — Tal 103
T_3 — Tal 377
T_4 — Allen 527

Method of Inoculation. — Four-hundred seeds of each variety were inoculated with each strain in separate containers containing a mixture of 3 ml of broth culture and 1 ml gum arabic solution. These were mixed thoroughly a few hours before planting to coat the seeds uniformly with the inoculants.

Planting. — Enough soybean seeds were sown 2-3 cm deep at a distance of 50 cm between rows and 30 cm between hills. Thinning was done 2 weeks after emergence leaving 2 plants/hill to attain the desired plant population of 20,000 plants/ha.

Cultural Management. — Fertilizers were applied at the rate of 30 kg/ha each of P_2O_5 and K_2O without nitrogen. Application was done in a narrow continuous band at the bottom of the furrow and covered with 2 cm layer of fine soil before the treated seeds were dropped. To control insect pests, Furadan 3G was applied at the rate of 15.7 kg/ha at planting time together with the

fertilizer. Thiodan was sprayed one month after planting and 2 weeks thereafter. Hand-weeding was done twice in all the treatments. Drainage canals were constructed to drain excess water especially during heavy rains.

Harvesting and Processing. — The crop was harvested when 85-90% of the plants in each plot turned yellow. Harvesting was done by cutting the plant 2 cm above the ground surface and allowing the remaining parts to decompose as a source of organic matter in the area. Harvested plants were sundried, threshed, and weighed.

RESULTS AND DISCUSSION

Agronomic Characteristics of Soybeans.

Days to Germination, Flowering, and Maturity. — Analysis of variance indicated no significant differences between inoculated and uninoculated varieties, among rhizobial strains, and their interactions as far as the germination period of the seeds was concerned. The same trend was observed on the number of days to flowering and maturity except that significant differences were noted among varieties. It appears that irrespective of the varieties, inoculation with any of the *Rhizobium* strains used had no influence on seed germination. Difference on flowering and maturity of the soybean varieties could be attributed more on their varietal characteristics and their reactions to the local climatic conditions than on the effect of inoculation (Table 1).

Plant Height (cm) at Maturity. — Inoculation with different rhizobial strains significantly influenced the plant height of the three soybean varieties. Variety UPLB Sy-2 inoculated with Allen 527 strain was the tallest (47.15 cm) among the treated varieties, followed by TK-5 (44.65 cm) inoculated wit the same strain. On the other hand, Clark 63 grew taller (39.47 cm) when treated with TAL 377. The control plants in each variety did not grow as tall as the treated ones.

The differences in height between inoculated and uninoculated plants must have been influenced by the varying levels of available nitrogen brought about by inoculation. In the inoculated ones, the variations among treatments could be attributed to the differences in the degree of symbiotic nitrogen-fixation between varieties and the *R. japonicum* strains. These varieties also inherently differed in plant height as manifested by their varying heights in the control plots.

Degree of Nodulation. Nodule formation in legumes indicates the presence of rhizobia which are capable of fixing nitrogen from the atmosphere and synthesizing it into simpler form for plant use. The presence of many nodules indicates that more nitrogen (N) can be potentially available for growth and development of plants, particularly on the succeeding crop.

In this study, inoculated plants developed many large nodules with pink-red color per plant indicating

Table 1. Agronomic characteristics of soybeans as influenced by inoculation with different strains of **Rhizobium japonicum.**

Variety	Rhizobial strains	Number of days from sowing to Germination	Flowering	Maturity	Plant ht. at maturity (cm)	No. of nodules/ plant	Ave. wt. of fresh herbage (t/ha)
Clark-63	Control	3.25	30.00	80.75	29.40	30.00	0.63
	Tal 102	3.25	29.25	80.50	30.92	46.50	0.82
	Tal 103	3.25	29.25	80.00	36.65	34.12	0.80
	Tal 377	3.50	29.75	80.00	39.47	53.87	0.96
	Allen 527	3.50	29.00	80.25	33.10	41.20	0.83
	Mean	3.35	29.45	80.50	33.91	40.89	0.81
	CV % (a)	11.77	1.91	0.98	15.27	31.33	17.99
	(b)	16.87	2.29	0.49	4.97	9.39	23.53
UPLB Sy-2	Control	3.50	28.25	79.75	36.15	44.37	0.83
	Tal 102	3.75	27.50	79.50	40.95	61.25	0.98
	Tal 103	3.50	27.75	79.50	41.10	53.13	0.94
	Tal 377	3.25	28.00	79.50	44.65	58.50	0.92
	Allen 527	3.25	28.00	79.50	47.15	77.93	1.19
	Mean	3.45	27.9	79.65	42.00	61.04	0.97
	CV % (a)	11.43	2.02	0.99	12.33	20.98	11.35
	(b)	16.38	2.42	0.49	4.02	6.29	19.65
TK-5	Control	3.50	26.75	79.25	32.95	37.37	0.71
	Tal 102	3.50	26.75	79.25	37.72	63.25	1.01
	Tal 103	3.75	26.50	79.50	34.75	44.50	0.82
	Tal 377	3.25	26.25	79.50	37.22	49.75	0.87
	Allen 527	3.25	26.75	79.50	40.05	53.87	0.93
	Mean	3.45	26.60	79.4	36.54	49.75	0.87
	CV % (a)	11.72	2.11	0.99	14.17	25.75	16.74
	(b)	16.87	2.53	0.50	4.61	7.72	21.19
	Grand Mean	3.41	27.28	79.78	37.48	50.64	0.88
	CV % (a)	11.56	2.01	0.99	13.82	25.3	12.51
	(b)	16.53	2.41	0.498	4.50	7.6	7.16
	LSD .05	1.05	1.25	0.73	3.13	15.89	0.85
	.01	1.55	1.85	1.09	4.64	23.50	1.24

