EFFECTS OF SOIL FERTILITY TREATMENTS ON GROWTH AND NODULE PARAMETERS OF WINGED BEAN [*PSOPHOCARPUS TETRAGONOLOBUS* (L) DC] WITH A DARK RED LATOSOL (TYPIC EUTRUSTOX) FROM JAIBA, MINAS GERAIS, BRAZIL

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CHAPTER I

INTRODUCTION

Edible legumes are excellent sources of dietary protein and lipids essential for human nutrition. Many nutritionists expect legumes to play an increasing role in meeting needs for food with increasing populations during times of food shortages, and widespread malnutrition. Protein malnutrition is especially acute for young children in many developing countries located in the humid tropics (2).

A large number of grain legumes are known to man, but only 20 species are used in the human diet and just six of them - soybean, peanut, dry bean, chick pea, pigeon pea, and cow pea - account for the bulk of world production (24). Among the less utilized legumes, the winged bean [*Psophocarpus tretagonolobus* (L) DC] has recently received much scientific attention for increasing vegetable protein production.

The entire winged bean plant can be utilized as a foodstaff. The young pods are a tender, crunchy vegetable that can be eaten raw or added to cooked vegetable dishes. The leaves, young shoots and flowers can be eaten either raw or cooked. Also the flowers are used to color dishes for the table. Mature seeds are not eaten raw and require cooking. Sometimes they are roasted and eaten like peanuts. The seeds have a similar composition to that of soybean. The tubers have a high protein content approximately 20% on a dry weight basis. The

tubers are eaten like potatoes, sometimes raw, but more frequently as a cooked vegetable. Medicinally, the leaves of the winged bean have been used in the treatment of small pox in Ghana. The roots have been used in Malaya as a poultice to cure vertigo (24).

In addition to value as human food, the leaves and stems can be used as forage, green manure, or as cover restorative crop (3, 41). Because of its capacity, through the nodules on its root, to fix nitrogen from the air into available soil nitrogen, it can grow in relatively poor soils (3) so common in the tropical areas.

Although the high protein content of winged bean has been known since 1929 (1), and its adaptability to the humid tropical environmental conditions considered excellent, this plant is presently farmed intensively in only a few countries in Asia (24). New areas that could benefit from the introduction and sound agronomic cultivation of this bean include large parts of Central and South America, the Caribbean, Africa, Oceania and West Asia (43).

The winged bean, if introduced and properly farmed in the humid area of northern Brazil, or if irrigated where required, could contribute toward alleviating the protein problems of a large area of the country.

The main objective of these studies was to determine the effect of soil fertility treatments on the winged bean growth and development, as well as on some selected nodule enzyme and carbohydrate components involved on the mechanism of symbiotic nitrogen fixation. A secondary objective was to determine if the enzyme activity data obtained conformed to the presently known pathways of ammonia incorporation into plant amino acids.

The soil used in these studies was a Dark Red Latosol (Typic Eutrustox) collected in Jaiba, in the northern part of Minas Gerais State, Brazil.

CHAPTER II

LITERATURE REVIEW

Because the winged bean is still a "backyard crop" in just a few countries of the humid tropics, scientific agronomic information about its cultivation is scarce and scattered in a number of publications sometimes hard to obtain. Information about nodulation and nodule enzyme parameters determinations are still more difficult to find.

Origin and Distribution

According to Masefield (42), the origin of the Psophocarpus tetragonolobus is still uncertain, for this species is not found anywhere in the world as a truly wild plant. Ramirez (55) claimed that today this plant is commonly cultivated in the Philippines, being introduced from India or Malaya. Agcaoili (1) citing Merril, acknowledged that the wing bean was cultivated in the Philippines prior to 1912. Merril speculated that the plant was introduced in that country from India and Malaya. Burkil (10) pointed out that winged beans have been farmed in Burma since 1897 and that the largest acreage planted to this crop occurred in 1902. According to Hymowitz et al. (25) this plant is mostly cultivated in Asia, especially India, Burma, Malaya, Thailand, the Philippines, Indo-China, China, Ceylon, Indonesia, Papua, New Guinea and several South Pacific Islands, and concluded that today the greatest diversity of P. tetragonolobus occurs in Papua New Guinea,

and this is probably the original geographical center of this genus. Other reports (50) cite the center as possibly Papua, New Guinea with the other species of the genus, Psophocarpus, being native to elsewhere in Africa.

Botany

Pospisil et al. (51) described the Psophocarpus tetragonolobus as a perennial herb usually grown as an annual. Masefield (42) also considered this plant to be a climbing herbaceous perennial. He further stated that Agcaoili (1) was mistaken when he described the winged bean as an annual. The plant when properly supported on poles or trellis, can grow up to three meters high or more, the leaves are trifoliate, 8-14 cm long, and the flowers of papilionaceous type, are blue or white (42, 51) or variations of purple (29). Pospisil et al. (51) observed that in Ghana, flowers opened between the hours of 8-10 a.m. and were pollinated by several types of bees, with a low setting of fruits when the population of insects was absent. On the other hand, Khan (29) in Papua, New Guinea, observed that flowering occurred between 48-90 days after planting, and that flowers opened after noon. He forwarded the hypothesis that under Papua, New Guinea, conditions, the winged bean was predominantly self-fertilized. Pods may be up to 30 cm long and bear from 10 to 16 seeds (42). The color of the seeds is influenced by genetics and environmental conditions. According to Masefield (42) they can be white, yellow, brown and black, but in Papua, New Guinea, Khan (29) found that variations of brown and tan were the most prevalent color. The winged bean root system has a tap root and several lateral roots which with time may thicken forming potato-like tubers.

Nodulation is abundant, even when the plant is grown for the first time in virgin lands, and Masefield (42) reported that in Malaya the fresh weight of nodules can attain 700 lb/acre.

The cycle of the plant, from seed to seed varies considerably, greatly influenced by genetics and environmental conditions. In Western Samoa, the winged bean produced seeds in 3 months after sowing, while in higher latitudes it may not flower at all (3).

Uses

A striking point about the winged bean is that the whole plant can be used as human foodstuff. The young pods, prepared like french beans, are the most popular part of the plant (24) and for this use they must be harvested 2 weeks after fertilization (51). After the third week the seeds mature, and can be consumed roasted like peanuts (24). Unripe seeds are also prepared for soups and curries (51). Like many other legume seeds, the winged bean seeds contain a pepsin inhibitor that can be easily destroyed by cooking (11), and no urease enzyme has been found to be present in the seeds (51).

The high protein content of ripe winged bean seeds has been known by Agcaoili (1) since 1929. He apparently was the first investigator to observe that the winged bean seeds are very similar in composition to those of soybean. He further indicated that winged bean seeds could have the same use as soybean.

In 1971, Cerny et al. (11) determined that the nutritive value of winged bean seeds were superior to peanuts and later (12) used it successfully to treat kwashiorkor, a disease caused by ingestion of extremely low amounts of protein in the human diet, commonly found in young growing children in Ghana.

Pospisil et al. (51) also determined that besides the high protein content, winged bean seeds have a favorable content of unsaturated fatty acids, with high concentration of vitamin E and A.

Several authors have performed protein and oil analysis of the winged bean seeds. Table I shows their results as cited by Masefield (42).

TABLE I

WATER, PROTEIN AND OIL CONTENT OF WINGED BEAN SEEDS

Author	% Water	% Protein	% Oil
Kong and Bromer	12.3	29.8	15.0
Hooper	9.5	37.4	15.5
Agcaoili	9.7	32.8	17.0
Tindall	14.0	33.0	16.0
Pospisil, et al.	n.a.	37.3	18.1

The roots of the winged bean are also edible and are most popular in Burma and in the South Pacific Islands (24). Unlike many other root crops with high carbohydrate-low protein content, the slightly sweet winged bean tuber contains around 20% protein on a dry weight basis. This is a remarkable 10-20 fold increase over the figures for such popular tropical staple root crops as cassava (1%), potatoes (2%) and yams (2%).

Pospisil et al. (51) recommended that in Ghana roots be dug out when they are little thicker than the human thumb.

Although the leaves of the winged bean have also a high protein content and in Malaya are used as a leaf vegetable (42), it has better possibilities as a forage crop. Pospisil, et al. (51) also pointed out that the winged bean makes a good green manure and a fallow restorative crop, the stem being very palatable to stock. However, according to Hyminowitz et al. (24) it is very difficult to incorporate into the soil the large mass of winged bean material. For this purpose the smaller Psophocarpus palustris is more suitable.

Cultivation

Extremely scarce data are available concerning the cultivation of the P. tetragonolobus. Pospisil, et al. in Ghana (51) seem to be the first investigators to set up winged bean cultivation experiments in the field. They found the 2 x 2 ft. spacing to be the best spacing seed production. Khan (29) in Papua, New Guinea planted several introductions of this plant spaced 1 x 1 m, and among various parameters determinations, recorded the root and seed yield per plant. He speculated that the 2 x 2 ft spacing suggested by Pospisil et al., could be successfully used in his trials without reducing the yield per plant. He further suggested then, that a dual purpose plant would produce 1322 Kg/ha of edible dry matter. However, recorded data for seed and root yields in Kg/ha varies to a great extent. Seed yields vary from 450 Kg/ha (10) to 11,000 Kg/ha every 5 months in Australia (3) and Hymowitz et al. (24) observed that higher yields could be achieved with improved varieties.

One of the major deterents for the cultivation of the winged bean on a large scale is the necessity for staking. Pospisil, et al. (51) recommended that plants be allowed to climb a hedge staking and not be permitted to grow very high, so that harvesting would not be difficult. The soil should be maintained free of weeds, so they will not become entwined with the winged bean, causing the harvesting to be difficult.

The winged bean is found commonly in the tropics and probably requires more than 250 mm of rainfall to thrive. In less rainy areas supplemental irrigation should be applied (50). The plant is found growing at sea level (50) and at 4000 meters altitude in the highlands of Papua, New Guinea (29).

Although it has been observed to be tolerant to insects and diseases, Khan (29) found several plants infected with root knot nematode (Meloidogyne sp), and a heavy infestation of Moruca testalis depressed the seed yields of his experiments. Pospisil et al. (51) in Ghana reported that flowers and pods can be sometimes attacked by caterpillars and the leaves are eaten by grasshoppers and spider mites. A minor attack of the fungus Sunchytrium psophocarpi was also observed in the young pods. Also, in Ghana (3), it was reported that a severe virus attack was observed on plants younger than 70 days.

In Brazil the plant was introduced in 1976 as a cover crop in rubber, cocoa and oil plantations in southern Bahia (3), and seeds were given out to some farmers to be used as a food crop. It was also reported that the winged bean was successfully introduced at IRI, Matao, São Paulo. Although one sprinker irrigation of about 50 mm was used because of drought, an unirrigated plot showed that irrigation

was of marginal value. No insect or disease problems were observed and the winged bean completely dominared the soil. Although the seed harvesting had not yet occurred at the time of this report, seed yields were expected to be heavy in both poled and unpoled crops (3).

Nodulation

A very high number of nodules in the winged bean roots was first observed by Thompstone and Sawyer (42) in 1914. Later Masefield (40) found that P. tetragonolobus was the most heavily nodulated legume plant in Nigeria and Malaya. He pointed out that in Malaya, individual plants of P. tetragonolobus may carry up to 440 nodules and their fresh weight can attain up to 784 Kg/ha. The winged bean nodules are unusually large, and individual nodules can weigh up to 0.6 g with a diameter up to 1.2 cm (40).

The plant-rhizobium association has been observed everywhere the crop has been planted, even in virgin soils of Malaya, when there was no artificial inoculation.

Although Dobereiner (15) has pointed out that soil management and fertilization studies may possibly indicate field practices which enhance nitrogen fixation, enzyme nodule parameters as influenced by nutrient levels in the soil has not been quantified for the winged bean.

CHAPTER II

MATERIALS AND METHODS

The soil used in these studies was the top layer of a Dark Red Latosol (Typic Eutrustox, isohyperthermic, fine, kaolinitic) previously described (18, 52). The chemical and particle size analysis of this soil is summarized in Table II.

Each experimental pot contained 100 g of soil diluted in 400 g of 0.1 N HCl washed white quartz sand, and planted with 1 winged bean [Psophocarpus tetragonolobus (L) DC] seedling. In the first experiment, the WB-21-8 Tinge was grown for 75 days during short day photoperiod (from 1-31-79 to 4-16-79). In the second experiment, the WB-12-11 Siempre was grown for 52 days during the long day photoperiod (from 5-4-79 to 6-25-79). Seeds for these experiments were obtained from Mayaguez Institute of Tropical Agriculture, Box 70, Mayaguez, Puerto Rico 00708. At planting, each pot culture was inoculated with 3 ml of Rhizobium leguminosarum, liquid medium, containing more than 10^8 viable cells ml^{-1} cultured from nodules of Strophostyles sp.

These experiments were carried out as a randomized complete block design, and fertility treatments consisted of a complete 2^5 factorial arrangement using P, S, Ca, Mg and K combinations. Each treatment was replicated three times.

The sources and nutrient levels for the first experiment, when WB-21-8 Tinge seeds were planted are presented in Table III.

TABLE II

SOIL ANALYSIS OF THE 0-20 CM DEPTH LAYER OF THE DARK RED LATOSOL USED IN THE GREENHOUSE EXPERIMENTS

Properties	
pH (H_2O)	6.1
Buffer index	6.8
CEC (NH_3EC), meq/100 g	25.4
Percent organic matter	3.3
Exchangeable cations meq/100 g	
Ca	13.8
Mg	2.5
K	0.4
Al	not detected
Available P (Bray P_1) ppm	7.5
Fe ppm	680.0
Mn ppm	208.0
Zn	1.0
% sand	24.5
% silt	19.5
% clay	56.0
Texture	Clay

Pot cultures not fertilized with $NH_4H_2PO_4$ received 11.2 mg NH_3 acetate to balance out the ammonium effect of the phosphate fertilizer.

