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NODULATION AND GROWTH OF *LABLAB PURPUREUS (DOLICHOS LABLAB)* IN RELATION TO RHIZOBIUM STRAIN, LIMING AND PHOSPHORUS

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KEY WORDS

Dolichos Greenhouse Growth Lablab Lime Nodulation Phosphorus requirement Rhizobium strain Soil acidity

SUMMARY

Greenhouse experiments were done with two purposes: (1) to identify strains of rhizobia effective and acid-tolerant in symbiosis with Lablab purpureus, and (2) to determine whether soil acidity or the symbiotic condition increased the phosphate requirement for growth.

Five rhizobia! strains were tested in one neutral soil, two acid soils, and the two acid soils limed to pH 6.6. In the neutral and limed soils, three of the strains were effective (CB1024, CB756, TAL169), but only two strains (CB756, TAL169) remained effective in acid soil.

Strain CB756 and plus-N treatments were further compared in a factorial trial involving combinations of five levels of P with lime, no lime and $CaCI_2$ treatments, applied to an acid soil. Some of the treatments were also applied to plants inoculated with (CB1024. Between the N-fertilized and CB756 treatments there was no clear difference in growth response to applied P, and the critical internal concentration of P for 95% of maximal growth was the same (0.22% shoot dry weight). Increasing P beyond levels needed for maximal growth increased nodulation and N concentration in plants inoculated with (CB756. It lowered N concentration in N-fertilized plants. There was evidence suggesting that the P requirement of symbiotic plants increased if the soil was acid, or if CB756 were replaced by CB1024 as microsymbiont: but the critical statistical interactions were not significant.

INTRODUCTION

Lablab purpurrus is a useful grain legume, rotational legume, and forage in tropical areas. Its nodulation and rhizobial strain requirements have received some attention[3,7,15,16] (J. *C.* Burton, personal communication), but apart from a field trial by Herridge and Roughley[7] there appear to have been no studies to assess influence of soil acidity or phosphate deficiency.

Some legume species are more tolerant of soil acidity and *P* deficiency than , others[4,9], and recent evidence suggests the same kind of variation for strains of

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rhizobium[11,14.] Such variation should be useful for selecting tolerant rhizobia and legumes. Considering this information and the importance *of Lablab purpureus,* greenhouse trials were conducted to: (1) select strains of rhizobium effective on this species under acid soil conditions and (2) evaluate the beneficial role of liming and phosphate fertilization on nodulation and plant growth.

Effects of rhizobial strain, liming and P were assessed by measurements of nodulation, acetylene reduction, dry matter production and N concentration in plant tops.

EXPERIMENTAL

Nodulation in soils of *different pH*

In a five x seven factorial experiment with two replicates, inoculation and N treatments were applied to each of five soils. The treatments were a check, an NH_4NO_3 treatment, and five inoculants each consisting of a separate strain of rhizobia. The strains were CB756 (= TAL309) and CB1024 (=TAL3I0) from CSIRO Division of Tropical Pastures, Brisbane, Queensland; and TAL169 (=176A22),TAL173(= 176A30)andTAL174(= 176A32)from the Nitragin Co.Inc., Milwaukee, Wisconsin via the University of Hawaii NifTAL Project, Paia, Hawaii. The soils were: (1) Tujunga sand, (Typic Xeropsamment)0-20cm. from Davis, California, pH 6.3(saturated aqueous paste); (2) Josephine loam (Typic Haploxerult)30-50 cm. from Calaveras, California, pH 5.6; (3) Josephine soil limed to pH 6.6 with 2 g $CaCO_3$ kg soil (pH measured a week after $CaCO_3$ addition): (4) Goldridge fine sandy loam (Typic Hapludult). 30- 60 cm from Sebastopol, California, pH 4.7;(5) Gold ridge soil limed to pH 6.6 with 3g $CaCO_3$ kg soil.

Undrained pots, lined with polyethylene, were filled with 1800g of air-dry soil. Each pot received a basal addition of S, P, Mo and Zn. After addition of lime and fertilizer, pots were brought to field capacity with distilled water, left one week, remixed and repotted.

Seed of Lablab purpureus (common Sudanese commercial) from the Department of Range Management, Khartoum, Sudan, was surface-sterilized with 30% H_2O_2, and rinsed with sterile water. Six seeds were planted per pot, then directly inoculated with 1 ml per seed of suspension containing 10^4 viable cells per ml. Seeds were then covered and the pots brought to field capacity by weight. After emergence, plants were thinned to three per pot. Directly after thinning. NH_4NO_3 was added to + N pots to supply 100 ppm N. and another equal dose was applied three weeks after emergence.

