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RESEARCH ARTICLES

LOW-INPUT IMPROVEMENT OF AGRICULTURE OF NORTHEAST THAILAND USING AQUATIC LEGUMES AND PHOSPHATE ROCK

PART 3: RELATIONS BETWEEN P-NUTRITION AND NODULATION UNDER UPLAND CONDITIONS

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ABSTRACT

Effects of triple superphosphate (TSP) and phosphate rock (PR) on growth of Sesbania rostrata *and* Aeshynomene afraspera *were examined by pot experiments. The plants were cultivated in a sandy soil under upland conditions during the dry season when the temperature was very high. In the initial growth stage, spontaneous nodulation was very poor for both plants, probably due to too high temperature and/or too low humidity. Inoculation of* Azorhizobbium caulinodans *ORS 571 on the stems of the plants easily induced many stem nodules. This situation revealed close interactions between nodules and P-nutrition. Even before inoculation, growth of* S. rostrata *was remarkably promoted with TSP but the yellow coloration of the plant indicated that they suffered from N-deficiency. After the inoculation, the plants became green again and it growth was further promoted. On the other hand, PR was somewhat suppressive to* S. rostrata *before inoculation. After the inoculation, growth of the plant was enhanced in the presence of PR. Similar results were obtained for* A. afraspera, *though the suppressing effect of PR was more serious for* A. afraspera *than for* S. rostrata. *Reasons for the difference is discussed.*

INTRODUCTION.

In one of the previous studies,[1] we found that application of P-fertilizers at the rate of 7.2 kg of available P/rai was one of the most effective measures to promote the growth of aquatic legumes planted in the submerged infertile sandy soil of Northeast Thailand. Among the P-fertilizers, triple superphosphate (TSP) was more effective than phosphate rock (PR). In addition, effectiveness of PR as a P-fertilizer for the aquatic legumes decreased in the following order: *Sesbania rostrata* > *S. aculeata* > *S. cannabina* > *S. speciosa* = *Aeschynomene afraspera*. Furthermore, it was recognized that *A. afraspera* produced a larger biomass, especially when it was cultivated in fertile soils.

One of our previous experiments[2] showed that PR was effective in promoting growth of both *S. rostrata* and *S. accculeata* under upland conditions as well as under submerged conditions. The initial objective of the present experiment was to clarify the differences between the effectiveness of TSP and PR for the two aquatic legumes cultivated under upland conditions. In the present study, spontaneous nodulation was inactive and inoculation of *Azorhizobium caulinodans* easily induced nodulation. This presented an opportunity to clarify the effect of nodulation on the growth of these plants in relation with their P-nutrition.

MATERIALS AND METHODS.

Materials:

Soil. Soil sample was collected from a surface horizon (0-15 cm) of an upland field in Ban Don Daeng, Khon Kaen Province. It was air dried and passed through a 1 cm screen. The soil was classified as a Khorat series (fine loamy mixed isohyperthermic, Oxic Paleustults). Some of its properties are listed in Table I.

Plant. Seeds of *S. rostrata* and *A. afraspera* were obtained from IRRI through the Department of Land Development, Ministry of Agriculture, Thailand.

P-fertilizer. Triple superphosphate (TSP) (Thai Central Fertilizer Co.) and rock phosphate (PR) (UPI Co.) were used as the P-fertilizers. Both were purchased from a shop in Khon Kaen.

Azorhizobium. *Azorhizobium caulinodans* ORS 571 which had been isolated from *S. rostrata* at ORSTOM, Dakar, Senegal,[3] was supplied by ORSTOM. It was aerobically cultivated on an yeast-mannitol agar in a 350 ml. glass cultivation bottle and kept in a refrigerator before use.

EXPERIMENTAL DESIGN:

For each plant, 3 treatments with 6 replications were set up. They were C treated (as obtained), PR treated (mixed with PR) and TSP treated (mixed with TSP). The P-fertilizers were added a rate equivalent to 7.2 kg. available P/rai, which was found to be the proper amount for pro-motion of the growth of the plants under study.[1]

Cultivation. One kilogram of the soil and the appropriate amount of fertilizer were placed in a plastic bag and mixed well. The treated soils were transferred to plastic containers (height 13 cm, diameter 12 cm), with tap water added to produce a moist soil comparable to field conditions. Ten day old seedlings of the plants were transplanted on April 2, 1991 and cultivated until May 31, 1991. During the cultivation, water was added to maintain the moisture at field conditions. The plants planted in the pots containing unfertilized soil, soil containing triple super phosphate or rock phosphate will be referred to in later discussion by the pots they are planted in, i.e., as C pots, TSP pots or PR pots, respectively.