After the harvesting of the WB-21-8 Tinge variety, the soils of the three replications were thoroughly mixed together, repotted, and then retreated with double nutrient levels and planted with 1 seed of WB-12-11 Siempre variety. The Base Cation Ratio was equal one for both experiments.

$$BCR = \frac{K}{\sqrt{\frac{Ca + Mg}{2}}} = 1$$

TABLE III

SOURCE AND NUTRIENT LEVELS FOR THE FIRST EXPERIMENT (SHORT DAY LENGTH) WB-21-8 TINGE VARIETY

Nutrient	Source	Level
Phosphorus	$NH_4H_2PO_4$	50 ppm
Sulphur	Na_2SO_4	50 ppm
Calcium	$CaCO_3$	6 meq/100 g of soil
Magnesium	$MgCl_2 \cdot 6H_2O$	2 meq/100 g of soil
Potassium	KCl	2 meq/100 g of soil

The 32 possible treatment combinations for the 2^5 complete P, S, Ca, Mg and K factorial are shown in Table IV.

Harvesting of these experiments took place between the hours of 8-10 a.m. Plant tops were clipped, oven dried at 105°C for 24 hours and weighed. The nodule-root system was carefully shaken free of soil, briefly washed (< 30 sec), blotted with paper toweling to remove excess water, and placed in serum cap bottles for nitrogenase (EC 1.7.99.2) activity (C_2H_2) reduction determinations (69).

Nitrogenase activity was measured according to the method described by Hardy et al. (23). The nodule-root system was incubated for one hour with 0.1 atm C_2H_2 (lab. spec, purified grade, Linde Div. Union Carbide, Inc.) at 27°C, and C_2H_4 production was detected with a Perkin-Elmer GC 3920 with 1.83 m x 3.2 mm Poropak N 80/100 column (Walters Assoc.). The ethylene standard utilized for calibration and monitoring

TABLE IV

TREATMENT COMBINATIONS FOR WINGED BEAN EXPERIMENTS

Treatment	Symbol	Treatment	Symbol
1	0	17	PKCa
2	P	18	PKMg
3	K	19	PKS
4	Ca	20	PCaMg
5	Mg	21	PCaS
6	S	22	PMgS
7	PK	23	KCaMg
8	PCa	24	KCaS
9	PMg	25	KMgS
10	PS	26	CaMgS
11	KCa	27	PKCaMg
12	KMg	28	PKCaS
13	KS	29	PKMgS
14	CaMg	30	PCaMgS
15	CaS	31	KCaMgS
16	MgS	32	PKCaMgS

of gas chromatography analysis was the Scott Ev. Tech. 1090 ppm ± 5% C_2H_4/N_2 (Supelco, Inc.).

After the C_2H_2 reduction analysis, the nodules were picked from the roots, counted and weighed. Nodule free roots were oven dried at 105°C for 24 hours and weighed.

Cell-free nodule extracts (cytosol) for enzyme activity determinations were obtained with the methods described by Grimes and Fottrell (21), and by Brown and Dilworth (9), with slight modifications. Samples of freshly picked nodules were crushed within glass tubes at -0.5°C with addition of 0.1 M of phosphate buffer, pH 7.41 at the ratio of 10 ml of the solution to each gram of nodule. The nodule homogenate was then subjected to ultransonification at 7.3 K pulse frequency in an ice bath for 30 sec. using a PT105T Williams Polytron (Brinkman Instruments, Inc.), followed by refrigerated centrifugation at 12 x 10^3 g for 10 minutes. The clear, cell-free nodule extract (cytosol) was asceptically transferred to sterile culture tubes and stored at 0-5°C.

Enzyme activities determined in the nodule cytosol extract are expressed as International Units (U), and defined as the amount of enzyme which causes transformation of 1.0 μ mole of specific substrate per minute (38) determined in 3.0 ml of reaction volume, 1 cm light path, at 27°C (57).

Enzyme determinations included glutamate-oxaloacetate transaminase (GOT) (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) (21), glutamate-pyruvate transaminase (GPT) (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) (57), glutamate dehydrogenase (GDH) (L-glutamate: NAD $(P)^+$ + oxidoreductase deaminating, EC 1.4.1.2) (33), glutamine synthetase (GS) (L-glutamate:ammonia ligase) (63), glutamate

synthase (GOGAT) (L-glutamine:2-oxoglutarate aminotransferase oxidoreductase NADH, EC 2.6.1.53) (57).

Nodule cytosol saccharide levels were determined for D-glucose and sucrose as described by Kidby (30), and starch was first hydrolysed to amylose-amylopectin by breaking both α-D-(1→4) and α-D-(1→6) glucan linkages and then analyzed by the methods of Keppler and Decker (28).

Levels of the tricarboxylic acid intermediate, alpha-ketoglutarate (αKG) (2-oxoglutarate), were determined with the method proposed by Bergmeyer and Bernt (7), and soluble protein was measured by the Folin phenol reagent as described by Lowry et al. (39). Nodule cytosol levels of Pyridoxal-Pyridoxamine Phosphates were determined using a fluorometric method adapted from Schreider (59).

The data obtained were analyzed according to the Statistical Analysis System (SAS) (58), using the PROCEDURE ANOVA for single degree of freedom comparisons for fertility treatments, PROCEDURE GLM for linear regression models, and PROCEDURE CORR to determine possible relationship between paired independent variables.

CHAPTER IV

RESULTS AND DISCUSSION

Experimental results for the first (winter) and for the second (spring) experiments are summarized in Tables V to XXXVII.

Most of the parameters determined in these studies were influenced by the soil fertility treatments used in these experiments. Generally, a large number of high order interactions were also significant and are listed at the bottom of each Table. Thus, fertility effects will be discussed separately for each parameter with emphasis on the main effects of P, S, Ca, Mg, and K.

To determine if the main effect of each nutrient had a significant effect on the parameters studied, the following hypothesis

$$HO: \Delta \text{ effect} = 0$$

$$HA: \Delta \text{ effect} \neq 0$$

were tested by the F statistical test obtained from the analysis of variance tables. The null hypothesis (no nutrient effect) was not accepted for $P \leq 0.1$ or less. The Δ effect was defined as the difference between the mean of the 48 pot cultures treated with a particular nutrient and the mean of the 48 pot cultures that did not receive that nutrient. Thus, the five possible Δ effects for each parameter can be represented as:

$$\Delta \text{ effect: } (\bar{P},\bar{S},\bar{Ca},\bar{Mg} \text{ or } \bar{K})_1 - (\bar{P},\bar{S},\bar{Ca}\bar{Mg} \text{ or } \bar{K})_0$$

Contrasting with earlier observations, the WB-21-8 Tinge variety flowered but did not produce tuber growth when cultivated for 75 days with short day length (winter) with the same experimental conditions utilized for the WB-12-11 Siempre variety (54).

Shoot Dry Weight

Both the WB-21-8 Tinge (Table V) and the WB-12-11 Siempre (Table VI) varieties showed a highly significant increase in shoot dry weight yields ($P \leq 0.001$) when the soil was fertilized with P. The WB-21-8 Tinge variety also responded to K application ($P \leq 0.1$), whereas the WB-12-11 Siempre had increased shoot growth when Ca was added to the soil ($P \leq 0.05$).

Root Dry Weight

The WB-21-8 Tinge variety root dry weight (Table VII) increased ($P \leq 0.001$) when the soil was fertilized with P. However, the root dry weight obtained for WB-12-11 Siempre variety (Table VIII), was depressed ($P \leq 0.05$) when the soil was fertilized with this nutrient. All of the other nutrient failed to have any significant effect on root growth of both winged bean varieties used in these experiments.

Fresh Nodule Weight

Results from Table IX (WB-21-8 Tinge variety) and Table X (WB-12-11 Siempre variety) show increased nodule weight when the soil was fertilized with P($P \leq 0.001$), S($P \leq 0.05$, 0.001 respectively), and K($P \leq 0.05$ and 0.001 respectively). Neither Ca nor Mg affected nodule weight of the two P. tetragonolobus cultivars in these studies.

TABLE V

WB-21-8 TINGE WINGED BEAN, SHOOT DRY WEIGHT WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.66	Mg	0.66	S	0.71	MgS	0.60
K	0.86	KMg	0.72	KS	0.75	KMgS	0.90
Ca	0.64	CaMg	0.66	CaS	0.74	CaMgS	0.63
KCa	0.60	KCaMg	0.83	KCaS	0.77	KCaMgS	0.79
P	0.94	PMg	0.97	PS	1.10	PMgS	0.56
PK	0.88	PKMg	0.65	PKS	1.25	PKMgS	1.26
PCa	1.21	PCaMg	1.03	PCaS	0.53	PCaMgS	1.07
PKCa	1.06	PKCaMg	0.90	PKCaS	0.94	PKCaMgS	1.05

Element Main Effect

	P	S	Ca	Mg	K
without	0.72	0.83	0.84	0.85	0.79
with	0.96	0.85	0.84	0.83	0.89
Δ effect	0.24***	0.02^{ns}	0.00^{ns}	-0.02^{ns}	0.10#

ns = not significant, #, *** significant at $P \leq 0.1$ and 0.001 respectively for Ho: Δ effect = 0.

The interactions K x S*, P x K x S*, P x Ca x Mg x S* and K x Ca x Mg x S* were significant for $P \leq 0.1$.

TABLE VI

WB-12-11 SIEMPRE WINGED BEAN, SHOOT DRY WEIGHT WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.78	Mg	1.21	S	1.50	MgS	0.95
K	1.00	KMg	0.81	KS	0.93	KMgS	1.50
Ca	0.91	CaMg	0.91	CaS	1.16	CaMgS	1.21
KCa	1.03	KCaMg	1.13	KCaS	2.03	KCaMgS	0.85
P	1.33	PMg	1.47	PS	1.38	PMgS	1.04
PK	1.29	PKMg	1.15	PKS	1.66	PKMgS	1.64
PCa	2.01	PCaMg	1.84	PCaS	0.88	PCaMgS	1.75
PKCa	1.92	PKCaMg	1.38	PKCaS	2.06	PKCaMgS	1.27

Element Main Effect

	P	S	Ca	Mg	K
without	1.12	1.26	1.23	1.37	1.27
with	1.51	1.36	1.40	1.26	1.35
Δ effect	0.39***	0.10^{ns}	0.17*	-0.11^{ns}	0.08^{ns}

ns = not significant, *, *** significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x K x S#, P x Ca x Mg, S# were significant at $P \leq 0.1$; K x Mg*, K x S* were significant at $P \leq 0.05$, P x S**, K x Ca x Mg** were significant at $P \leq 0.01$; and K x Ca x Mg x S*** was significant at $P \leq 0.001$.

TABLE VII

WB-21-8 TINGE WINGED BEAN, ROOT DRY WEIGHT WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.18	Mg	0.19	S	0.17	MgS	0.20
K	0.22	KMg	0.26	KS	0.26	KMgS	0.22
Ca	0.17	CaMg	0.25	CaS	0.24	CaMgS	0.23
KCa	0.19	KCaMg	0.20	KCaS	0.21	KCaMgS	0.29
P	0.32	PMg	0.28	PS	0.31	PMgS	0.25
PK	0.17	PKMg	0.24	PKS	0.28	PKMgS	0.30
PCa	0.34	PCaMg	0.27	PCaS	0.27	PCaMgS	0.29
PKCa	0.28	PKCaMg	0.23	PKCaS	0.21	PKCaMgS	0.33

Element Main Effect

	P	S	Ca	Mg	K
without	0.22	0.24	0.24	0.24	0.25
with	0.27	0.25	0.25	0.25	0.24
Δ effect	0.05***	0.01^{ns}	0.01^{ns}	0.01^{ns}	-0.01^{ns}

ns = not significant, *** significant at $P \leq 0.001$ for Ho: Δ effect = 0.

The interaction P x K* was significant for $P \leq 0.05$ and K x Ca x Mg x S# for $P \leq 0.1$.

TABLE VIII

WB-12-11 SIEMPRE WINGED BEAN, ROOT DRY WEIGHT WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.45	Mg	0.46	S	0.46	MgS	0.49
K	0.44	KMg	0.49	KS	0.43	KMgS	0.37
Ca	0.42	CaMg	0.42	CaS	0.41	CaMgS	0.57
KCa	0.44	KCaMg	0.47	KCaS	0.47	KCaMgS	0.52
P	0.35	PMg	0.37	PS	0.34	PMgS	0.44
PK	0.42	PKMg	0.37	PKS	0.45	PKMgS	0.45
PCa	0.39	PCaMg	0.44	PCaS	0.54	PCaMgS	0.38
PKCa	0.57	PKCaMg	0.40	PKCaS	0.35	PKCaMgS	0.41

Element Main Effect

	P	S	Ca	Mg	K
without	0.46	0.43	0.42	0.43	0.43
with	0.42	0.44	0.45	0.44	0.44
Δ effect	- 0.04**	0.01^{ns}	0.03^{ns}	0.01^{ns}	0.01^{ns}

ns = not significant, ** significant at $P \leq 0.01$ for Ho: Δ effect = 0.

The interactions P x Ca x Mg#, K x Ca x Mg x S#, P x K x Ca x Mg x S# were significant at $P \leq 0.1$; P x Ca x S*, P x K x Mg x S* were significant at $P \leq 0.05$.