Six weeks after emergence. plants were cut at the soil surface, tops were dried, and roots were used to determine nodule number and fresh weight.

Effects of *phosphorus, $CaCl_2$ and $CaCO_3$*

A five x three x two factorial experiment with two replications was set up with the Goldridge soil. Five levels of P were applied, as KH_2PO_4 solution (Table 2). These were factorially combined with treatments supplying 3.0 g $CaCO_3$/kg soil, 0.3g $CaCl_2$ /kg soil, or no addition of calcium; and with two N treatments, via inoculation with strain CB756 and + N. In all other respects, soil preparation, basal treatment, planting and inoculation were as in the previous experiment.

There was a sub-experiment with 14 pots inoculated with strain CB1024, run concurrently. Its purpose was to compare the P responses of CB1024 and CB756 in the presence of $CaC0_3$, and to compare their response to $CaCl_2$ and acidity at the highest P level.

At harvest, six weeks from emergence, tops were cut, dried arid weighed. Roots were washed out of the soil, blotted and immediately assayed for acetylene reduction (1 hour, 0.1 atmosphere acetylene, 25ºC). Percent N was determined by a micro-Kjeldahl procedure[12]and total P according to Johnson and Ulrich[8].

RESULTS AND DISCUSSION

Strain performance in different soils

From early growth, visual observation of plant size and color separated the five strains into three groups. Strains TAL169 and CB756 were effective in all soil treatments. Strain CB1024 was effective except in the acid Goldridge soil. Strains TAL173 and TAL174 were ineffective. These observations were generally supported by data on the presence of nodules, plant weight, and plant N (as compared with checks. Table 1). The three effective strains differed little in nodule weight, except for poorer nodulation by CB1024 in the acid Goldridge soil. Except for plants grown in the Tujunga sand, yields were significantly greater with + N treatments compared to any other treatment. The three effective strains gave plant N concentration similar to N-fertilized controls in the naturally neutral Tujunga soil. In the limed acid soils although yield was depressed relative to N-fertilized controls, yet N concentration in the shoots remained high. Reduction in plant yields and N concentrations by soil acidity was largest for CB1024 and smallest for CB756 (Table 1). Strain CB756 is an established wide spectrum strain for tropical legumes (R. A. Diatloff and R. A. Date, personal communication). It has been shown capable of effectively nodulating *Lablub* purpureus in soils low in Ca and P[15].

The ineffective strains produced less than five nodules per plant, all very small. Both strains are effective on some other tropical legumes and tolerate soil acidity and aluminium in culture media and in symbiosis with cowpea in soil[10,11.]

The differences in symbiotic tolerance of soil acidity between the three effective strains agree with observations on their relative tolerance of acidity factors in culture media and in symbiosis with cowpea[10] and Vigna trilobata (M. G. Zaroug, unpublished) in soil. These results differ from those of Herridge and Roughley [10], who concluded from a field trial with *Lablab* purpureus that CB 1024 formed more nodules per plant in an acid soil than CB756 and gave plant yields that were (not significantly) higher. However, the discrepancy between their results and those presented here may arise from points of technique. They used larger inocula (10^7 cells per seed as against 10^4 in this study), and they applied 500 kg/ha of a 50:50 mixture of lime and superphosphate, concentrated in the

Table 1. Nodulation, dry matter and percent nitrogen of *Lablab purpureus* as affected by Rhizobium strain, soil type and liming

	Nodule fresh weight g/plant	Dry matter yield g/plant	Percent nitrogen
Tujunga			
Check	–	2.21	1.49
Plus nitrogen	–	3.94	3.03
CB756	0.68	3.81	3.03
CB1024	0.68	3.62	3.17
TAL169N	0.83	3.76	3.51
TAL173N	–	2.36	1.20
TAL174N	–	2.15	1.15
Josephine			
Check	–	2.20	0.91
Plus nitrogen	–	3.33	3.65
CB756	0.61	2.52	3.17
CB1024	0.68	2.47	2.74
TAL169N	0.63	2.65	2.83
TAL173N	–	2.24	1.25
TAL174N	–	2.26	1.58
Josephine + lime			
Check	–	2.05	1.25
Plus nitrogen	–	3.92	3.46
CB756	0.73	2.81	3.07
CB1024	0.67	2.75	3.36
TAL169N	0.73	2.74	3.12
TAL173N	–	2.17	1.16
TAL174N	–	2.02	1.39
Goldridge			
Check	–	2.06	1.02
Plus nitrogen	–	3.60	2.93
CB756	0.56	2.78	3.46
CB1024	0.18	2.27	2.93
TAL169N	0.48	2.77	2.98
TAL173N	–	2.22	0.98
TAL174N	0.11	2.22	1.07
Goldridge + lime			
Check	–	2.29	0.87
Plus nitrogen	–	4.00	3.17
CB756	0.68	2.85	3.03
CB1024	0.74	3.07	3.12
TAL169N	0.81	3.01	3.70
TAL173N	–	2.15	1.31
TAL174N	–	2.23	1.44
$LSD_{0.05}$		0.51	0.51
$LSD_{0.01}$		0.61	0.61