Inoculation. Inoculation was done four weeks after the transplanting. About 100 ml of distilled water was added to the cultivation bottle containing the *A. caulinodans* and was shaken by hand to produce a water suspension of the bacterium. For each treatment, three pots were randomly selected and the stems of the plants were inoculated with the bacterium by gently rubbing them with a piece of adsorbent cotton which had been soaked with the water suspension.

OBSERVATION AND MEASUREMENT:

The state of plant growth was observed every day and the plant height was measured every week. At harvest time, the plant height, the stem diameter at the crown level and the plant color were measured first. After collecting soil samples using a soil profile sampler, the plant roots were thoroughly washed with tap water to remove all soil particles. The soil samples were air dried and then measured for their pH and the content of available P (Bray 2 method). The plant samples were measured for the fresh and dry weight of both the above ground part and the root parts and for the amounts of N, P, K, Ca and Mg in them.

RESULTS AND DISCUSSION.

Nodulation:

In the initial growth stage, nodulation was very poor in every pot. This was thought to be due mainly to high temperatures (> 40° C) and/or low humidity of the green house during this time. About one week after inoculation, many stem nodules were observed at the inoculated parts of the stems (similar to what was previously reported[4]). About three weeks after the inoculation, a few stem nodules appeared in the uninoculated parts of *Aeschtnomene*. About six weeks after the transplanting, spontaneous nodulation appeared in all plants, even in the uninoculated ones. This was especially true for the TSP treated pots.

I. The state of growth as affected by the nodulation:

Taking the time-course of plant height as a representative index of the growth pattern of the plants (Figs. 1 -4 and Table 2), we find for;

a. *S. rostrata*

A lag of about three weeks between the growth patterns of the inoculated and uninoculated plants for the C and PR pots. No lag was seen between plants in the TSP pot. As expected, the TSP was more effective than PR in enhancing the plant growth. The remarkable growth enhancing effect of TSP was believed to be due to the elimination of the lag phase. This should be compared to the effect of PR, no detectable growth enhancement in the initial period, slight suppression of growth in the middle period and a small enhancement in the later period. This is quite different from what was earlier reported.[1,2] There it was reported that the PR enhanced the plant growth throughout the entire period, from the initial to the final. These results suggest that the lag period is caused by the suppression of uptake of P by the young seedling from the soil and/or the phosphate rock. The suppression