TABLE IX

WB-21-8 TINGE WINGED BEAN, FRESH NODULE WEIGHT WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.07	Mg	0.24	S	0.21	MgS	0.27
K	0.31	KMg	0.18	KS	0.11	KMgS	0.92
Ca	0.15	CaMg	0.24	CaS	0.13	CaMgS	0.43
KCa	0.26	KCaMg	0.29	KCaS	0.47	KCaMgS	0.35
P	0.56	PMg	0.85	PS	0.96	PMgS	0.15
PK	0.50	PKMg	0.58	PKS	1.45	PKMgS	1.14
PCa	0.96	PCaMg	0.83	PCaS	0.28	PCaMgS	0.98
PKCa	0.84	PKCaMg	0.46	PKCaS	0.65	PKCaMgS	0.90

Element Main Effect

	P	S	Ca	Mg	K
without	0.29	0.46	0.53	0.49	0.46
with	0.75	0.59	0.51	0.55	0.59
Δ effect	0.46***	0.13*	-0.02^{ns}	0.06^{ns}	0.13*

ns = not significant; *, *** significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interactions K x Ca# and P x Mg# were significant for $P \leq 0.1$; Ca x S*, P x Ca x Mg*, K x Ca x Mg*, P x K x S*, Ca x Mg x S*, K x Ca x Mg x S* were significant for $P \leq 0.05$; and P x Ca x Mg x S*** was significant for $P \leq 0.001$.

TABLE X

WB-12-11 SIEMPRE WINGED BEAN, FRESH NODULE WEIGHT WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	g/pot	Trt	g/pot	Trt	g/pot	Trt	g/pot
0	0.45	Mg	0.57	S	1.06	MgS	0.56
K	0.42	KMg	0.42	KS	0.48	KMgS	2.27
Ca	0.40	CaMg	0.47	CaS	0.45	CaMgS	0.73
KCa	0.72	KCaMg	0.72	KCaS	1.96	KCaMgS	0.71
P	1.36	PMg	1.30	PS	1.59	PMgS	0.55
PK	1.82	PKMg	1.30	PKS	1.98	PKMgS	1.91
PCa	1.20	PCaMg	1.34	PCaS	0.34	PCaMgS	2.03
PKCa	1.64	PKCaMg	1.68	PKCaS	1.92	PKCaMgS	1.70

Element Main Effect

	P	S	Ca	Mg	K
without	0.78	0.99	1.15	1.15	0.90
with	1.48	1.27	1.11	1.13	1.37
Δ effect	0.70***	0.28***	- 0.04^{ns}	0.02^{ns}	0.47***

ns = not significant, *** significant at $P \leq 0.001$ for Ho: Δ effect = 0.

The interaction P x K x Mg# was significant at $P \leq 0.1$; P x S***, K x S***, P x Ca x Mg***, P x Ca x Mg x S***, K x Ca x Mg x S*** were significant at $P \leq 0.001$.

Number of Nodules

While the fresh nodule weights of both varieties were significantly increased by P, S, and K fertilization, the data obtained for number of nodules (Tables XI and XII), indicated that only P and K had a significant effect on this parameter. Thus, these observations suggest that whereas S was important for increasing nodule growth, it had no effect on the number of nodules set during root growth. Similarly to fresh nodule weight, neither Ca nor Mg influenced the number of nodules per plant.

Nitrogenase Activity

The acetylene reduction (C_2H_2 red) technique was employed in these studies to assay nitrogenase activity. Data obtained for the WB-21-8 Tinge and WB-12-11 Siempre are summarized in Tables XIII to XV. A sharp increase ($P \leq 0.001$) in $C_2H_4 g^{-1}$ fresh nod hr^{-1} was observed when the WB-21-8 Tinge variety was fertilized with P and a significant response ($P \leq 0.05$) was also observed when K was added to the soil. Contrarywise, K decreased ($P \leq 0.05$) acetylene reduction ($C_2H_4 g^{-1}$ fresh nod hr^{-1}) in the WB-12-11 Siempre variety, with no P effect being observed in this case. Apparently, S, Ca and Mg did not affect nitrogenase activity of the winged bean when activity was measured as production of $C_2H_4 g^{-1}$ fresh nod hr^{-1}. However, a different pattern is shown in Table XV when nitrogenase activity was measured as production of C_2H_4 pot^{-1} hr^{-1} for the WB-12-11 Siempre variety. Measurement of nitrogenase activity in terms of production of $C_2H_4 g^{-1}$ fresh nod hr^{-1} indicates the efficiency of the R. leguminosarum to fix nitrogen in

TABLE XI

WB-21-8 TINGE WINGED BEAN, NUMBER OF NODULE WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot
0	6.33	Mg	9.00	S	6.33	MgS	17.33
K	19.67	KMg	18.33	KS	9.67	KMgS	17.33
Ca	23.33	CaMg	14.67	CaS	8.67	CaMgS	15.67
KCa	15.33	KCaMg	22.67	KCaS	14.00	KCaMgS	20.00
P	12.67	PMg	20.00	PS	15.67	PMgS	7.00
PK	48.00	PKMg	12.33	PKS	26.00	PKMgS	27.67
PCa	38.00	PCaMg	22.00	PCaS	21.67	PCaMgS	25.67
PKCa	15.00	PKCaMg	19.00	PKCaS	25.33	PKCaMgS	15.00

Element Main Effect

	P	S	Ca	Mg	K
without	14.90	19.77	17.08	19.11	16.50
with	21.94	17.06	19.75	17.73	20.33
Δ effect	7.04**	- 2.71^{ns}	2.67^{ns}	- 1.38^{ns}	3.83#

ns = not significant, #, ** significant at P $\leq$ 0.1 and 0.01 respectively for Ho: Δ effect = 0.

The interaction P x K x Ca x Mg x S# was significant for P $\leq$ 0.1; P x Mg* and P x K x Ca* were significant for P $\leq$ 0.05; and K x Ca** and K x Ca x Mg x S** were significant for P $\leq$ 0.01.

TABLE XII

WB-12-11 SIEMPRE WINGED BEAN, NUMBER OF NODULE WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot	Trt	$\bar{x}$/pot
0	4.00	Mg	8.00	S	7.00	MgS	5.67
K	6.00	KMg	4.66	KS	6.33	KMgS	13.33
Ca	3.33	CaMg	6.33	CaS	5.33	CaMgS	2.67
KCa	8.33	KCaMg	6.00	KCaS	12.00	KCaMgS	7.67
P	8.33	PMg	8.00	PS	6.33	PMgS	6.67
PK	9.33	PKMg	11.33	PKS	11.00	PKMgS	9.00
PCa	7.33	PCaMg	7.67	PCaS	6.33	PCaMgS	9.00
PKCa	11.67	PKCaMg	16.00	PKCaS	13.67	PKCaMgS	11.33

Element Main Effect

	P	S	Ca	Mg	K
without	7.06	7.76	7.85	7.94	6.75
with	9.43	8.71	8.69	8.55	9.80
Δ effect	2.37^{ns}	0.95^{ns}	0.79^{ns}	0.61^{ns}	3.05^{***}

ns = not significant, ***, significant at $P \leq 0.001$ for Ho: Δ effect = 0.

The interactions K x Ca x Mg# was significant at $P \leq 0.1$; P x S*, P x K x Mg x S*, K x Ca x Mg x S*were significant at $P \leq 0.05$.

TABLE XIII

WB-21-8 TINGE WINGED BEAN, NODULE NITROGENASE (C_2H_2 REDUCTION) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}
0	4.7	Mg	21.7	S	11.7	MgS	10.7
K	33.7	KMg	8.0	KS	5.3	KMgS	97.3
Ca	18.7	CaMg	16.0	CaS	6.3	CaMgS	7.0
KCa	10.3	KCaMg	13.0	KCaS	118.6	KCaMgS	8.3
P	30.0	PMg	31.0	PS	90.3	PMgS	3.3
PK	79.0	PKMg	33.3	PKS	63.0	PKMgS	132.0
PCa	119.7	PCaMg	72.3	PCaS	9.3	PCaMgS	61.0
PKCa	48.3	PKCaMg	55.0	PKCaS	37.0	PKCaMgS	124.3

Element Main Effect

	P	S	Ca	Mg	K
with	24.5	37.2	40.9	42.9	32.1
without	61.8	49.1	45.4	43.4	54.2
Δ effect	37.3***	11.9^{ns}	4.5^{ns}	0.5^{ns}	22.1*

ns = not significant, *, ***, significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x Ca x Mg*, K x S*, P x Ca x Mg x S* were significant at $P \leq 0.05$ and K x Ca x Mg x S** at $P \leq 0.01$.

TABLE XIV

WB-12-11 SIEMPRE WINGED BEAN, NODULE NITROGENASE (C_2H_2 REDUCTION) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}	Trt	μ moles C_2H_4 g^{-1} fresh nod. hr^{-1}
0	76.67	Mg	110.67	S	78.00	MgS	83.00
K	74.00	KMg	65.00	KS	59.00	KMgS	98.67
Ca	74.33	CaMg	113.33	CaS	126.00	CaMgS	62.00
KCa	56.67	KCaMg	63.00	KCaS	66.67	KCaMgS	63.67
P	79.33	PMg	316.33	PS	102.33	PMgS	57.33
PK	116.00	PKMg	53.33	PKS	49.33	PKMgS	69.33
PCa	92.33	PCaMg	91.67	PCaS	66.00	PCaMgS	67.00
PKCa	44.67	PKCaMg	55.50	PKCaS	60.33	PKCaMgS	65.67

Element Main Effect

	P	S	Ca	Mg	K
without	79.53	93.89	93.42	76.40	99.77
with	87.32	73.40	73.43	90.45	66.37
Δ effect	7.79^{ns}	-20.49^{ns}	-19.99^{ns}	14.05^{ns}	-33.4^{*}

ns = not significant, * significant at $P \leq 0.05$ for Ho: Δ effect = 0.

The interaction K x Mg x S* was significant for $P \leq 0.05$.

TABLE XV

WB-12-11 SIEMPRE WINGED BEAN, NODULE NITROGENASE (C_2H_2 REDUCTION) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	μ moles C_2H_4 pot^{-1} hr^{-1}	Trt	μ moles C_2H_4 pot^{-1} hr^{-1}	Trt	μ moles C_2H_4 pot^{-1} hr^{-1}	Trt	μ moles C_2H_4 pot^{-1} hr^{-1}
0	34.33	Mg	64.00	S	63.33	MgS	45.67
K	21.67	KMg	30.33	KS	28.00	KMgS	219.00
Ca	28.67	CaMg	50.33	CaS	37.33	CaMgS	45.33
KCa	40.33	KCaMg	45.33	KCaS	132.67	KCaMgS	41.00
P	108.67	PMg	119.33	PS	188.33	PMgS	29.33
PK	208.33	PKMg	64.00	PKS	97.33	PKMgS	137.33
PCa	70.00	PCaMg	119.33	PCaS	30.67	PCaMgS	192.00
PKCa	73.66	PKCaMg	66.33	PKCaS	115.33	PKCaMgS	109.00

Element Main Effect

	P	S	Ca	Mg	K
without	57.96	71.54	91.19	79.92	73.54
with	109.94	91.35	71.70	82.98	89.35
Δ effect	46.98***	19.81#	- 19.49**	3.06^{ns}	15.81^{ns}

ns = not significant, #, *** significant at P $\leq$ 0.1 and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x K#, P x Ca x Mg x S# were significant at P $\leq$ 0.1; K x S*, K x Mg x S* were significant at P $\leq$ 0.05; P x Ca x Mg***, K x Ca x Mg x S*** were significant at P $\leq$ 0.001

relation to the amount of nodule tissue. On the other hand, nitrogenase activity measurements as production of C_2H_4 pot^{-1} hr^{-1} represents an estimation of the total amount of N available for incorporation into plant amino acids. A good evidence of this is the data obtained for P for the WB-12-11 Siempre variety. In this cultivar P did not influence the measurements of nitrogenase activity when C_2H_2 reduction was determined as $C_2H_4 g^{-1}$ fresh nodule hr^{-1} indicating that P did not influence the Rhizobium efficiency to fix N. Nonetheless, a large increase in C_2H_2 reduction took place within the nodule root system of plants fertilized with P, since acetylene reduction as production of C_2H_4 pot^{-1} hr^{-1} sharply increased ($P \leq 0.001$) in this case. These trends can be explained by the fact that, whereas P did not increase the Rhizobium efficiency, it did increase nodule fresh weight (Table X) and number of nodules (Table XII) of the winged bean plants, so that the increased amount of N fixed within the nodules of P fertilized plants can be accounted for. Apparently, these data are very similar to those obtained for Cratylia floribunda, Benth, as reported by Purcino (53).

Nodules of WB-12-11 Siempre plants fertilized with S, also increased the amount of N fixed/pot ($P \leq 0.1$), while fertilization with Ca depressed availability of N ($P \leq 0.05$).

Alpha Ketoglutarate Levels

Results obtained for measurement of Alpha Ketoglutarate (α KG) levels in nodule cytosol of both WB-21-8 Tinge and WB-12-11 Siempre varieties are reported in Tables XVI and XVII. Levels of this tricarboxylic acid intermediate, which is an essential component for NH_3 assimilation into plant amino acids, were depressed in nodule cytosol of WB-21-8 Tinge

TABLE XVI

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL ALPHA KETOGLUTARATE (αKG) LEVELS WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	μ mole/g nod.	Trt	μ mole/g nod.	Trt	μ mole/g nod.	Trt	μ mole/g nod.
0	0.65	Mg	0.54	S	0.62	MgS	0.62
K	0.56	KMg	0.62	KS	0.72	KMgS	0.51
Ca	0.29	CaMg	0.71	CaS	0.67	CaMgS	0.23
KCa	0.35	KCaMg	0.27	KCaS	0.46	KCaMgS	0.24
P	0.48	PMg	0.51	PS	0.61	PMgS	0.36
PK	0.44	PKMg	0.20	PKS	0.54	PKMgS	0.54
PCa	0.46	PCaMg	0.43	PCaS	0.56	PCAMgS	0.21
PKCa	0.51	PKCaMg	0.38	PKCaS	0.42	PKCaMgS	0.39

Element Main Effect

	P	S	Ca	Mg	K
without	0.50	0.46	0.53	0.52	0.50
with	0.44	0.48	0.41	0.42	0.45
Δ effect	- 0.06#	0.02^{ns}	- 0.12**	- 0.10**	$- 0.05^{ns}$

ns = not significant, #, **, significant at $P \leq 0.1$ and 0.01 respectively for Ho: Δ effect = 0.