Table 2. Influence of pH, calcium, and phosphorus nutrition on nodule fresh weight of *Lablab purpureus* (Goldridge soil)

Strain	CB756			CB1024		
pH	4.7	4.4	6.6	4.7	4.4	6.6
Phosphorus applied – ppm	No Ca	$+CaCl_2$	$+CaCO_3$	No Ca	$+CaCl_2$	$+CaCO_3$
0	0.38	0.34	0.71	–	–	0.32
20	0.70	0.71	0.74	–	–	0.64
40	0.99	0.69	0.89	–	–	0.72
80	1.18	0.80	0.93	–	–	0.87
160	1.27	1.42	1.48	0.22*	0.37*	1.01
	$LSD_{(.05)} = 0.54$ $LSD_{(.01)} = 0.75$			$LSD_{(.05)} = 0.53$		

* Values not included in the analysis of variance. Nodule fresh weight is given in g/plant.

Table 3. Influence of pH, calcium and phosphorus nutrition on nodule activity of *Lablab purpureus* (expressed as μmol of ethylene formed per g nodule fresh weight per hour)

Strain	CB756			CB1024		
pH	4.7	4.4	6.6	4.7	4.4	6.6
Phosphorus applied – ppm	No Ca	$+CaCl_2$	$+CaCO_3$	No Ca	$+CaCl_2$	$+CaCO_3$
0	18.15	13.32	12.15	–	–	15.89
20	21.06	15.58	32.73	–	–	19.18
40	31.64	29.08	30.67	–	–	31.76
80	42.18	33.22	44.26	–	–	32.16
160	39.39	29.32	28.12	56.48*	50.71*	40.87
	$LSD_{(.05)} = 21.31$			No significant difference		

* Values not included in the analysis of variance.

furrows. Both points would tend to minimize expression of acid and phosphate stress in their experiments.

Apart from acidity, there were effects of soil on plant yield that may relate to nutrient supplying abilities of the soils. Thus, strains CB756. CB1024 and TA L 169 produced yields of dry matter and N only about 70% of the appropriate N controls in the limed acid soils, but almost 100% of the N controls in the Tujunga soil. Better fertility of the latter, in some respect not identified, may have been responsible for narrowing the difference between N treatment and the most effective strains.

Effects of phosphate, time and $CaCI_2$

Nodule number per plant increased gradually from 14 per plant with no added P to 35 with the addition of 160 ppm P, with strain CB756 in the $CaCI_2$ treatment.

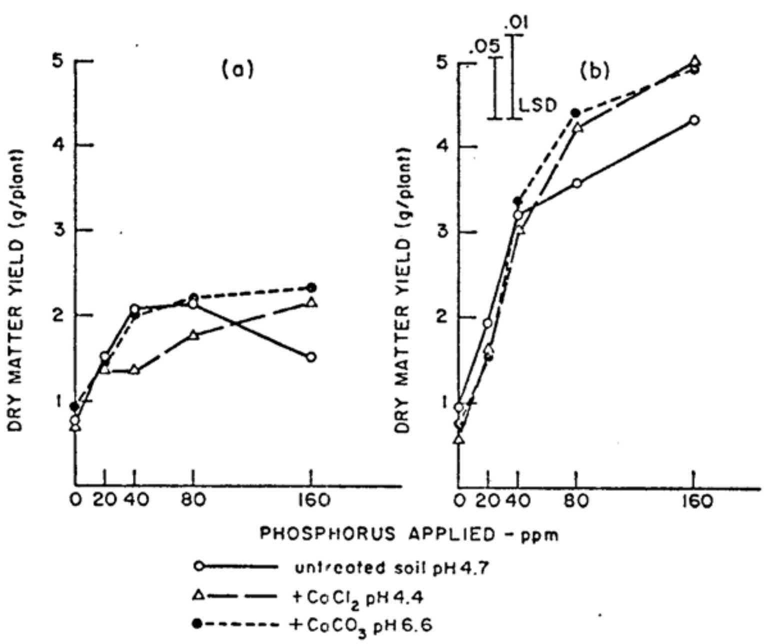

Fig. 1. Effects of phosphorus, pH and calcium on dry matter yield of *Lablab purpureus*. (a) symbiotic plants. (b) N-fertilized plants.