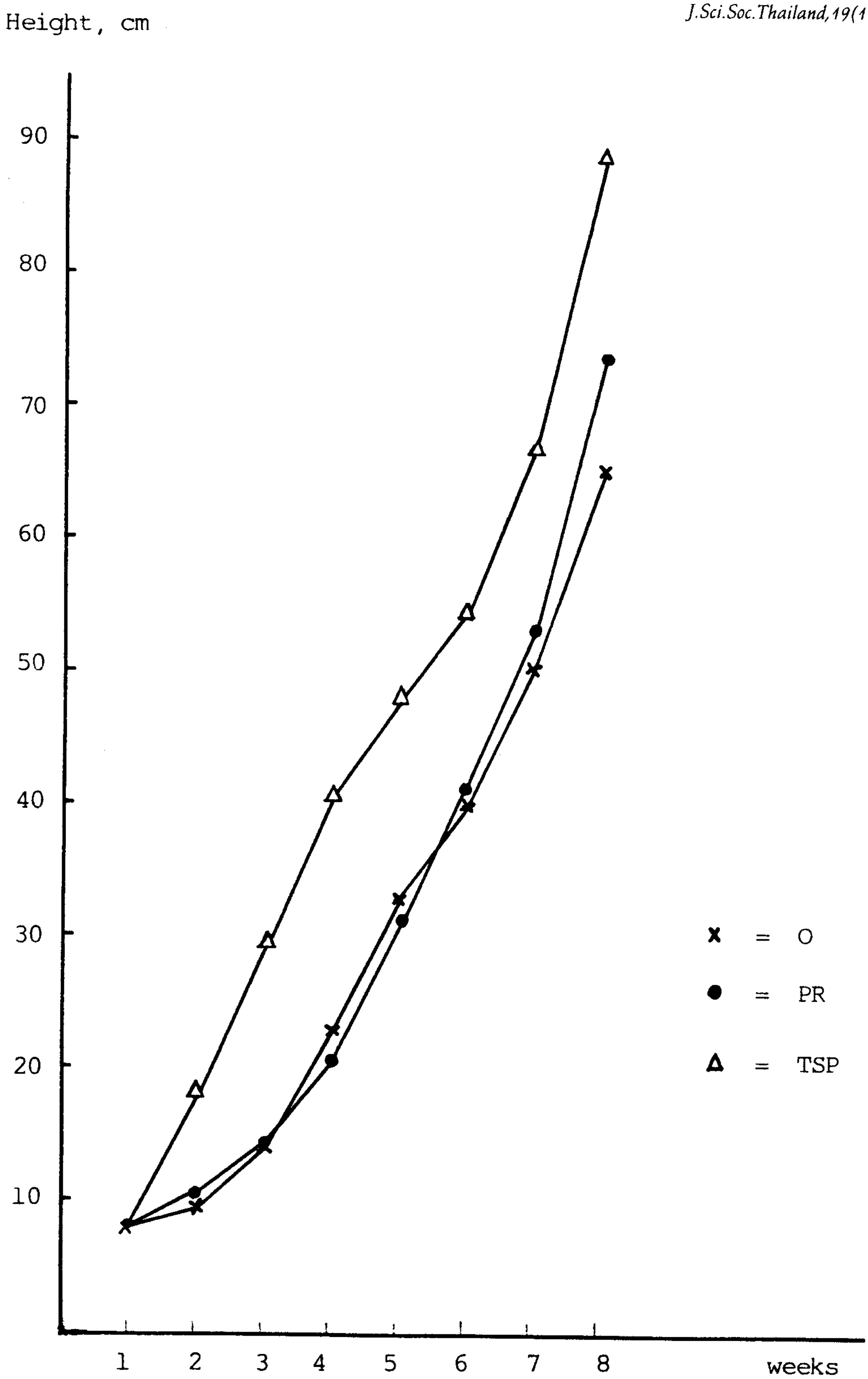

Fig.1. Height of *S. rostrata* (+Inoculation) under Upland condition (Kt soil).

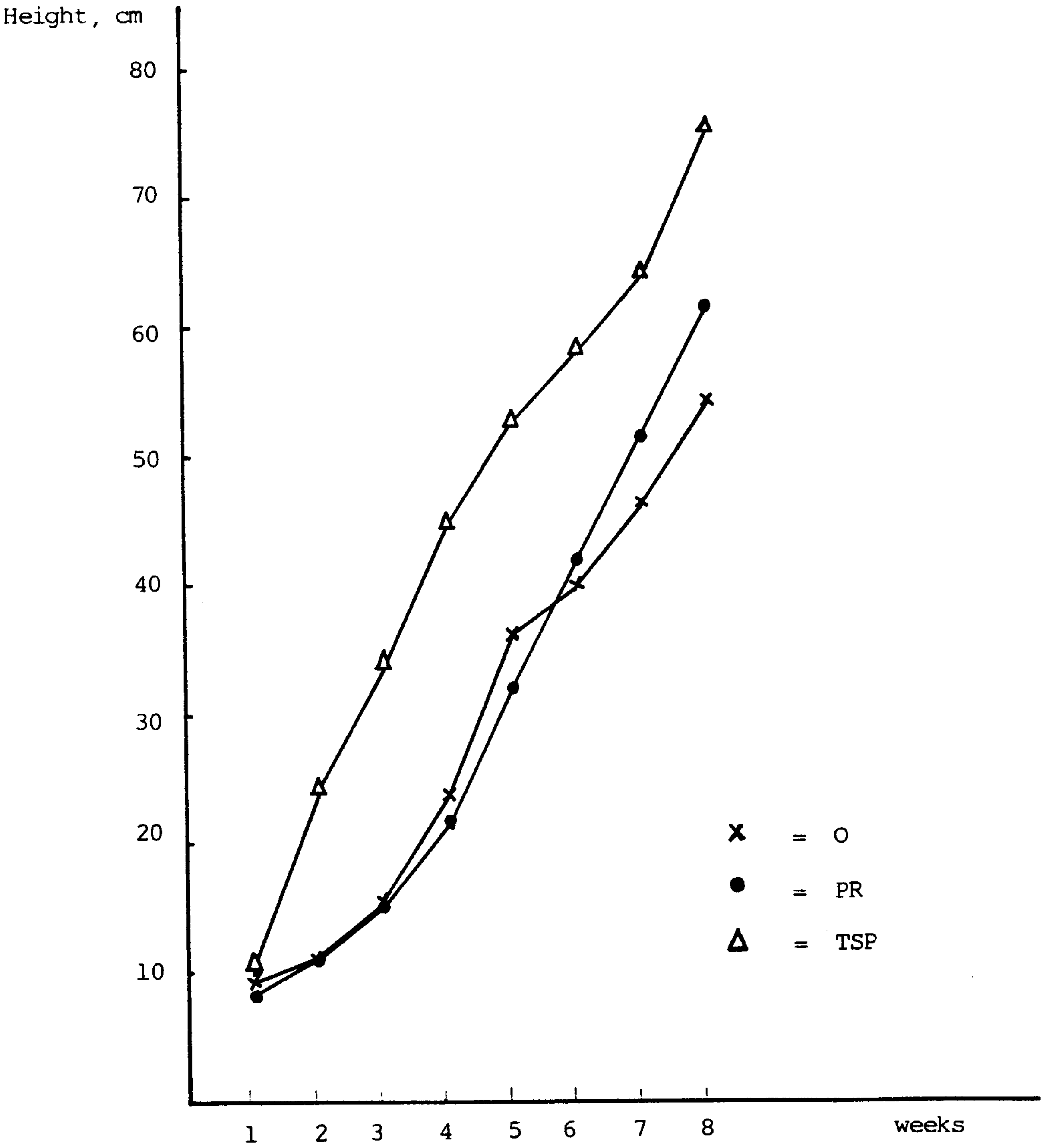

Fig.2. Height of *S. rostrata* (- Inoculation) under Upland condition (Kt soil).