The interactions Ca x Mg x S#, K x Ca x Mg x S# were significant at $P \leq 0.1$; P x Ca*, K x mg*, P x K x Ca x Mg x S* were significant at $P \leq 0.05$; and Mg x S** was significant at $P \leq 0.01$.

TABLE XVII

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL ALPHA KETOGLUTARATE (αKG) LEVELS WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	μ mole/g nod.	Trt	μ mole/g nod.	Trt	μ mole/g nod.	Trt	μ mole/g nod.
0	0.36	Mg	0.30	S	0.28	MgS	0.23
K	0.26	KMg	0.19	KS	0.21	KMgS	0.23
Ca	0.20	CaMg	0.29	CaS	0.26	CaMgS	0.24
KCa	0.23	KCaMg	0.23	KCaS	0.18	KCaMgS	0.21
P	0.11	PMg	0.10	PS	0.15	PMgS	0.22
PK	0.14	PKMg	0.35	PKS	0.25	PKMgS	0.22
PCa	0.11	PCaMg	0.10	PCaS	0.27	PCAMgS	0.27
PKCa	0.30	PKCaMg	0.41	PKCaS	0.24	PKCaMgS	0.22

Element Main Effect

	P	S	Ca	Mg	K
without	0.24	0.23	0.23	0.22	0.22
with	0.22	0.23	0.23	0.24	0.24
Δ effect	-0.02^{ns}	0.00^{ns}	0.00^{ns}	0.02^{ns}	0.02^{ns}

ns = not significant for Ho: Δ effect = 0.

The interactions P x Ca#, K x Ca x S# were significant at $P \leq 0.1$; K x S* P x K x S*, P x K x Mg x S* were significant at $P \leq 0.05$ and P x K*** was significant at $P \leq 0.001$.

plants fertilized with P, Ca, and Mg ($P \leq 0.1$, 0.05 and 0.05 respectively, with no effect being observed for S and K fertilizations. No fertility effect was observed on levels of this keto acid on nodule cytosol of the WB-12-11 Siempre variety.

The relevance of soil fertility effects on the levels of α KG as well as its relationship with the other nodule enzymes, which are involved in the pathways of N incorporation into amino acids, will be discussed later in this chapter.

Glutamate Dehydrogenase Activity

Nodule cytosol activity levels of glutamate dehydrogenase (GDH) are shown in Tables XVIII and XIX for varieties WB-21-8 Tinge and WB-12-11 Siempre, respectively. GDH activity increased when the former was fertilized with P ($P \leq 0.001$) and in the latter when fertilized with Mg ($P \leq 0.05$). Changes in GDH activity caused by fertilization with the other nutrients were not statistically significant for these two varieties.

Glutamine Synthetase Activity

Phosphorus was a very important nutrient for increasing ($P \leq 0.001$) glutamine synthetase (GS) activity in the nodule cytosol of both varieties used in these experiments (Tables XX and XXI). However, a depressive effect ($P \leq 0.1$) was also observed when these plants were fertilized with Mg, an effect also detected for Ca fertilization ($P \leq 0.05$) for the WB-12-11 Siempre plants. Whereas K increased GS activity in the WB-12-11 Siempre variety, no such effect was observed for WB-21-8 Tinge variety, and S had an innocuous effect in both experiments.

TABLE XVIII

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUTAMATE DEHYDROGENASE (GDH) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	14.0	Mg	13.0	S	9.5	MgS	23.0
K	22.5	KMg	23.5	KS	26.5	KMgS	22.5
Ca	24.0	CaMg	22.0	CaS	30.5	CaMgS	19.0
KCa	29.0	KCaMg	18.0	KCaS	23.5	KCaMgS	17.5
P	25.0	PMg	43.0	PS	36.5	PMgS	30.0
PK	18.5	PKMg	17.0	PKS	28.5	PKMgS	37.5
PCa	33.5	PCaMg	23.5	PCaS	29.5	PCaMgS	30.5
PKCa	26.0	PKCaMg	23.0	PKCaS	20.0	PKCaMgS	44.5

Element Main Effect

	P	S	Ca	Mg	K
without	21.13	23.47	24.41	24.81	25.41
with	29.16	26.81	25.88	25.47	24.88
Δ effect	8.03***	3.34^{ns}	1.47^{ns}	0.66^{ns}	-0.53^{ns}

ns = not significant, *** significant for $P \leq 0.001$ for Ho: Δ effect = 0.

The interactions P x Mg#, P x K x S#, P x K x Mg x S# and P x K x Ca x Mg x S# were significant for $P \leq 0.1$; P x K*, P x Ca x Mg x S* were significant for $P \leq 0.05$; and P x K** was significant for $P \leq 0.01$.

TABLE XIX

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUTAMATE DEHYDROGENASE (GDH) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	1.60	Mg	4.30	S	3.20	MgS	3.05
K	3.15	KMg	3.00	KS	3.20	KMgS	1.30
Ca	2.75	CaMg	3.05	CaS	3.65	CaMgS	1.85
KCa	3.35	KCaMg	2.35	KCaS	1.10	KCaMgS	2.95
P	1.20	PMg	3.35	PS	1.25	PMgS	1.90
PK	1.05	PKMg	2.70	PKS	2.60	PKMgS	2.25
PCa	2.70	PCaMg	1.60	PCaS	3.40	PCaMgS	3.20
PKCa	0.85	PKCaMg	6.95	PKCaS	2.70	PKCaMgS	3.45

Element Main Effect

	P	S	Ca	Mg	K
without	2.74	2.76	2.44	2.33	2.60
with	2.55	2.55	2.85	2.95	2.68
Δ effect	-0.19^{ns}	-0.22^{ns}	0.41^{ns}	0.62**	0.08^{ns}

ns = not significant, ** significant at $P \leq 0.05$ for Ho: Δ effect = 0.

The interactions P x K#, P x K x Ca x S# were significant at $P \leq 0.1$; P x K x Mg*, P x K x Ca x Mg x S* were significant at $P \leq 0.05$; P x Ca**, P x Mg**, Mg x S** were significant at $P \leq 0.01$; and P x K x Mg x S*** was significant at $P \leq 0.001$.

TABLE XX

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUTAMINE SYNTHETASE (GS) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	1.80	Mg	1.15	S	1.55	MgS	1.40
K	1.70	KMg	2.40	KS	2.15	KMgS	3.80
Ca	1.85	CaMg	0.85	CaS	1.25	CaMgS	1.95
KCa	1.05	KCaMg	1.00	KCaS	4.10	KCaMgS	1.10
P	3.95	PMg	5.05	PS	4.80	PMgS	1.40
PK	7.20	PKMg	1.70	PKS	3.90	PKMgS	4.40
PCa	6.75	PCaMg	4.40	PCaS	1.35	PCaMgS	4.30
PKCa	4.80	PKCaMg	3.55	PKCaS	2.40	PKCaMgS	2.95

Element Main Effect

	P	S	Ca	Mg	K
without	1.82	3.08	3.02	3.16	2.73
with	3.93	2.68	2.73	2.59	3.01
Δ effect	2.11***	- 0.40^{ns}	- 0.29^{ns}	- 0.57#	0.28^{ns}

ns = not significant, #, ***, significant at P $\leq$ 0.1 and 0.001 respectively for Ho: Δ effect = 0.

The interactions Mg x S#, P x K x Ca x Mg x S# were significant at P $\leq$ 0.1; K x S*, P x Ca x Mg*, P x K x Mg x S* were significant at P $\leq$ 0.05; P x S** was significant at P $\leq$ 0.01; and K x Ca x Mg x S*** was significant at P $\leq$ 0.001.

TABLE XXI

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUTAMINE SYNTHETASE (GS) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	3.10	Mg	3.50	S	3.05	MgS	2.45
K	2.25	KMg	3.30	KS	2.90	KMgS	7.00
Ca	3.50	CaMg	1.50	CaS	3.30	CaMgS	2.70
KCa	2.75	KCaMg	7.65	KCaS	5.00	KCaMgS	3.90
P	9.00	PMg	7.80	PS	8.00	PMgS	3.60
PK	8.40	PKMg	5.60	PKS	7.60	PKMgS	4.70
PCa	5.20	PCaMg	4.20	PCaS	5.20	PCaMgS	3.60
PKCa	6.20	PKCaMg	5.30	PKCaS	6.40	PKCaMgS	4.50

Element Main Effect

	P	S	Ca	Mg	K
without	3.62	4.95	5.14	5.11	4.33
with	5.98	4.60	4.41	4.46	5.22
Δ effect	2.36***	- 0.35^{ns}	- 0.73*	- 0.65#	0.89**

ns = not significant, **, ***, significant at P $\leq$ 0.01 and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x S#, P x Ca x S# were significant at P $\leq$ 0.1; K x Mg*, K x Ca x S* were significant at P $\leq$ 0.05; P x Ca**, K x Ca x Mg x S** were significant at P $\leq$ 0.01; P x mg*** was significant at P $\leq$ 0.001

Glutamate Synthase Activity

Glutamate synthase activities (GOGAT) obtained in these experiments are summarized in Tables XXII and XXIII. Similar to GS activities, GOGAT activity was higher ($P \leq 0.001$) in nodule cytosol of plants fertilized with P. No effect was observed for fertilization with either Mg or K, but Ca and S depressed GOGAT activities in nodules of WB-21-8 Tinge and WB-12-11 Siempre varieties, respectively.

Glutamate-Oxaloacetate Transaminase Activity

Glutamate-oxaloacetate transaminase (GOT) activities for both varieties are summarized in Tables XXIV and XXV. Apparently, GOT activity in the nodule cytosol of these plants was greatly enhanced ($P \leq 0.001$) when the soil was fertilized with P. A beneficial effect on the activity of this enzyme was also observed when WB-21-8 Tinge variety was fertilized with Ca ($P \leq 0.05$) and K ($P \leq 0.001$), no effect being observed for fertilizing this variety with either S or Mg. However, an opposite effect was observed with WB-12-11 Siempre variety. A depressive effect on GOT activity was observed when these plants were fertilized with the bases Ca ($P \leq 0.01$) and Mg ($P \leq 0.01$) and the anion S ($P \leq 0.001$).

Glutamate-Pyruvate Transminase Activity

In these studies, nodules cytosol activities of the enzyme glutamate-pyruvate transaminase (GPT) (Tables XXVI and XXVII) were not as high as GOT activities. Thus, these observations suggest that larger amounts of aspartic acid are formed within the nodule cytosol of the

TABLE XXII

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUTAMATE SYNTHASE (GOGAT) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	1.05	Mg	1.25	S	1.65	MgS	1.35
K	0.85	KMg	1.45	KS	1.65	KMgS	0.75
Ca	1.65	CaMg	1.45	CaS	1.25	CaMgS	1.40
KCa	0.95	KCaMg	1.90	KCaS	0.90	KCaMgS	1.05
P	1.70	PMg	5.20	PS	3.95	PMgS	1.40
PK	2.85	PKMg	2.05	PKS	2.45	PKMgS	2.20
PCa	4.00	PCaMg	2.50	PCaS	1.90	PCaMgS	1.30
PKCa	2.20	PKCaMg	2.45	PKCaS	1.30	PKCaMgS	4.45

Element Main Effect

	P	S	Ca	Mg	K
without	1.28	2.09	1.99	1.89	2.06
with	2.62	1.81	1.92	2.01	1.84
Δ effect	1.34***	- 0.28#	- 0.07^{ns}	0.12^{ns}	0.22^{ns}

ns = not significant, #, ***, significant at P $\leq$ 0.1 and 0.001 respectively for Ho: Δ effect = 0.

The interactions K x Mg#, K x S#, Mg x S#, P x K x Mg x S# were significant at P $\leq$ 0.1; K x Mg x S*, K x Ca x Mg x S*, P x K x Ca x Mg* were significant at P $\leq$ 0.05; P x K x S**, P x Ca x Mg x S** were significant at P $\leq$ 0.01; and K x Ca x Mg***, Ca x Mg x S***, P x K x Mg x S*** were significant at P $\leq$ 0.001.

TABLE XXIII

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUTAMATE SYNTHASE (GOGAT) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	1.30	Mg	0.70	S	1.40	MgS	1.00
K	1.55	KMg	1.55	KS	1.50	KMgS	1.65
Ca	1.45	CaMg	1.75	CaS	1.10	CaMgS	1.20
KCa	0.85	KCaMg	1.65	KCaS	1.25	KCaMgS	1.20
P	1.50	PMg	1.40	PS	2.20	PMgS	1.75
PK	1.90	PKMg	1.10	PKS	1.70	PKMgS	3.15
PCa	2.20	PCaMg	1.55	PCaS	1.80	PCaMgS	1.50
PKCa	1.30	PKCaMg	2.05	PKCaS	1.40	PKCaMgS	1.45

Element Main Effect

	P	S	Ca	Mg	K
without	1.32	1.49	1.58	1.52	1.48
with	1.75	1.57	1.47	1.54	1.58
Δ effect	0.43***	0.08^{ns}	- 0.11#	0.02^{ns}	0.10^{ns}

ns = not significant, #, ***, significant at $P \leq 0.1$ and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x K x Mg#, P x K x Ca x S# were significant at $P \leq 0.1$; P x Ca x Mg*, P x Ca x S*, P x Mg x S*, P x K x Mg x S* were significant at $P \leq 0.05$; P x S**, P x K x Ca x Mg x S** were significant at $P \leq 0.01$; and K x Ca***, K x Mg***, Ca x S***, Ca x Mg x S***, K x Ca x Mg x S*** were significant at $P \leq 0.001$.