effective nodulation. Uninoculated plants formed small-sized nodules with green or white interior.

Highly significant differences among the varieties and strains and their interactions were noted indicating that nodulation in each variety was associated with different rhizobial strains. Table 1 shows that among the soybean-*R. japonicum* strain combinations, UPLB Sy-2 inoculated with Allen 527 had the highest number of nodules that developed per plant (77.93), followed by TK-5 inoculated with TAL 102 (63.25), and Clark-63 inoculated with TAL 377 (53.87).

Yield of Fresh Herbage (t/ha). Significant effects of inoculation were observed among the different strains of *R. japonicum*, soybean varieties, and their interactions. As in the degree of nodulation, the same patterns of treatments and their effects, were noticed. UPLB Sy-2 inoculated with Allen 527 produced the heaviest fresh herbage (1.19 t/ha), followed by TK-5 inoculated with TAL 102 (1.01 t/ha), and Clark-63 inoculated with TAL 377 (0.96 t/ha). The uninoculated plants in the three varieties had the lowest fresh herbage weight.

Among the inoculated plants, the difference in weight of fresh herbage might be partly due to the available polysaccharide produced by the soybean plant as a source of energy so that the *R. japonicum* can fix N at a maximum rate. Alexander (1961) reported that these bacteria depend on the host plant for carbohydrates (sugars) as source of energy and, in turn, supply the plant with N fixed from the atmosphere, promoting rapid vegetative growth resulting in a significant increase in weight of fresh herbage.

Yield and Yield Components.

Number of Pods per Plant. Differences in the number of pods formed were significant among varieties, *Rhizobium* strains, and their interactions. The inoculated plants produced more pods than the uninoculated ones (Table 2). This could have been influenced partly by the presence of *R. japonicum* in the treated plots which, through symbiotic N-fixation, produced additional N leading to the development of more pods per plant. It could have also been influenced by the characteristic of the variety.

Number of Productive and Unproductive Pods per Plant. Plants which were not inoculated developed fewer productive pods compared to the inoculated ones. Abel and Erdman (1964) mentioned that uninoculated soybean formed fewer productive pods due to lesser amount of N available for plant use, thus affecting the flowering processes and hampered fruit setting and seed development. On the other hand, the differences in the number of productive pods among the inoculated varieties could be due to the differences in their symbiotic reaction with the *R. japonicum* strains. Nitrogen fixation is dependent on the kind and amount of substrate that the host plant could provide in symbiotic relation between *R. japonicum* and the soybean plant.

It can be noted in Table 2 that it does not necessarily follow that more productive pods mean higher grain yield. There were pods with only one seed per pod and still were considered productive. On the other hand, there were varieties with only few productive pods such as UPLB Sy-2 and TK-5 but developed more seeds/pod and therefore out-yielded the rest of the varieties.

Uninoculated plants produced more unproductive pods than the inoculated ones. Less available N contributed largely to the formation of unproductive pods.

Table 2. Yield and yield components and harvest index of soybeans as affected by inoculation with different strains of **Rhizobium japonicum.**