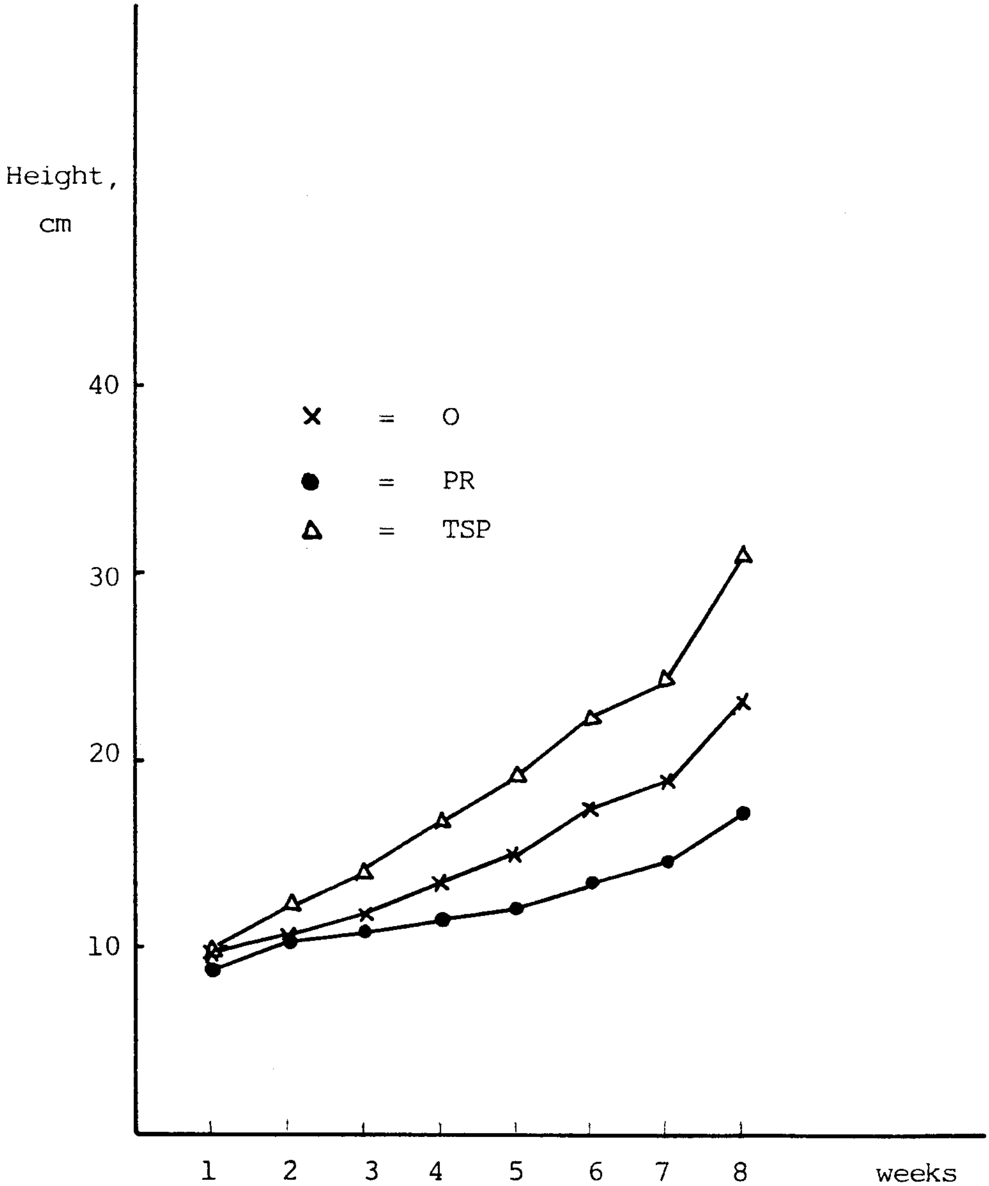

Fig.3. Height of *A. afraspera* (- Inoculation) under Upland condition (Kt soil).