TABLE XXIV

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUTAMATE-OXALOACETATE TRANSAMINASE (GDT) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	22.5	Mg	23.0	S	24.0	MgS	25.0
K	29.0	KMg	30.5	KS	29.5	KMgS	80.0
Ca	27.0	CaMg	28.5	CaS	38.0	CaMgS	37.5
KCa	46.5	KCaMg	50.0	KCaS	68.0	KCaMgS	39.0
P	36.5	PMg	85.0	PS	104.0	PMgS	35.5
PK	82.0	PKMg	68.0	PKS	80.5	PKMgS	78.5
PCa	140.0	PCaMg	71.5	PCaS	41.5	PCaMgS	66.0
PKCa	50.0	PKCaMg	63.5	PKCaS	96.5	PKCaMgS	75.0

Element Main Effect

	P	S	Ca	Mg	K
without	37.38	53.34	52.09	57.22	50.34
with	73.38	57.41	58.66	53.53	60.41
Δ effect	36.00***	4.07^{ns}	6.57*	- 3.69^{ns}	10.07***

ns = not significant, *, *** significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interaction P x K x Ca# was significant at $P \leq 0.1$; Ca x S*, K x Ca*, Ca x Mg*, P x S*, P x K x S*, K x Ca x S*, Ca x Mg x S* and P x K x Ca x Mg* were significant at $P \leq 0.05$; P x K**, P x Mg** were significant at $P \leq 0.01$; and K x S*** was significant at $P \leq 0.001$.

TABLE XXV

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUTAMATE-OXALOACETATE TRANSAMINASE (GOT) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	4.40	Mg	7.40	S	8.20	MgS	12.00
K	3.50	KMg	9.00	KS	6.00	KMgS	16.50
Ca	13.60	CaMg	5.00	CaS	7.90	CaMgS	12.80
KCa	11.10	KCaMg	7.25	KCaS	8.50	KCaMgS	5.50
P	36.00	PMg	42.50	PS	23.00	PMgS	6.65
PK	49.00	PKMg	28.50	PKS	14.50	PKMgS	6.05
PCa	40.00	PCaMg	10.50	PCaS	9.20	PCaMgS	4.00
PKCa	16.00	PKCaMg	20.50	PKCaS	17.70	PKCaMgS	16.50

Element Main Effect

	P	S	Ca	Mg	K
without	8.67	19.05	17.11	17.06	15.42
with	21.71	10.99	13.00	13.16	14.76
Δ effect	13.04***	- 8.06***	- 4.11**	- 3.90**	- 0.66^{ns}

ns = not significant, **, ***, significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interaction Ca x Mg x S# was significant at $P \leq 0.1$; Ca x S*, K x Ca x Mg* were significant at $P \leq 0.05$; P x Ca**, P x K x Ca x S** were significant at $P \leq 0.01$; and P x Mg***, P x S***, P x Ca x S***, P x K x Ca x Mg***, K x Ca x Mg x S*** were significant at $P \leq 0.001$.

TABLE XXVI

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUTAMATE-PYRUVATE TRANSAMINASE (GPT) ACTIVITY WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	2.20	Mg	2.40	S	2.90	MgS	1.55
K	2.05	KMg	1.85	KS	2.00	KMgS	2.00
Ca	1.65	CaMg	1.60	CaS	1.75	CaMgS	1.95
KCa	2.25	KCaMg	1.80	KCaS	1.80	KCaMgS	2.15
P	3.10	PMg	2.85	PS	2.65	PMgS	2.40
PK	4.85	PKMg	2.05	PKS	2.90	PKMgS	2.90
PCa	3.30	PCaMg	2.90	PCaS	1.40	PCaMgS	2.25
PKCa	2.90	PKCaMg	2.90	PKCaS	1.35	PKCaMgS	2.70

Element Main Effect

	P	S	Ca	Mg	K
without	1.99	2.54	2.54	2.44	2.30
with	2.71	2.16	2.17	2.27	2.40
Δ effect	0.72***	- 0.38*	- 0.37*	- 0.17^{ns}	0.10^{ns}

ns = not significant, *, *** significant at $P \leq 0.05$ and 0.001 respectively for Ho: Δ effect = 0.

The interaction P x K x Ca# was significant at $P \leq 0.1$; P x S*, Mg x S*, P x Mg x S*, K x Mg x S*, P x K x Ca x Mg*, K x Ca x Mg x S* were significant at $P \leq 0.05$; and Ca x Mg** was significant at $P \leq 0.01$.

TABLE XXVII

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUTAMATE-PYRUVATE TRANSAMINASE (GPT) ACTIVITY WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.	Trt	U/g nod.
0	0.50	Mg	1.65	S	1.45	MgS	1.60
K	0.40	KMg	1.45	KS	1.70	KMgS	1.30
Ca	1.45	CaMg	1.70	CaS	1.65	CaMgS	1.50
KCa	1.50	KCaMg	1.85	KCaS	0.80	KCaMgS	1.60
P	1.30	PMg	1.40	PS	1.50	PMgS	1.20
PK	1.20	PKMg	1.50	PKS	1.30	PKMgS	0.80
PCa	2.00	PCaMg	0.80	PCaS	1.80	PCaMgS	0.80
PKCa	0.80	PKCaMg	1.50	PKCaS	1.20	PKCaMgS	0.80

Element Main Effect

	P	S	Ca	Mg	K
without	1.38	1.31	1.27	1.27	1.38
with	1.23	1.30	1.35	1.39	1.23
Δ effect	- 0.15*	- 0.01^{ns}	0.08^{ns}	0.07^{ns}	- 0.15*

ns = not significant, * significant at $P \leq 0.05$ for Ho: Δ effect = 0.

The interactions Ca x Mg#, P x Ca x Mg x S# were significant at $P \leq 0.1$; P x S*, P x K x Mg*, P x K x Ca x S*, P x K x Mg x S*, P x K x Ca x Mg x S* were significant at $P \leq 0.05$; P x Ca**, K x Mg**, Ca x S**, P x Ca x S**, Ca x Mg x S** were significant at $P \leq 0.01$; P x Mg***, K x Ca x Mg*** were significant at $P \leq 0.001$.

winged bean cultivars used in these experiments, as compared to alanine.

A very interesting contrast was observed for P fertilization in these studies. GPT activity was greatly enhanced ($P \leq 0.001$) when WB-21-8 Tinge plants were fertilized with this nutrient, but a significant inhibitory ($P \leq 0.005$) effect was observed for the WB-12-11 Siempre variety. This inhibitory effect was also observed when the former variety was fertilized with S ($P \leq 0.05$) and Ca ($P \leq 0.05$) and the latter one when K ($P \leq 0.05$) was added to the soil. Apparently, Mg fertilization had no effect on GPT activity in these experiments.

Soluble Protein Levels

Soluble protein as measured by the folin phenol method (39) for both varieties is shown in Tablex XXVIII and XXIX. More soluble protein was detected in the nodule cytosol of the WB-21-8 Tinge variety when either S ($P \leq 0.05$) or Mg ($P \leq 0.01$) were added to the soil. A different response was observed for the WB-12-11 Siempre variety since its nodule cytosol had higher ($P \leq 0.001$) soluble protein content when fertilized with P. Nonetheless, a negative ($P \leq 0.05$) effect was observed for Ca fertilization in this variety.

In these experiments soluble protein levels in the nodule cytosol were not affected by K fertilization.

Pyridoxyl Phosphates Levels

In these experiments, the pyridoxal-pyridoxamine phosphates complex was assayed by a fluorometric method modified from the procedure described by Schreider (59). In this report, they are collectively referred to as pyridoxyl phosphates (PLP's) (vitamin B6).

TABLE XXVIII

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL SOLUBLE PROTEIN WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	%	Trt	%	Trt	%	Trt	%
0	0.80	Mg	1.58	S	1.46	MgS	1.08
K	0.54	KMg	1.16	KS	1.20	KMgS	2.07
Ca	1.20	CaMg	1.26	CaS	1.27	CaMgS	1.33
KCa	1.55	KCaMg	1.50	KCaS	1.53	KCaMgS	1.29
P	1.14	PMg	1.69	PS	1.65	PMgS	1.30
PK	1.22	PKMg	0.99	PKS	1.25	PKMgS	1.61
PCa	1.30	PCaMg	0.97	PCaS	0.98	PCaMgS	1.38
PKCa	0.92	PKCaMg	1.74	PKCaS	1.15	PKCaMgS	1.13

Element Main Effect

	P	S	Ca	Mg	K
without	1.30	1.22	1.29	1.20	1.27
with	1.27	1.37	1.28	1.38	1.30
Δ effect	-0.03^{ns}	0.13*	-0.01^{ns}	0.18**	-0.03^{ns}

ns = not significant, *, ** significant at $P \leq 0.05$ and 0.01 respectively for Ho: Δ effect = 0.

The interaction K x Ca#, was significant for $P \leq 0.1$; P x Ca* and P x K x Ca x Mg* were significant for $P \leq 0.05$, Ca x S**, P x Ca x Mg**, K x Ca x S were significant for $P \leq 0.01$; and K x Ca x Mg*** was significant for $P \leq 0.001$.

TABLE XXIX

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL SOLUBLE PROTEIN WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	%	Trt	%	Trt	%	Trt	%
0	1.30	Mg	1.50	S	1.24	MgS	1.70
K	1.40	KMg	1.46	KS	1.15	KMgS	2.88
Ca	1.49	CaMg	1.67	CaS	1.21	CaMgS	1.18
KCa	0.92	KCaMg	1.53	KCaS	1.56	KCaMgS	0.85
P	3.05	PMg	2.44	PS	2.15	PMgS	1.53
PK	2.41	PKMg	2.03	PKS	1.42	PKMgS	1.92
PCa	2.20	PCaMg	2.00	PCaS	1.60	PCaMgS	1.60
PKCa	1.92	PKCaMg	1.63	PKCaS	2.42	PKCaMgS	1.53

Element Main Effect

	P	S	Ca	Mg	K
without	1.44	1.81	1.85	1.71	1.74
with	2.00	1.62	1.58	1.71	1.68
Δ effect	0.56***	- 0.19^{ns}	- 0.27*	0.00^{ns}	0.06^{ns}

ns = not significant, *, ***, significant at P $\leq$ 0.05 and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x S#, K x S#, K x Ca x Mg# were significant at P $\leq$ 0.1; P x Mg*, Ca x Mg x S*, P x Ca x S*, K x Ca x Mg x S* were significant at P $\leq$ 0.05.

PLP's function in a large number of enzymatic reactions, but the most common type involves the transfer of the α amino group of an amino acid to the α carbon atom of an α keto acid. Enzymes catalyzing such reactions are called transaminases (such as GOT and GPT).

Transaminases are found in the mitochondria and in the cytosol of eukaryotic cells (38), and the figures reported on Tables XXX and XXXI refer to nodule cytosol levels of PLP's for the two winged bean varieties in these experiments. These data indicated that P fertilization enhanced PLP's levels on both varieties, but a depressive effect was observed for Ca ($P \leq 0.001$) with the WB-21-8 Tinge variety.

Carbohydrate Levels

Glucose, sucrose and starch were the carbohydrates determined on the nodule cytosol of the two winged bean varieties. Results for each of these saccharides are summarized on Tables XXXII to XXXVII.

The importance of these sugars on the mechanism of nitrogen fixation is not well understood. Nonetheless, Tempest and co-workers (66) have suggested that glucose plays an important role on the pathways of glutamate synthesis (discussed in more detail later in this chapter). These writers, working with several genera of gram positive and gram negative bacteria were able to determine that the GS-GOGAT pathway for glutamate synthesis was only active when glucose was not a limiting factor in the assaying medium. Apparently, glucose-limited organisms possessed an active glutamate dehydrogenase substituting for the GS-GOGAT couple action. They pointed out to the fact that the GS-GOGAT couple can function in low ammonia environments, but this is only possible with the expenditure of energy for synthesis of glutamine by glutamine synthetase.

TABLE XXX

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL PYRIDOXYL PHOSPHATES (PLP'S) WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	µg/g nod.	Trt	µg/g nod.	Trt	µg/g nod.	Trt	µg/g nod.
0	19.50	Mg	11.00	S	13.50	MgS	14.00
K	14.00	KMg	16.50	KS	17.50	KMgS	16.50
Ca	15.00	CaMg	14.00	CaS	14.50	CaMgS	13.50
KCa	14.00	KCaMg	13.00	KCaS	12.00	KCaMgS	13.50
P	17.00	PMg	22.50	PS	25.00	PMgS	12.50
PK	16.00	PKMg	13.00	PKS	14.50	PKMgS	18.00
PCa	17.00	PCaMg	14.50	PCaS	13.00	PCaMgS	13.50
PKCa	14.00	PKCaMg	15.00	PKCaS	13.00	PKCaMgS	18.50

Element Main Effect

	P	S	Ca	Mg	K
without	14.50	15.38	16.31	15.59	15.63
with	16.10	15.19	14.25	14.97	14.94
Δ effect	1.60**	- 0.18^{ns}	- 2.06***	- 0.62^{ns}	- 0.69^{ns}

ns = not significant, **, *** significant at P $\leq$ 0.01 and 0.001 respectively for Ho: Δ effect = 0.

The interactions P x K*, Ca x Mg*, K x S*, K x Mg x S* and Ca x Mg x S* were significant at P $\leq$ 0.05; P x Ca x Mg x S** at P $\leq$ 0.01; and K x Mg***, P x K x Ca***, P x K x Mg x S*** and P x K x Ca x Mg x S*** at P $\leq$ 0.001.