With strain CB1024, $CaCl_2$ significantly lowered the number of nodules ($P < 0.01$) as compared with lime. In the presence of lime or in the untreated soil. P had little effect on nodule number with either CB756 or CB1024.

There were large effects of P on nodule weight and activity (Table 2 and 3), as well as on plant growth and nitrogen concentrations (Figs. I and 2). For both rhizobia strains nodule weight increased gradually with addition of P up to 160 ppm. There was no significant depression of nodule weight by acidity, except in CB1024 treatments (Table 2). Nodule activity, measured as acetylene reduction rate per unit nodule weight, was similar for both rhizobia and similarly tended to increase with addition of P, as also observed in *St ylosanthes humilis*[5]. Unlike nodule weight, nodule activity tended to increase with soil acidity in CB1024 (Table 3). This increase did not correspond with adverse effects of acidity on growth of the plants. For strain CB756, correlation studies showed significant relation of the P concentration in the plant shoots with nodule weight ($r = 0.91$, $P < 0.01$) and with activity ($r = 0.74.$, $P < 0.05$).

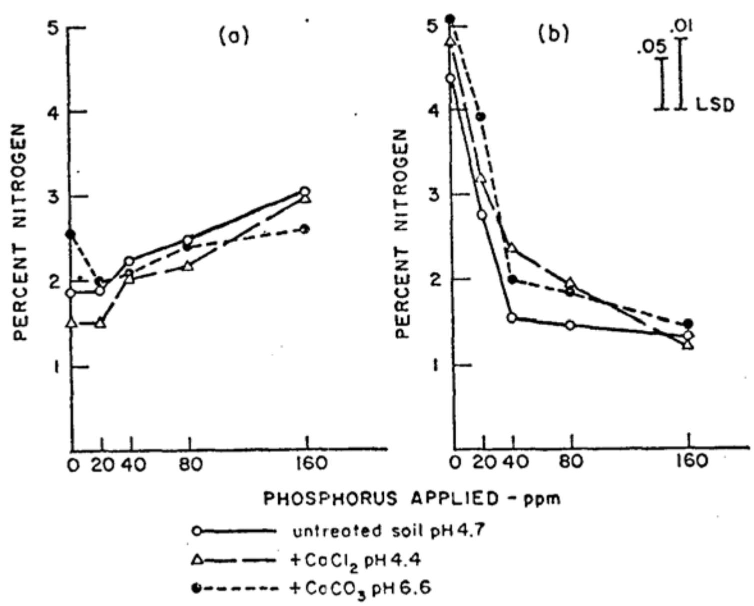

Fig. 2. Effects of phosphorus, pH and calcium on N concentration in shoots of *Lablab purpureus*. (a) symbiotic plants. (b) N-fertilized plants.

Dry matter yield with CB756 and with fertilizer N increased most sharply with P addition up to 40 and 80 ppm P, respectively, in limed treatments (Fig. 1). There was no clear indication of a difference in P requirements between N fertilized and symbiotic plants. A determination of critical internal P value confirms this interpretation.

Critical value is considered here as the P concentration of the plant tops required to produce 95% of the maximal plant yield. Curves of the form $y = a + bx + cx^2$ were fitted to the data of dry matter yield and P concentration in the shoots (Fig. 3). The resulting critical P values were identical, 0.22% for N-treated and symbiotic plants.

The maximal yield of symbiotic plants was substantially less than N-fertilized plants in this trial (Fig. I). Clearly P was no longer limiting at the higher P levels supplied, at least for plants symbiotic with CB756. Nor was N, since its concentration at P levels above 40 ppm, was higher in symbiotic than in N-treated plants.

Nitrogen concentrations in N-treated plants declined with increasing P (Fig.