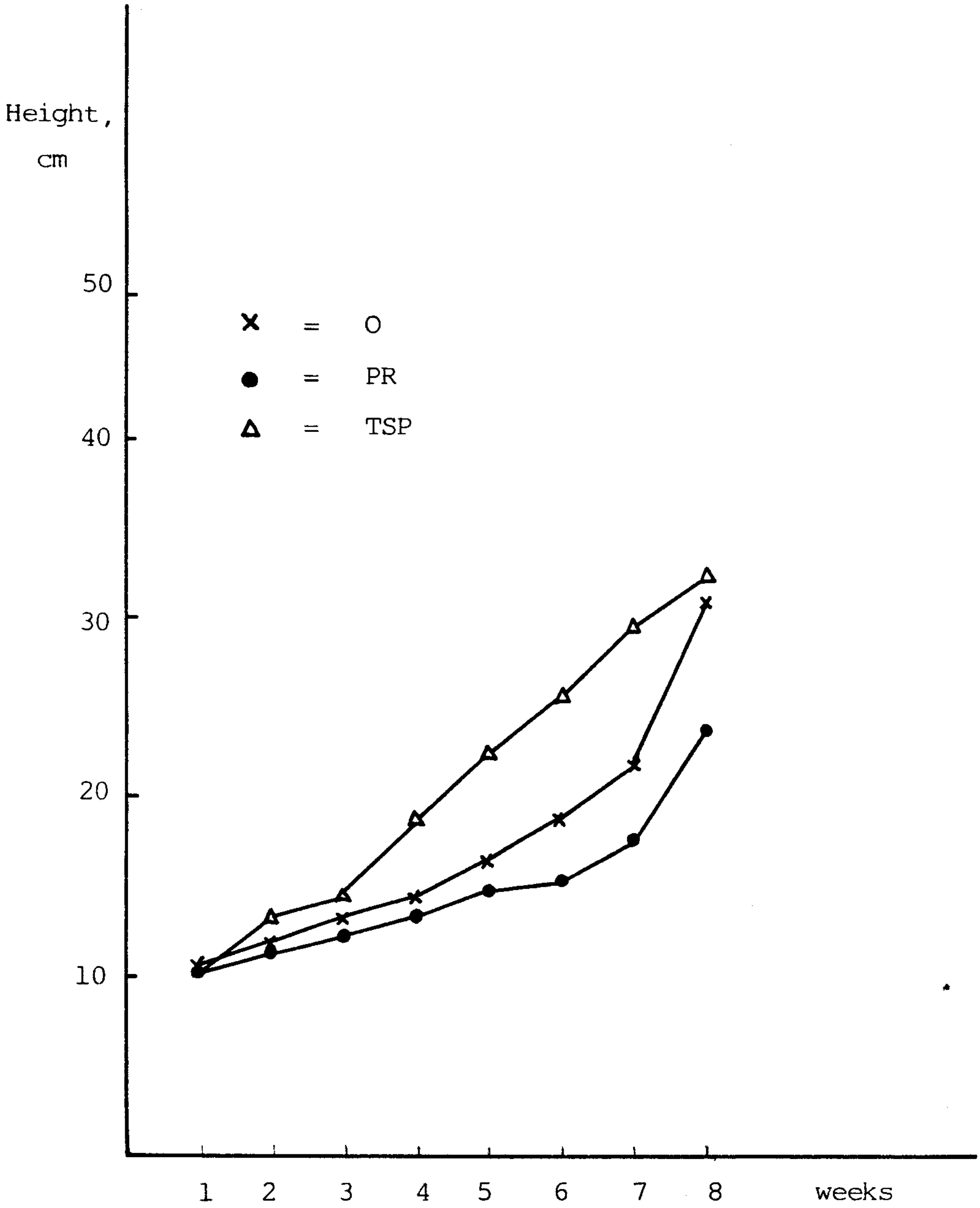

Fig.4. Height of *A. afraspera* (+ Inoculation) under Upland condition (Kt soil).

Table 1. Chemical properties of the soil

Soil	pH 1:2.5	EC 1:2.5 mS	O.M. %	P ppm	K	Na	Mg	Ca	CEC	N %
					me/100 g					
Kt	5.97	0.03	0.21	7.28		0.129	0.185	0.362	1.171	1.30.013

Table 2. Height (cm) of sesbania under upland condition (Kt soil)

7.2 kg P rai^{-1}	**+ Inoc. wks**								**-Inoc. wks**							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
								S. rostrata								
0	7.7	9.5	14	23	32.7	40	50.3	65.3	9.2	11.3	15.5	23.7	36.3	40	46.2	54
PR	8.3	10.5	14.2	20.5	31.2	41	53.3	73.7	8.2	11	15.3	21.7	32	42	51.3	61.7
TSP	8.0	18.2	29.3	40.5	45	54.5	66.8	88.7	10.7	23.6	33.5	44.7	52.3	57.7	64	75.3
								A. afraspera								
0	10.5	11.8	13.2	14.3	16.3	18.7	21.7	31	9.8	10.8	11.8	13.3	15.3	17.3	19.3	23.7
PR	10.2	11.3	12.2	13.3	14.8	15.3	17.7	23.7	9.0	10.2	10.8	11.3	12.3	13.5	14.7	17.3
TSP	10.7	13.3	14.5	18.7	22.3	25.7	29.3	40.3	10.0	12.2	14	16.7	19	22.3	24.7	31.3

wks = week after transplanting

of the P-uptake, in turn, may have been due to too high of a temperature of the drained soil and/or poor nodulation.