TABLE XXXI

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL PYRIDOXYL PHOSPHATES (PLP'S) WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	µg/g nod.	Trt	µg/g nod.	Trt	µg/g nod.	Trt	µg/g nod.
0	14.0	Mg	12.00	S	11.50	MgS	11.00
K	13.00	KMg	13.50	KS	13.00	KMgS	14.50
Ca	15.00	CaMg	10.50	CaS	14.50	CaMgS	14.00
KCa	11.00	KCaMg	17.50	KCaS	13.50	KCaMgS	10.00
P	14.50	PMg	14.50	PS	13.50	PMgS	15.00
PK	13.00	PKMg	15.00	PKS	13.00	PKMgS	13.00
PCa	13.00	PCaMg	14.50	PCaS	21.00	PCaMgS	12.50
PKCa	13.50	PKCaMg	14.00	PKCaS	15.00	PKCaMgS	11.50

Element Main Effect

	P	S	Ca	Mg	K
without	13.03	13.66	13.36	13.65	13.58
with	13.94	13.29	13.58	13.31	13.37
Δ effect	0.91#	- 0.37^{ns}	0.22^{ns}	- 0.34^{ns}	- 0.21^{ns}

ns = not significant, # significant at $P \leq 0.1$ for Ho: Δ effect = 0.

The interactions Mg x S#, K x Ca x S#, K x Mg x S# were significant at $P \leq 0.1$, and P x K x Ca x Mg x S** was significant at $P \leq 0.05$.

TABLE XXXII

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL GLUCOSE WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	1.88	Mg	1.74	S	1.27	MgS	2.73
K	2.23	KMg	2.23	KS	2.84	KMgS	1.90
Ca	2.16	CaMg	2.08	CaS	2.11	CaMgS	2.46
KCa	1.27	KCaMg	2.16	KCaS	1.75	KCaMgS	2.12
P	1.82	PMg	1.52	PS	1.95	PMgS	2.53
PK	1.65	PKMg	2.15	PKS	2.13	PKMgS	1.75
PCa	1.59	PCaMg	1.75	PCaS	2.48	PCaMgS	1.95
PKCa	2.52	PKCaMg	2.10	PKCaS	2.00	PKCaMgS	1.50

Element Main Effect

	P	S	Ca	Mg	K
without	2.06	1.93	2.02	1.98	2.00
with	1.96	2.09	2.00	2.04	2.02
Δ effect	-0.10^{ns}	$0.16^{\#}$	-0.02^{ns}	0.06^{ns}	0.02^{ns}

ns = not significant, # significant at $P \leq 0.1$ for Ho: Δ effect = 0.

The interactions K x Ca#, P x Mg#, P Ca x Mg# were significant at $P \leq 0.1$; K x S*, P x K x S* P x K x Ca* were significant at $P \leq 0.05$; K x Mg x S**, K x Ca x Mg x S**, P x K x Ca x Mg** were significant at $P \leq 0.01$.

TABLE XXXIII

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL GLUCOSE WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	3.00	Mg	2.43	S	2.58	MgS	1.89
K	2.04	KMg	1.95	KS	2.06	KMgS	3.18
Ca	2.37	CaMg	1.69	CaS	2.40	CaMgS	1.74
KCa	1.79	KCaMg	1.83	KCaS	3.53	KCaMgS	2.49
P	2.02	PMg	2.51	PS	2.08	PMgS	1.23
PK	2.33	PKMg	2.23	PKS	3.35	PKMgS	2.74
PCa	2.75	PCaMg	3.88	PCaS	1.43	PCaMgS	3.38
PKCa	2.89	PKCaMg	2.80	PKCaS	2.88	PKCaMgS	2.92

Element Main Effect

	P	S	Ca	Mg	K
without	2.31	2.40	2.35	2.50	2.36
with	2.62	2.52	2.58	2.43	2.56
Δ effect	0.31^{ns}	0.12^{ns}	0.23^{ns}	-0.07^{ns}	0.20^{ns}

ns = not significant for Ho: Δ effect = 0.

The interactions P x K x Ca#, P x K x Mg#, P x Ca x Mg# were significant at $P \leq 0.1$; P x Ca* was significant at $P \leq 0.05$; and K x S*** was significant at $P \leq 0.001$.

TABLE XXXIV

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL SUCROSE WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	0.11	Mg	0.34	S	0.11	MgS	0.22
K	0.30	KMg	0.06	KS	0.10	KMgS	0.10
Ca	0.11	CaMg	0.38	CaS	0.21	CaMgS	0.11
KCa	0.20	KCaMg	0.40	KCaS	0.10	KCaMgS	0.3
P	0.61	PMg	0.69	PS	1.40	PMgS	0.20
PK	0.44	PKMg	0.32	PKS	0.16	PKMgS	0.30
PCa	0.88	PCaMg	0.19	PCaS	0.29	PCaMgS	0.25
PKCa	0.23	PKCaMg	0.10	PKCaS	0.20	PKCaMgS	1.19

Element Main Effect

	P	S	Ca	Mg	K
without	0.18	0.33	0.34	0.34	0.38
with	0.46	0.32	0.31	0.31	0.27
Δ effect	0.28***	- 0.01^{ns}	- 0.03^{ns}	- 0.03^{ns}	- 0.11*

ns = not significant, *, ***, significant at P $\leq$ 0.05 and 0.01 respectively for Ho: Δ effect = 0.

The interactions P x S#, P x K x S#, P x Mg x S#, K x Ca x Mg x S# were significant for P $\leq$ 0.1; P x K*, P x Mg*, Ca x Mg*, P x K x Ca*, P x K x Mg x S* were significant at P $\leq$ 0.05; K x Ca**, K x Mg**, K x Ca x S**, K x Mg x S**, Ca x Mg x S**, P x K x Ca x S** were significant at P $\leq$ 0.01; and P x K x Mg***, P x Ca x Mg x S*** were significant at P $\leq$ 0.001.

TABLE XXXV

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL SUCROSE WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	3.65	Mg	1.89	S	1.88	MgS	1.71
K	2.32	KMg	1.25	KS	1.82	KMgS	0.28
Ca	2.46	CaMg	1.76	CaS	3.13	CaMgS	1.77
KCa	1.36	KCaMg	2.70	KCaS	0.80	KCaMgS	2.16
P	0.22	PMg	0.28	PS	0.17	PMgS	1.43
PK	0.23	PKMg	0.26	PKS	0.40	PKMgS	0.90
PCa	0.35	PCaMg	0.60	PCaS	2.57	PCaMgS	0.57
PKCa	0.64	PKCaMg	0.61	PKCaS	0.16	PKCaMgS	0.55

Element Main Effect

	P	S	Ca	Mg	K
without	1.93	1.29	1.13	1.39	1.49
with	0.52	1.19	1.35	1.14	0.99
Δ effect	- 1.41***	- 0.10^{ns}	0.22^{ns}	- 0.20^{ns}	- 0.50**

ns = not significant, **, ***, significant at $P \leq 0.01$ and 0.001 respectively for Ho: Δ effect = 0.

The interaction P x S* was significant at $P \leq 0.05$; P x Ca x Mg**, K x Ca x Mg**, K x Ca x Mg x S** were significant at $P \leq 0.01$.

TABLE XXXVI

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL STARCH WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	0.52	Mg	0.56	S	0.51	MgS	0.65
K	0.61	KMg	0.68	KS	0.54	KMgS	0.50
Ca	0.85	CaMg	0.68	CaS	0.46	CaMgS	0.37
KCa	0.48	KCaMg	0.52	KCaS	0.60	KCaMgS	0.69
P	0.99	PMg	0.93	PS	1.72	PMgS	0.65
PK	1.17	PKMg	0.63	PKS	0.54	PKMgS	0.70
PCa	1.38	PCaMg	0.53	PCaS	0.65	PCaMgS	0.55
PKCa	0.58	PKCaMg	0.60	PKCaS	0.85	PKCaMgS	1.49

Element Main Effect

	P	S	Ca	Mg	K
without	0.57	0.73	0.74	0.78	0.75
with	0.87	0.72	0.70	0.67	0.70
Δ effect	0.30**	- 0.01^{ns}	- 0.04^{ns}	- 0.11^{ns}	- 0 05^{ns}

ns = not significant, *** significant at P $\leq$ 0.001 for Ho: Δ effect = 0.

The interactions P x Mg#, P x K x Ca#, Ca x Mg x S#, P x K x Mg x S#, P x K x Ca x Mg x S# were significant for P $\leq$ 0.1; P x K x Mg* was significant for P $\leq$ 0.05; K x Mg** was significant for P $\leq$ 0.01; and K x Ca x S*** was significant for P $\leq$ 0.001.

TABLE XXXVII

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL STARCH WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.	Trt	mg/g nod.
0	3.70	Mg	2.15	S	2.00	MgS	1.80
K	2.76	KMg	1.55	KS	2.52	KMgS	0.56
Ca	2.79	CaMg	1.51	CaS	1.53	CaMgS	1.97
KCa	1.16	KCaMg	0.10	KCaS	0.98	KCaMgS	2.46
P	0.49	PMg	0.28	PS	0.45	PMgS	0.19
PK	0.45	PKMg	0.64	PKS	0.56	PKMgS	0.62
PCa	0.63	PCaMg	0.85	PCaS	2.47	PCaMgS	1.23
PKCa	0.92	PKCaMg	1.00	PKCaS	0.75	PKCaMgS	1.11

Element Main Effect

	P	S	Ca	Mg	K
without	1.85	1.31	1.29	1.48	1.46
with	0.73	1.28	1.30	1.12	1.13
Δ effect	- 1.12***	- 0.03^{ns}	0.01^{ns}	- 0.36***	- 0.33***

ns = not significant, *** significant at P $\leq$ 0.001 for Ho: Δ effect = 0.

The interactions K x Ca#, P x S#, P x K x Ca x Mg#, P x K x Mg x S# were significant and P $\leq$ 0.1; K x Ca x Mg*, P x Ca x S*, Ca x Mg x S*, P x K x Ca x Mg x S* were significant at P $\leq$ 0.05; P x Mg**, Ca x Mg**, Mg x S**, P x Ca x Mg**, P x K x Ca x S**, P x Ca x Mg x S**, K x Ca x Mg x S** were significant at P $\leq$ 0.01; and P x K***, P x Ca***, Ca x S***, P x K x S***, P x Mg x S*** were significant at P $\leq$ 0.001.

The same findings were later observed for glutamate synthesis in Rhizobium cultures and nodule bacteroids (9).

The importance of glucose in the GS-GOGAT pathway is that it can ultimately be oxidized to produce ATP, essential for GS activity. Thus, it is not improbable that other carbohydrates can perform this function.

In these experiments, contrasting results were obtained for fertility effects on nodule carbohydrate of the two varieties used. Sulphur increased ($P \leq 0.1$) glucose levels on the WB-21-8 Tinge plants, but no fertility effect was observed on the WB-12-11 Siempre variety. Both sucrose and starch increased in the WB-21-8 Tinge variety when the soil was fertilized with P ($P \leq 0.001$ and 0.001 respectively) but the opposite was observed in the WB-12-11 Siempre plants ($P \leq 0.001$ and 0.001 respectively). Potassium depressed sucrose levels on both varieties ($P \leq 0.05$ and 0.01), but increased starch levels on the WB-12-11 variety. Magnesium decreased ($P \leq 0.001$) starch levels on the latter variety, and Ca failed to influence carbohydrate levels on both experiments.

Fixed N Incorporation Into Plant Amino Acids

It is presently accepted that the abundant atmospheric N can be fixed as NH_3 by the nitrogenase activity of rhizobium bacteroid cells when in symbiosis with a host leguminous plant (4, 6, 26, 27). Nonetheless, no enzymatic system is found within the bacteroid cells with activity sufficiently high to match that of nitrogenase, and thus, NH_3 incorporation into amino acids is believed to be accomplished by the plant enzymatic systems (9, 33).

Glutamate dehydrogenase (GDH), prior to discovery of glutamate synthase (GOGAT) was believed to be the most important mechanism for NH_3 incorporation into glutamate in the animal cell (38) and in root nodules (21). GDH catalyses the reaction:

$$\alpha \text{ KG} + NH_3 + NAD(P)H + H \rightleftarrows \text{L-glutamate} + NAD(P)^+ + H_2O$$

The reaction rate can be affected by addition of Zn^{2+}, CA^{2+}, or MN^{2+} (48). Plant GDH acts with both NAD and NADP (48), and the balance between its aminating and deaminating activity is believed to be controlled by the NAD/NADH ratio (14, 48).

Alanine dehydrogenase (ADH) (EC 1.4.1.1) and aspartate dehydrogenase (Asp DH) are found in some plants (38), but are not considered to be important mechanisms of NH_3 utilization in plant root nodules. Dunn and Klucas (17) speculated that ADH could be important during ammonium assimilation only under certain conditions. They found that soybean root nodules had a ten fold higher ADH activity than GDH.

The reaction catalyzed by ADH is:

$$\text{pyruvate} + NH_3 + NAD\ (P)\ H + H^+ \rightleftarrows \text{L - alanine} + NAD(P)^+\ H_2O$$

and AspDH catalysis:

$$\text{oxaloacetate} + NH_3 + NAD(P)H + H^+ \rightleftarrows \text{aspartate} + NAD(P)^+ + H_2O\ .$$

For many years it was well known that the enzyme glutamine synthetase could catalyse the reaction indicated below:

$$\text{ATP} + \text{L-glutamate} + NH_3 \rightleftarrows \text{L - glutamine} + \text{ADP} + \text{Pi}$$

which can be affected by the presence of divalent cations. Glutamine synthetase exists in two states: taut and relaxed. The relaxed state is achieved by removing the divalent cations and renders

the enzyme inactive more liable to degradation (31, 48, 62).