Variety	Rhizobial strains	Total no. of pods/ plant	Ave. no. of productive pods/plant	Ave. no. of unproductive pods/ plant	Weight of 100 seeds (g)	Grain yield (t/ha)	Harvest Index
Clark-63	Control	13.62	6.30	7.32	17.75	0.54	0.46
	Tal 102	17.30	12.70	4.60	18.10	0.74	0.45
	Tal 103	17.70	13.58	4.12	18.80	0.64	0.46
	Tal 377	22.35	17.05	5.30	18.92	0.87	0.45
	Allen 527	15.02	12.32	2.72	19.70	0.68	0.45
	Mean	17.20	12.39	4.81	18.78	0.69	0.45
	CV % (a)	12.17	16.83	14.89	6.84	15.58	2.26
	(b)	8.81	11.32	23.05	3.42	13.05	2.53
UPLB Sy-2	Control	14.05	5.85	8.20	18.55	0.75	0.45
	Tal 102	23.45	18.82	4.62	20.47	0.85	0.46
	Tal 103	21.72	18.70	3.03	21.25	0.83	0.45
	Tal 377	21.60	18.80	2.80	19.27	0.83	0.45
	Allen 527	19.15	17.68	1.47	20.03	1.13	0.45
	Mean	19.99	15.97	4.02	19.82	0.88	0.45
	CV % (a)	21.93	13.06	17.82	6.48	12.19	2.26
	(b)	11.48	8.78	27.58	3.24	10.24	2.53
TK-5	Control	15.27	6.72	8.55	15.92	0.64	0.44
	Tal 102	22.15	17.20	4.95	17.87	0.92	0.45
	Tal 103	22.50	18.12	4.37	18.07	0.72	0.45
	Tal 377	20.37	15.40	4.97	17.17	0.77	0.45
	Allen 527	15.32	12.55	2.77	16.97	0.85	0.45
	Mean	19.12	14.00	5.12	17.19	0.78	0.45
	CV % (a)	10.95	14.89	13.99	7.47	13.76	2.26
	(b)	7.92	10.02	21.65	3.73	11.54	2.53
	Grand Mean	18.77	14.12	4.65	18.59	0.78	0.45
	CV % (a)	11.15	14.77	15.39	6.9	13.76	2.26
	(b)	8.07	9.94	23.82	3.46	11.55	2.53
	LSD .05	2.82	2.61	2.06	1.19	0.09	0.02
	.01	4.16	3.82	3.05	1.76	3.65	0.03

Weight of 100 Seeds (g). Marked differences in weight of seeds were noted among varieties, rhizobial strains, and their instructions. Among the variety-rhizobial strain combination, UPLB Sy-2/TAL 103 had the highest weight of 100 seeds (21.25 g), followed by TK-5/TAL 103 combination (18.07 g) and Clark-63/Allen 527 combination (19.7 g).

The differences in weight of seeds among inoculated plants were due to the same reaction between varieties and rhizobial strains affecting the degree of nodulation and pod formation. Heavier seeds were larger than the lighter seeds.

Grain Yield (t/ha). Significant differences in grain yield were observed among varieties, rhizobial strains, and their interactions. UPLB Sy-2 inoculated with Allen 527 had the highest grain yield at 1.13 t/ha while TK-5 inoculated with TAL 102 and Clark-63 inoculated with TAL 137 produced grain yields of 0.92 and 0.87 t/ha, respectively. The yields of the latter two varieties were not significantly different from each other. Production of fewer nodules and productive pods/plant contributed to their lower yields.

The results of this study show that inoculation could increase the grain yield of soybean because of the ability of *R. japonicum* to fix and convert atmosphere N into useable form for plant use. The crop, therefore, grew and developed faster and produced more pods per plant, and formed larger and heavier seeds, which contributed to increased grain production. The results further suggest that specific strains of microorganisms (*R. japonicum*) can be associated with specific varieties.

Harvest Index. This parameter indicates the efficiency of the plant to convert the absorbed nutrient into economic yield. Statistical analysis showed no significant differences among inoculated plants within a variety.

LITERATURE CITED

ABEL, C.H., and ERIMAN, L.W. 1964. Response of Lee soybean to different strains of *Rhizobium japonicum*. *Agron. Jour.* 56: 423-424.

ALEXANDER, M. 1961. *Introduction to Soil Microbiology*. John Wiley & Sons, Inc. New York and London.

BUNOAN, J.C. JR., and PROTACIO, M.E. 1973. Field response of mungo to fertilization and inoculation. Proc. of the 4th Ann. Conf., College, Laguna, UPCA.

CALENDACION, H.N. 1975. Inoculation increases soybean yield. *Crop and Soils* 6(1): 5.

ISHAWARAN, V. 1974. *A manual for the proper use of inoculation and pelleting for legumes*. Bureau of Plant Industry. Manila.

KANG, B.T. 1974. Effect of inoculation and nitrogen fertilization on soybean in Western Nigeria. *Expt. Agric.* 11: 23-31.

NITROGEN FIXATION OF TROPICAL AGRICULTURE LEGUMES. 1976. Exploiting the legume-*Rhizobium* symbiosis in tropical agriculture. *Workshop Proceedings*, August 23-28, 1976. Univ. of Hawaii, Honolulu, U.S.A.

PHILIPPINE CONCIL FOR AGRICULTURE AND RESOURCES RESEARCH. 1976. The Philippines Recommends for Soybean.