The color of uninoculated sesbania was yellowish. This was especially evident in the TSP pot where the color of the stems and leaves become yellow after only a few weeks after the transplanting. The color gradually become green after nodulation on the stems began. This was clearly noticeable in the new leaves. Inoculation was effective in promoting plant growth (as measured by the plant height), especially in the later period in all pots. In this period, the growth rate of the plant varied in the follow-ing order: PR > TSP . C, +inoculation > -inoculation.

The results suggests that;

i. Water soluble P in TSP is easily taken up *S. rostrata*. This led to a remarkable promotion of photosynthesis in the plant, resulting in N-deficiency in the plant.

ii. N fixed by stem nodules of *S. rostrata* is quite effective in remedying the N-deficiency of the plant.

iii.PR had some adverse effects on *Sesbania* if nodulation of the plant is poor or absent.

iv.The enhancing effect of PR on plant growth, especially of the inoculated plants, would become clearer if the plants were cultivated for a longer period.

b. *A. afraspera*.

Rather similar results to the above were obtained for *A. afraspera*. The following differences were seen;

i. The lag periods were not clearly evident.

ii. PR was always suppressive.

iii. The growth rate in the later period decreased in the following order; PR = TSP > C.

The results suggest that *A. afraspera* is more sensitive to the adverse effects of PR than is *S. rostrata*. The inoculation of *A. caulinadans* ORS 571 on the stem of *A. afraspera* did not affect nodulation initially but appeared to induce nodulation in the later period, which in turn lead to an enhancement of the growth of the plant. This enhancement is in conflict with the belief that *A. caulinodans* would not infect aquatic legumes other than sesbania.[5] The stem nodules of the inoculated *A. afraspera* are presently being examined to find the bacterium causing the nodules.

II. Plant Growth at Harvest-time.

Stem diameter (Table 3)

The diameter of the stem at the crown level appears to affected by the application of the P-fertilizer and the inoculation. The effect was different for the two plants. In the case of *S. rostrata*, TSP caused a decrease of the stem diameter, suggesting that TSP causes a elongation of the plant height at the cost of the thickness of the stem. Inoculation increased the stem diameter of the C and PR pot plants, but not the stem diameters of the TSP pot plants. This may be caused by a too rapid of an elongation by TSP even in the presence of nodules on the plant. In the case of *A. afraspera*, TSP caused an increase in the stem diameter, especially in combination with inoculation of the plant. On the other hand, PR caused a decrease in the stem diameter.

Fresh weight and dry weight of above ground parts (Table 4 & 5)

For *S. rostrata* and *A. afraspera*, both the fresh weight and the dry weight of the above ground part of the plants increased remarkably when TSP and inoculation were both used. This was in accord with the increases in the plant heights and stem diameters of the two plants seen. The dry weight of the PR pot of *S. rostrata* was more or less the same as that for the C pot, though the ordering of the fresh weight was the same as that of the plants height. This was due to fact that the ratio of fresh weight to dry weight was higher for the TSP pots and the PR pots than for the c pots. At harvest time, the TSP and PR potted plants would be more succulent (less immature) than the C pot grown plants. This would be due to the higher growth rates in the later period of the TSP and PR pots over that of the C pot. PR causes a decrease in the fresh weight and dry weight of the above ground part of *A. afraspera*. This is a consequence of the decrease in both the plant height and stem diameter brought upon by PR.

Fresh weight and dry weight of the roots (Tables 6 & 7)

Among the replications pots of the same treatment, both the fresh weight and dry weight of the roots fluctuated more widely that the dry weights of the above ground parts. This could be caused by three things; (1) loss of some of the roots during the washing process, (2) inclusion of unremoved soil particles in the weighing or (3) actual differences in the roots biomass by slight changes in the growing conditions. Nevertheless, inoculation and TSP, in combination, definitely increased the root biomass of both *S. rostrata* and *A. afraspera*.