In 1970, a group of British researchers (45, 66) reported that a novel enzyme'glutamine (amide): 2-oxoglutarate amino transferase oxido - reductase (NADP)' (later reclassified as EC 1.4.1.13, L - glutamate: $NADP^+$ oxido-reductase (transaminating), often referred to by its acronym GOGAT (48) could catalyse the following reaction:

$$\alpha \text{ KG} + \text{NAD(P)(H)} \text{ (or Fd red)} + \text{L - glutamine} \rightarrow 2 \text{ L - glutamate} + \text{NAD(P)}^+ \text{ (or } Fd_{ox})$$

Afterward, it became apparent that the coupled action of GS-GOGAT could play an important role in NH_3 assimilation in many organisms. These enzymes have been suggested to be present in the animal cell (38), several gram positive and gram negative bacteria (13, 45, 46, 49, 66), chloroplasts (37), shoot of halophytes (65), and root nodules of leguminous plants (9, 33, 48, 49, 57, 60, 61, 64). Good evidence has been presented in the literature indicating that the nodule cytosol (9) and not the bacteroid (9, 33), is the site of NH_3 utilization. Thus, nodule enzyme activity in this report refers to measurements obtained in the nodule cytosol fraction.

Glutamate dehydrogenase has a higher K_m value for NH_3 than the GS-GOGAT couple, and usually shows activity levels that can not match those of nitrogenase. Therefore, more recently, it has been accepted that the GS-GOGAT route is the most important pathway for NH_3 incorporation into plant amino acids, and that GDH is active only under certain conditions. Usually, the GDH pathway is operative under conditions of excess of ammonia or limited supply of carbohydrate. In the case of low nodule glucose levels, GS is inactivated since it requires ATP for

functioning, favoring the GDH pathway. High levels of glutamine, alanine or Mg^{2+} are also known to repress GS, thus, rendering the GS-GOGAT couple inoperative. However, when operative, both enzyme systems produce glutamate as their end product.

Table XXXVIII shows the correlation coefficients for nitrogenase, plant growth parameters and nodulation of the two winged bean varieties used in these experiments.

The correlation coefficients for the WB-21-8 Tinge variety clearly indicates that nitrogenase activity (μ moles C_2H_4 g^{-1} nod hr^{-1}) was positively related to nodule weight and to nodule number, as well as to plant growth and development. Therefore, a practical indication of this experiment is that maximization of plant growth and nitrogen fixation for the WB-21-8 Tinge variety requires P,K, and S fertilization of this dark red latosol.

Although nitrogenase activity of the WB-21-8 Tinge nodules during plant flowering was lower than that of the WB-12-11 Siempre before plant flowering, it was still higher than the average activity observed for the latter variety in a flowering stage, as previously observed (54).

The correlation coefficients obtained for the WB-12-11 Siempre indicate no relationship between nitrogenase (μ moles C_2H_4 g^{-1} nod hr^{-1}) and either nodule weight, nodule number or even shoot growth. A negative, significant correlation ($P \leq 0.05$), was observed between nitrogenase (μ moles C_2H_4 g^{-1} nod hr^{-1}) and root growth.

Perhaps a better understanding of N fixation within nodules of the WB-12-11 Siempre plants can be obtained when nitrogenase activity is expressed as a measure of total N fixed per pot culture (μ moles C_2H_4 pot^{-1} hr^{-1}). This data indicate that more N was fixed within pot

TABLE XXXVIII

CORRELATION COEFFICIENTS FOR NITROGENASE, PLANT GROWTH PARAMETERS AND NODULATION OF TWO WINGED BEAN VARIETIES

WB 12-11 Siempre

	WB 21-8 Tinge					
	Nase-μ moles pot^{-1} hr^{-1}	Nase-μ moles g^{-1} nod hr^{-1}	Nod. Wt.	Nod. No.	Shoot Wt.	Root Wt.
Nase μ moles C_2H_4 pot^{-1} hr^{-1}						
Nase μ moles C_2H_4 g^{-1} nod hr^{-1}	0.23757 *		0.58497 ***	0.21718 *	0.51208 ***	0.36090 ***
Nod. Wt.	0.74806 ***	0.00436 ns		0.39318 ***	0.65904 ***	0.42877 ***
Nod. No.	0.40625 ***	- 0.00431 ns	0.68083 ***		0.33369 ***	0.20297 *
Shoot Wt.	0.47082 ***	0.09532 ns	0.66968 ***	0.46407 ***		0.60190 ***
Root Wt.	- 0.15356 ns	- 0.20432 *	-0.13517 ns	0.05100 ns	0.09314 ns	

ns = not significant, *, ***, significant at $P \leq 0.05$ and 0.001 respectively for Ho: r = 0; 96 observations/variable.

Nase = nitrogenase

cultures with larger plant shoots and increased nodule weight and number. Plants with these characteristics were produced when the soil was fertilized with P, S, Ca, and K, with total nitrogenase activity not being correlated with root growth.

Tables XXXIX and XL show the nodule cytosol enzymic specific activity means for the 32 soil fertility treatments used in these experiments. Enzyme specific activity is a measure of enzyme units expressed per mg protein (specific activity = μ moles specific substrate conversion min^{-1} mg^{-1} protein).

A summary of the statistical analysis for these data appears in Table XLI for both varieties. Treatment effect is expressed in terms of Δ effect (as defined earlier) for P, S, Ca, Mg, and K. The null hypothesis HO: Δ effect = 0 was rejected any time $P \leq 0.1$. The null hypothesis indicates that the increase in enzymic specific activity caused by a particular nutrient cannot be considered different from zero, on the other hand, the alternative hypothesis HA: Δ effect $\neq$ 0 indicates that the nutrient effect was different from zero.

For both varieties, all enzymic specific activities, but WB-12-11 Siempre nitrogenase, were significantly increased when this dark red latosol was fertilized with P. Sulphur in these experiments was found to be required for nodule growth, but apparently in many situations depressed enzyme activity. It had a negative effect ($P \leq 0.001$ in all cases) on GS, GOGAT, GOT, and GPT activities in the WB-21-8 Tinge plants, but did not affect both nitrogenase and GDH. A less pronounced effect was noted in the WB-12-11 Siempre plants, but it strongly depressed ($P \leq 0.001$) GOT while favoring ($P \leq 0.05$) GOGAT activity.

Except for the fact that if increased ($P \leq 0.05$) GDH activity on the WB-12-11 Siempre plants, Ca effect was mostly noted on the transaminases.

TABLE XXXIX

WB-21-8 TINGE WINGED BEAN, NODULE CYTOSOL ENZYMIC SPECIFIC ACTIVITY MEANS WITH SHORT DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

Trt	Nase	GDH	GS	GOGAT	GOT	GPT
0	0.0094	1.7500	0.2250	0.1312	2.8125	0.2750
K	0.0395	4.2570	0.3179	0.1624	5.4556	0.3854
Mg	0.0138	0.8045	0.0752	0.0778	1.5313	0.1503
MgK	0.0134	2.0387	0.2108	0.1256	2.6417	0.1614
Ca	0.0260	2.0018	0.1578	0.1387	2.2399	0.1358
CaK	0.0069	1.8761	0.0680	0.0621	3.0378	0.1468
CaMg	0.0283	1.7457	0.0725	0.1155	2.3202	0.1282
CaMgK	0.0177	1.2078	0.0692	0.1250	3.3658	0.1213
S	0.0200	0.6507	0.1061	0.1130	1.6438	0.1986
SK	0.0039	2.1521	0.1886	0.1337	2.4971	0.1685
SMg	0.0191	2.1920	0.1300	0.1208	2.3175	0.1436
SMgK	0.0697	1.0976	0.1821	0.0370	3.8265	0.0952
SCa	0.0154	2.7126	0.1053	0.1053	3.3011	0.1573
SCaK	0.1530	1.5262	0.2731	0.0591	4.5874	0.1193
SCaMg	0.0089	1.4174	0.1464	0.1044	2.8065	0.1464
SCaMgK	0.0087	1.3592	0.0861	0.0827	3.0484	0.1676
P	0.0511	2.2644	0.3147	0.1416	3.1945	0.2627
PK	0.1280	1.5175	0.5924	0.2333	6.7284	0.3982
PMg	0.0339	2.5458	0.2982	0.3081	5.0433	0.1696
PMgK	0.0771	1.7163	0.1716	0.2072	6.8622	0.2067
PCa	0.2088	2.6146	0.5333	0.3149	11.1292	0.2630
PCaK	0.1144	2.8551	0.5534	0.2410	5.4356	0.3177
PCaMg	0.0916	2.5360	0.4477	0.2705	7.5320	0.3040
PCaMgK	0.0688	1.3306	0.2175	0.1442	3.7428	0.1667
PS	0.0988	2.2102	0.2907	0.2395	6.3003	0.1604
PSK	0.0823	2.2868	0.3168	0.1991	6.4999	0.2339
PSMg	0.0050	2.5259	0.1175	0.1088	2.8856	0.1974
PSMgK	0.1633	2.3583	0.2733	0.1355	5.0323	0.1822
PSCa	0.0210	3.0849	0.1374	0.1876	4.2589	0.1412
PSCaK	0.0732	1.7391	0.2086	0.1130	8.3913	0.1173
PCaMgK	0.502	2.1801	0.3019	0.0993	4.9689	0.1621
PSCaMgK	0.2517	4.0715	0.2623	0.4027	6.7562	0.2415
=						
x	0.0614	2.0820	0.2323	0.1575	4.4427	0.1945
s	0.0622	0.9705	0.1579	0.0912	2.3340	0.0816

μ moles min^{-1} mg^{-1} protein

TABLE XL

WB-12-11 SIEMPRE WINGED BEAN, NODULE CYTOSOL ENZYMIC SPECIFIC ACTIVITY MEANS WITH LONG DAY LENGTH AS AFFECTED BY SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL, JAIBA, BRAZIL

Trt	Nase	G04	GS	GOGAT	GOT	GPT
0	0.0911	0.1229	0.2403	0.1003	0.3406	0.0387
K	0.0879	0.2237	0.4611	0.1115	0.2490	0.0290
Mg	0.1391	0.3184	0.2621	0.0532	0.5322	0.1216
MgK	0.0609	0.2087	0.2287	0.1081	0.6234	0.1005
Ca	0.1056	0.1901	0.2512	0.1019	0.9914	0.1058
CaK	0.1049	0.3672	0.3008	0.0928	1.2150	0.1643
CaMg	0.1091	0.2171	0.1074	0.1262	0.3548	0.1214
CaMgK	0.0723	0.1594	0.5237	0.1109	0.4861	0.1245
S	0.1034	0.2628	0.2520	0.1165	0.6770	0.1181
SK	0.0902	0.2947	0.2642	0.1374	0.5567	0.1521
SMg	0.0941	0.1936	0.1525	0.0631	0.7656	0.0981
SMgK	0.0542	0.0559	0.2955	0.0697	0.6628	0.0559
SCa	0.2504	0.3016	0.2821	0.0912	0.6557	0.1379
SCaK	0.0826	0.0693	0.3251	0.0851	0.5209	0.0506
SCaMg	0.0717	0.1607	0.2397	0.1033	1.1060	0.1350
SCaMgK	0.1047	0.3581	0.4751	0.1430	0.6845	0.1945
P	0.0504	0.0401	0.3200	0.0510	1.2291	0.0483
PK	0.0973	0.0453	0.3570	0.0821	2.1665	0.0514
PMg	0.3341	0.1432	0.3205	0.0562	1.7963	0.0588
PMgK	0.0559	0.1299	0.3040	0.0563	1.4752	0.0773
PCa	0.0839	0.1266	0.2710	0.1011	1.8729	0.0947
PCaK	0.0334	0.0476	0.3390	0.0696	0.9279	0.0453
PCaMg	0.0930	0.0839	0.2135	0.0792	0.5577	0.0416
PCaMgK	0.0599	0.4179	0.3244	0.1268	1.2491	0.0919
PS	0.1082	0.0587	0.3802	0.1054	1.1024	0.0726
PSK	0.0563	0.1989	0.5541	0.1241	1.0468	0.0923
PSMg	0.0576	0.1267	0.2408	0.1159	0.4362	0.0798
PSMgK	0.0799	0.1170	0.2424	0.1650	0.3138	0.0415
PSCa	0.0635	0.2125	0.3250	0.1125	0.5750	0.1125
PSCaK	0.0435	0.1113	0.2696	0.0578	0.7324	0.0495
PCaMgK	0.0824	0.1988	0.2109	0.0930	0.2634	0.0492
PSCaMgK	0.0823	0.2352	0.3039	0.0954	1.0983	0.0494
= x	0.0949	0.1807	0.2911	0.0968	0.8564	0.0872
s	0.0844	0.0752	0.1194	0.0278	0.3827	0.0319

μ moles min^{-1} mg^{-1} protein.

TABLE XLI

WINGED BEAN NODULE CYTOSOL SPECIFIC ENZYMATIC ACTIVITY Δ EFFECTS FOR SOIL FERTILITY COMBINATIONS TO A DARK RED LATOSOL JAIBA, BRAZIL

		P	S	Ca	Mg	K
		Δ Effect μ moles/min/mg protein				
Nase	Tinge	0.066***	0.008^{ns}	0.019^{ns}	-0.007^{ns}	0.036*
	Siempre	-0.013^{ns}	-0.010^{ns}	0.005^{ns}	0.006^{ns}	-0.043*
GDH	Tinge	0.566***	0.332^{ns}	0.118^{ns}	-0.274^{ns}	0.010^{ns}
	Siempre	0.078***	0.007^{ns}	0.444^{+}	0.029^{ns}	0.019*
GS	Tinge	0.163***	-0.074**	-0.012^{ns}	-0.082**	0.032^{ns}
	Siempre	0.037^{ns}	0.017^{ns}	0.011^{ns}	0.027^{ns}	0.077**
GOGAT	Tinge	0.103***	-0.034**	0.005^{ns}	-0.007^{ns}	-0.007^{ns}
	Siempre	0.008^{ns}	0.016*	0.004^{ns}	0.002^{ns}	0.011^{ns}
GOT	Tinge	2.956***	-0.249^{ns}	0.73**	-0.804**	0.853**
	Siempre	0.417***	-0.300***	0.033^{ns}	-0.165#	0.039^{ns}
GPT	Tinge	0.051***	-0.060***	-0.035**	-0.046**	0.015^{ns}
	Siempre	0.044***	0.011^{ns}	0.021**	0.006^{ns}	0.003^{ns}

Δ effect = as defined in Results and Discussion.