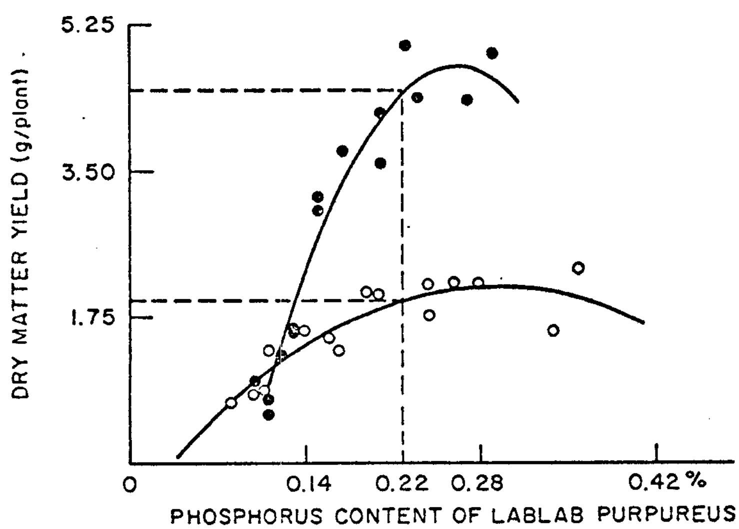

Fig. 3. Relations between dry matter yield and concentration of phosphorus in *Lablab purpureus* tops.

● Plus nitrogen $r = 0.943$.
○ Inoculated $r = 0.790$.

2), and concentrations of N and P in the tops were negatively correlated ($r = -0.80$, $P < 0.01$). This reflects a common tendency for a nutrient in excess supply to accumulate in stunted plants and became diluted when a growth limitation is relieved. In symbiotic plants (CB756), N concentration increased with addition of P, regardless of lime treatment, up to the highest (160 ppm) level of P (Fig. 2); and concentrations of N and P in the plants correlated positively ($r=0.79$, $P < 0.01$). This is consistent with the similar progressive increase in nodule weight (Table 2) and with Gates's's[5] evidence that P promotes nodulation. Improvement of P nutrition beyond levels necessary to maximize dry matter production commonly improves protein content of effectively symbiotic legumes[1,2,6].

The decrease in yield of plants symbiotic with CB756 at the highest level of P in limed soil was statistically significant (Fig. la). It may have arisen from a phosphate induced deficiency of some other nutrient. It was accompanied by formation of a large mass of nodule tissue of low activity (Tables 2 and 3). perhaps produced and maintained at the expense of plant growth:

Nodule activity and weight measurements in general failed to reflect the poor performance of strain C11 1024 indicated by yield and N concentration data. The activity might have been high because it was measured on young nodules. formed late; or there may have been poor energetic efficiency Or some failure to translocate N to the shoots with CB1024.

An effect of soil acidity on P requirement is not demonstrated with certainty. Acidity clearly had little effect on N-fertilized plants (Figs. 1, 2); but there is some suggestion that the most acid treatment ($CaCl_2$) had deleterious effects on growth and N concentration that were partly overcome by P addition in the CB756-symbiosis (Figs. 1,2). This can be regarded as an increase in P requirement in the soil, consequent upon $CaCl_2$ addition, and would be consistent

Table 4. The effect of the addition of phosphorus at different soil pH values on dry matter yield and nitrogen concentration of *Lablab purpureus* inoculated with CB1024

Phosphorus applied ppm	pH	Dry matter yield g plant	Percent nitrogen
0	6.6	0.73	2.60
20	6.6	1.39	2.23
40	6.6	1.51	2.21
80	6.6	1.50	2.53
160	6.6	1.70	2.40
160	4.4	0.74	1.97
160	4.7	0.61	2.48
$LSD_{.05}$		0.73	

with salt effects on soil pH and AI-activity and with precipitation of toxic concentrations of Al by added P[13,14]. However, the interaction between P treatment and lime/Ca treatments was not statistically significant.

Likewise the data do not establish that the symbiosis with CB1024 needed higher P than the symbiosis. with CB756, as might be expected from evidence that CB1024 has a high P requirement for growth in culture media[11]. In the limed soil, CB756-inoculated plants approached maximal yield at 40 ppm added P (Fig. 1) while CB 1024-inoculated plants might have needed more than 160 ppm (Table 4), but interactions between P treatment and rhizobial strain were not significant.

The data do confirm the finding of the first experiment that CB 1024 was more acid-sensitive than CB756. At high levels of P, there was only slight decrease in yield of the no-lime treatment as compared with lime or $CaCl_2$ on the part of plants inoculated with CB756 or given N (Fig. I). But plants inoculated with CB1024 failed completely at low pH, with or without calcium (Table 4), in keeping with the severe depression in nodule weight.

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