Nutrient uptake (Table 8)

Inoculation increased both the total amount of nitrogen and the amount per unit weight in the two plants. The increase was especially large for *S. rostrata*. This was probably due to the remarkable effectiveness of inoculation to induce nodulation in this plant. Nodulation appeared to cause a decrease in amount of phosphorus per unit weight, though it did cause the total amount of phosphorus to increase. This suggests that the increase in growth rate induced was greater than was not accompanied by an equivalent increase in the phosphorus uptake.

TSP increased both the total amount and the amount per unit weight of phosphorus in the plants. PR, however, caused a decrease in both the total amount and the amount per unit weight of phosphorus in *A. afraspera*. This was due mainly to the suppression of the plants growth in the presence of PR.

Table 3. Diameter (cm) at the crown of *sesbania* under upland condition.

Tmts	***S. rostrata***		***A. afraspera***	
7.2 kg P/rai	**+ Inoc.**	**- Inoc.**	**+ Inoc.**	**- Inoc.**
0	1.0	0.63	0.87	0.9
PR	1.0	0.73	0.77	0.6
TSP	0.67	0.63	1.40	1.0

Table 4. Fresh weight of above ground part of *sesbania* (g/pot) under upland condition (Kt soil).

Tmts	***S. rostrata***		***A. afraspera***	
7.2 kg P/rai	**+ Inoc.**	**- Inoc.**	**+ Inoc.**	**- Inoc.**
0	11.29	5.96	4.56	2.90
PR	12.16	6.41	2.17	1.37
TSP	22.48	11.41	13.71	5.85

Table 5. Dry weight above ground part of sesbania (g/pot) under upland condition (Kt soil).

Tmts	***S. rostrata***		***A. afraspera***	
7.2 kg P/rai	**+ Inoc.**	**- Inoc.**	**+ Inoc.**	**- Inoc.**
0	3.03	1.76	0.78	0.86
PR	2.84	1.54	0.80	0.28
TSP	5.28	2.80	2.93	1.20

Table 6. Fresh weight of root of sesbania (g/pot) under upland condition (Kt soil).

Tmts	*S. rostrata*		*A. afraspera*	
7.2 kg P/rai	+ Inoc.	- Inoc.	+ Inoc.	- Inoc.
0	9.10	8.26	5.19	4.56
PR	15.00	8.04	3.59	3.18
TSP	20.17	7.32	13.77	5.44

Table 7. Dry weight of root of sesbania (g/pot) under upland condition (Kt soil).

Tmts	*S. rostrata*		*A. afraspera*	
7.2 kg P/rai	+ Inoc.	- Inoc.	+ Inoc.	- Inoc.
0	1.19	0.68	0.86	0.49
PR	1.67	0.77	0.33	0.16
TSP	2.94	0.57	1.45	0.72

Table 8. Nutrients uptake (mg plant^{-1}) of sesbania under upland condition (Kt soil).

7.2 kg P rai^{-1}	+ Inoc. Biomass* g/plant	N	P	K	Ca	Mg	- Inoc. Biomass* g/plant	N	P	K	Ca	Mg
			S. rostrata						*S. rostrata*			
0	4.22	75.5	4.22	50.6	32.1	11.4	2.44	12.7	3.42	3.88	9.76	6.34
PR	4.51	79.8	4.96	50.5	31.1	14.0	2.31	16.2	4.39	4.34	12.2	5.78
TSP	8.22	171.0	9.86	55.1	30.4	21.4	3.37	47.2	12.50	5.12	14.8	10.1
			A. afraspera						*A. afraspera*			
0	1.64	29.7	1.97	21.3	9.18	5.08	1.35	20.9	2.43	17.1	7.97	4.59
PR	1.13	19.0	1.58	18.0	9.04	4.18	0.44	6.69	0.57	5.59	4.36	1.89
TSP	4.38	93.7	9.20	44.2	26.7	13.1	1.93	32.2	1.95	23.7	12.2	6.76

Table 9. pH and P content in soil after harvesting *sesbania* under upland condition (Kt soil).

Tmts 7.2 kg P/rai	*S. rostrata* pH + Inoc.	*S. rostrata* pH - Inoc.	*A. afraspera* pH + Inoc.	*A. afraspera* pH - Inoc.
0	6.70	6.80	6.76	6.54
PR	6.90	7.28	6.79	6.85
TSP	6.78	7.31	6.92	6.64
	ppm P		ppm P	
0	4.26	3.41	4.14	4.61
PR	22.05	28.32	22.13	38.13
TSP	17.50	11.44	20.30	18.53

Soil properties (Table 9)

For *S. rostrata*, the pH's of the soils in the pots containing the ino-culated plants were lower than those of the soil in the pots containing the uninoculated plants. This was especially noticeable for the PR and TSP treated soils. No changes in the pH's was found for the pots containing *A. afraspera*. This difference could be caused by excretion of an acid substance by *S. rostrata* but not by *A. afraspera*. This acid substance, if really present, could account for the fact that *S. rostrata* is helped by PR which would be dissolved by the acid, making into a form which could be utilized by the plant. Moreover, if we assume that nodulation promotes the release of this acid substance, we could explain the ineffectiveness of PR in promoting the growth of *S. rostrata* when the nodulation on the plant was poor or absent.

Similar assumptions regarding the uptake of iron from the soil for rhizobia/legume symbioses have been proposed recently,[6] i.e., that the nodules excreted siderophores to dissolve the iron in the soil. Some of the siderophores, such as citric acid, would be able to dissolve the insoluble phosphorus contained in the PR and also in the soil. The import-ance of the iron uptake in the rhizobia/legume symbiosis suggests that part of the detrimental effect of PR on aquatic legumes was caused by the Fe deficiency due to high soil pH's. This consideration regarding the adverse effect of PR on the plant growth is probably more appropriate than what was proposed in the previous paper,[1] that some toxic Al-organic compounds were formed in the rhizosphere of the plants when PR was around.

Rather large amount of available phosphorus remained in the soil after harvesting. This was particularly true for the PR pots. In the case of the PR pots, most of the residual phosphorus remained in the form of PR. The residual PR would therefore be available for the succeeding aquatic legumes and so the cost of the PR application per crop would become less.

GENERAL DISCUSSION

1. The effects of PR on plant growth as affected by nodulation.

As pointed out above, nodulation is beneficial to growth of the plantsthrough its enhancement of the uptake of phosphorus from the soil and fromthe PR and its negation of the adverse effect of PR. The mechanism forthe beneficial effects may be the increase in the eexcretion by the nodules of some organic acids which helps to dissolve the PR and to lower of the pH of the soil. This mechanism may account for why PR is sometimes suppressive to the growth of *s. rostrata*, especially under upland conditions.[7] PR easily increases the pH of infertile sandy soil have low buffering capacity[8] and may induce deficiency of some micronutrients such as Fe. This would be remarkable for the drained soils. (The buffering capacity of the soil large and the availability of some micronutrients in the soil is increased when the soil is submerged.)

2. A favorable prospect of utilizing *A. caulinodans* in biotechnology of *S.rostrata.*

Many people in the world have been trying to introduce new alien genes responsible for some desirable plant properties to the host plants in the hope that they will have novel man-made plants with the desirable properties. At present, this is the principle direction of research in the field of plantbiotechnology.[9] If the above premise that *A. caulinodana* has the ability to modify the physiology of *S. rostrata* is really true, we have a new direction to pursue in plant biotechnology. In the first step, *A.caulinodans*(insteadof*S.rostrata*) is manipulated for its genes. The gene engineered*A. caulinodana*wouldthenmodify thephysiology of *S. rostrata* in a desired direction. Advantages of this approach to obtain plants with desirable characteristics are;

a. Gene-manipulation of micro organism is easier and more advance than the manipulation of plant genes.

b. Inoculation of *A. caulinodans*, a host micro organism, has been widely practiced without any problems in the field.

The second step is utilize most of the rhizobia/legume symbiosis and final step is to utilize the microbe/plant relationships.

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บทคัดย่อ

ศึกษาผลของ Triple super phosphate (TSP) และ Phosphate Rock (PR) ที่มีต่อการเจริญเติบโตของ *S. rostrata* และ *A. afraspera* ในกระถาง โดยปลูกโสนในสภาพที่มีน้ำท่วมขัง และสภาพไม่มีน้ำท่วมขัง ในระหว่างฤดูแล้งที่อุณหภูมิค่อนข้างสูง โดยระยะแรกของการเจริญเติบโต การเกิดปมเองน้อยมากในโสนทั้ง 2 พันธุ์ อาจเนื่องมาจากอุณหภูมิสูงและความชื้นต่ำ การใส่เชื้อ *A. caulinodans* ที่ลำต้นช่วยให้เกิดปมมากมายสถานการณ์เช่นนี้แสดงว่ามีความสัมพันธ์ระหว่างปมและธาตุอาหาร P อย่างใกล้ชิด แม้กระทั่งก่อนการใส่เชื้อ การเจริญเติบโตของ *S. rostrata* ในที่ซึ่งใส่ TSP จะเป็นไปได้อย่างดี แต่พบว่าพืชค่อนข้างเหลืองเนื่องจากขาด N หลังจากใส่เชื้อแล้วพืชจะเขียวขึ้น สำหรับ PR ก่อนใส่เชื้อดูเหมือนจะกดการเจริญเติบโตของพืช หลังจากใส่เชื้อแล้วการเจริญเติบโตจะดีขึ้น ใน *A. afraspera* ก็พบเช่นเดียวกัน แต่ว่า *A. afraspera* ค่อนข้างแย่กว่า *S. rostrata* ในแง่ของปุ๋ย PR