Nase = nitrogenase

ns = not significant, #, *, **, ***, significant at $P \leq 0.1$, 0.05, 0.01 and 0.001 respectively for Ho: Δ effect = 0.

It had a positive effect ($P \leq 0.01$) on the WB-21-8 Tinge GOT activity, and a negative one ($P \leq 0.01$) on the GPT activity. A positive effect ($P \leq 0.01$) was also observed in the WB-12-11 Siempre GPT activity.

Unlike P, whenever detected to have a significant effect on enzymic specific activity, magnesium was found to have depressive effects. In the WB-21-8 Tinge variety, it depressed GS ($P \leq 0.01$), GOT ($P \leq 0.01$), and GPT ($P \leq 0.01$), whereas for the WB-12-11 Siempre variety it decreased ($P \leq 0.1$) GOT activity.

Nitrogenase and GOT activity of WB-21-8 Tinge plants benefited ($P \leq 0.05$ and 0.01 respectively) from K fertilization. However, for the WB-12-11 Siempre plants, this nutrient depressed ($P \leq 0.05$) nitrogenase, while favoring GDH ($P \leq 0.05$) and GS ($P \leq 0.05$).

Comparisons of the nutrient effects on plant growth and nodulation (nodule weight and number) and on enzymic specific activities, suggest two facts are worth noting.

The first one, indicates that the optimum fertility status of the soil that brings about improved plant growth and nodulation is not necessarily conducive to higher enzymic activity levels. In these experiments, a good indication was obtained that P fertilization was beneficial for both varieties. It consistently improved plant growth, nodule weight, number of nodules, and all enzymic specific activities, except nitrogenase and GS of the WB-12-11 Siempre plants. Opposite results were noted when the soil was fertilized with Mg. Despite the fact that this nutrient is an essential nutrient for all living cells and contributes toward increased activity levels and stability of several enzymes (GS for example), this soil, apparently has sufficient available Mg to support adequate plant growth, nodulation

and high enzymic specific activities. Indeed, additions of this nutrient to the pot cultures failed to improve plant growth and nodulation and usually depressed the enzymic activities determined in these experiments.

The second important point obtained in these experiments indicates that a given nutrient will have different degrees of effect on several of these enzymes, and consequently, can possibly affect the route of NH_3 assimilation by differentially affecting the activity of GDH, GS and GOGAT. This can be illustrated by treatments PCaMgK and PS from Table XL.

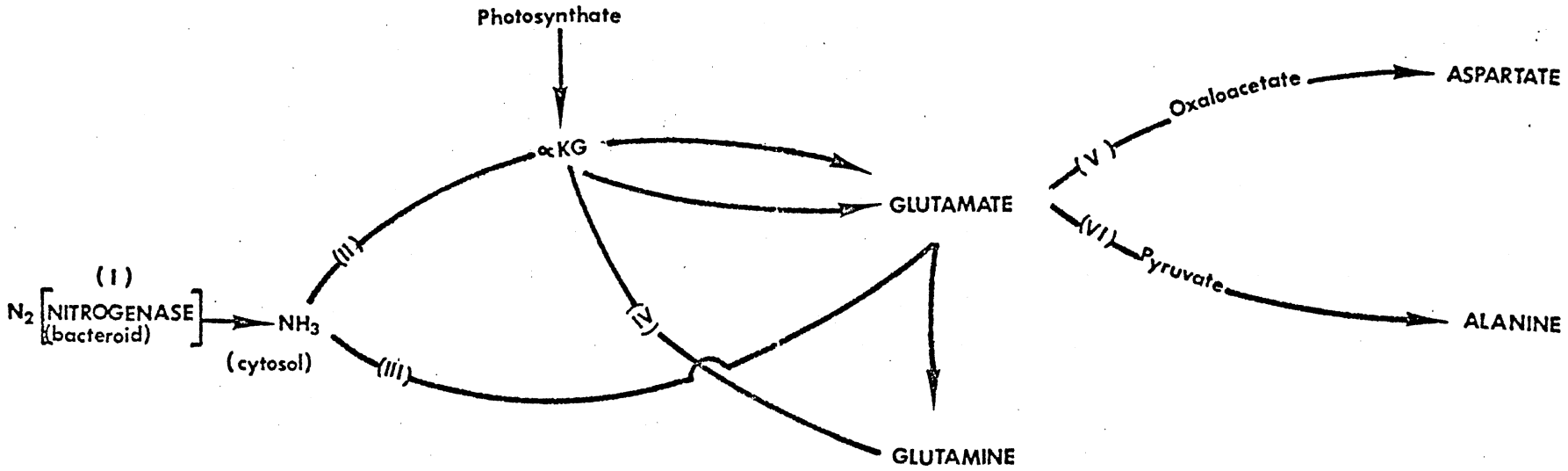
The figures obtained indicate that when the anion P is combined with the cations Ca, Mg and K, NH_3 was possibly incorporated via the GDH pathway, but the combination of two anions P and S, switched the assimilation pathway to the GS-GOGAT couple.

The enzymatic sequences for ammonia assimilation determined in these studies are shown in Figure 1.

In these enzyme sequences, N is believed to be fixed by the nodule bacteroid nitrogenase as NH_3 which is translocated into the cytosol where it can be assimilated by plant enzymes. Both GDH and GS-GOGAT pathways are shown, as well as the transaminases GOT and GPT. These are apparently the two most important transaminases in utilizing the α amino group of glutamic acid (22, 57, 60, 68) for the synthesis of other plant amino acids.

Correlation coefficients obtained for enzymic specific activities, and nodule cytosol carbohydrates, PLP's, protein and α KG for the two winged bean varieties used in these experiments are shown in Table XLII.

It is readily noticeable from this table that the relationship among the cytosol components was not identical for the two varieties.



I. Nitrogenase (Nase) (EC 1.7.99.2)
II. Glutamate dehydrogenase (GDH) - (L-glutamate:$NAD(P)^+$ oxidoreductase deaminating EC, 1.4.1.3)
III. Glutamine synthetase (GS) (L-glutamate: ammonia ligase (ADP), EC 6.3.1.2)
IV. Glutamate Synthetase (GOGAT) (L-glutamate: $NAD(P)^+$ oxidoreductase (transaminating)
V. Glutamate-oxaloacetate transaminase (GOT)(L-apartate:2-oxoglutarate aminotransfrase, EC 2.6.1.1)
VI. Glutamate-pyruvate transaminase (GPT) L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2)
αKG. alpha ketoglutarate (2-oxoglutarate)

Figure 1. A Schematic Composite of Enzymatic Pathways Proposed for N Assimilation Within Legume Nodules

TABLE XLII

CORRELATION COEFFICIENTS FOR ENZYME SPECIFIC ACTIVITY AND CARBOHYDRATE COMPONENTS OF WINGED BEAN NODULE CYTOSOL

WB 12-11 Siempre \ WB 21-8 Tinge	Nase	αKG	GDH	GS	GOGAT	GOT	GPT	PROT	PLP's	Glucose	Sucrose	Starch
Nase		0.08	0.33**	0.38**	0.53***	0.51***	0.21#	0.10	0.26*	-0.28*	0.46***	0.44***
αKG	-0.23#		0.07	0.01	0.03	-0.22#	0.05	0.01	0.33**	0.05	0.08	0.08
GDH	0.29*	0.35**		0.32**	0.56**	0.41***	0.45***	-0.50***	0.11	0.09	0.30**	0.29**
GS	0.08	0.03	0.17		0.54***	0.64***	0.71***	-0.21#	0.26*	-0.20	0.25	0.39***
GOGAT	-0.02	0.07	0.41***	0.29*		0.69***	0.51***	-0.25*	0.40***	-0.11	0.58***	0.54***
GOT	0.29**	-0.22#	0.00	0.40***	-0.11		0.45***	-0.23#	0.14	-0.18	0.37**	0.43***
GPT	0.23#	0.10	0.67***	0.36**	0.43***	0.13		-0.55***	0.07	-0.09	0.14	0.23#
PROT	-0.17	-0.23#	-0.62***	-0.26*	-0.60***	0.11	0.63***		0.24*	-0.32**	0.10	0.04
PLP's	-0.20	-0.01	-0.27*	0.01	-0.20	-0.09	-0.24*	0.30**		-0.22#	0.57***	0.52***
Glucose	0.00	-0.02	-0.17	0.02	-0.18	-0.09	-0.35**	0.14	0.07		-0.22#	-0.15
Sucrose	-0.04	0.28*	0.30**	-0.13	0.15	-0.43***	0.30**	-0.45***	0.14	-0.20		0.83***
Starch	-0.05	0.27*	0.34**	-0.31**	0.16	-0.38***	0.22#	-0.45***	-0.08	0.01	0.71***	

#, *, **, *** significant for $P \leq 0.1$, 0.05, 0.01 and 0.001 respectively for Ho: r = 0. 64 observations/variable.

Specific nitrogenase activity of the variety WB-21-8 Tinge was positively co-related with all other enzymes, PLP's, sucrose and starch, but not to α KG levels. A negative relationship was also observed for glucose and this enzyme.

For this variety, α KG levels seemed only to be negatively related to GOT activity and positively to PLP's levels.

It was interesting to observe that GDH activity was also positively correlated to activities of GS and GOGAT, as well as to GOT, GPT and the carbohydrates sucrose and starch.

According to the evidences reviewed by Miflin and Lea (47), the GDH pathway is active when there is an excess of ammonia or a limitation of glucose in the growing medium, and on the other hand, the GS-GOGAT pathway is active in the mostly common occurrence of limited supply of NH_3. Allosteric inhibition by end product or substrate concentration prevents that both pathways be active at the same time (56, 67).

Therefore, the fact that the GDH and GS-GOGAT pathways were not negatively co-related can be taken as an indication that these pathways are compartmentalized within the nodule cytosol; the GDH system functioning in response to a high NH_3 concentration pool and GS-GOGAT couple in response to a low concentration pool.

Further support for this can be drawn from the specific activity levels observed for WB-21-8 Tinge plants as shown in Table XXXIX. These data show that activity levels for GS are within ranges reported by McParland et al. (44) for soybean root nodules, and although GOGAT activities are somewhat lower than those of GS, it is usually higher than nitrogenase, and hence, does not preclude the action of the GS-GOGAT couple. However, activity levels for GDH are approximately 33.9-fold

higher than nitrogenase and higher than GS and GOGAT by 9- and 13.2-fold, respectively. Thus, it is unlikely that these highly increased GDH activity levels were brought about by the level of the ammonia pool derived from nitrogenase activity.

Lawrie and Wheeler (36) have demonstrated that in Pisum sativum a 60% reduction for nitrogenase activity and nodule ^{14}C- photosynthates occurred after flowering, whereas photosynthesis of the plant doubled. They also observed that this reduced nitrogenase activity could be alleviated by removal of flowers as they were formed, thus reestablishing a continuous supply of photosynthate to the nodules.

Apparently, the nodules do not have priority over flowering and pod filling concerning the partitioning of available photosynthate during this stage of plant development. Thus, it can be suggested that nitrogenase activity can not meet the nitrogen requirements of the plant after the flowering stage due to the lack of available nodule carbohydrates (34).

The data obtained in these experiments indicate that nitrogenase activity of the WB-21-8 Tinge plants during flowering was only 64.6% of the nitrogenase activity of WB-12-11 Siempre plants before flowering. Flowering WB-21-8 Tinge plants had also 18% less available glucose, 76% less sucrose and 45% less starch when compared with the non-flowering WB-12-11 Siempre plants. Thus, it is possible to interpret the 35.4% reduction in nitrogenase activity of the flowering plants as due to lower carbohydrate availability on the nodule cytosol of these plants.

The negative correlations obtained between glucose and nitrogenase and the enzymes of ammonia assimilation, and the positive correlation between these and sucrose and starch, are in agreement with the findings

of Lawrie and Wheeler (35) for Vicia faba. They concluded in their work that photosynthates are rapidly metabolized on arrival in the nodules. Therefore, higher nitrogenase activity in WB-21-8 Tinge nodules was associated with decreased glucose levels, and positively associated with rates of ingressing sucrose and starch. An inorganic phosphate activated invertase capable of breaking sucrose to glucose and fructose has been reported to occur in root nodules of Lupinus luteus L (30).

As discussed above, during flowering, a shortage of carbohydrates appears to inhibit nitrogenase activity shortly before a period in which the nitrogen requirement of the plant is expected to be high, since pod filling is to take place, Apparently, in the case of the WB-21-8 Tinge plants, this requirement was being met by the soil NH_3 pool, and this can at least partially explain the extremely high GDH activity levels obtained. The GDH pathway is known to be active under conditions of low carbohydrate levels and excess ammonia, and the fact that half of the pot cultures were fertilized with $NH_4H_2PO_4$ and the other half balanced out with 11.4 mg of NH_4 acetate seems to satisfy both of these requirements. A rapid increase in activity levels of NADH-GDH (aminating) has been detected in roots of rice seedlings when NH_4 was added to the growing medium (25). The study of Ca effects in these experiments prevented the use of $CaH_4\ (PO_4)_2 \cdot H_2O$ as a P source.

Thus, the observation that GS-GOGAT activities can account for the symbiotically fixed N with the simultaneous presence of a highly active glutamate dehydrogenase suggests that these pathways are compartmentalized within the root nodules of flowering WB-21-8 Tinge plants.

Some support for this hypothesis can be derived from regression equations established based on the enzyme sequences of Figure 1. Several

linear models were tested to explain variations in GOT and GPT activities levels as:

(GOT, GPT) = f(Nase, α KG, GDH, GS, GOGAT, PLP's, glucose, sucrose, starch, prot.).

Best nodules were selected based on r^2 improvement and the size of the standard variation associated with the mean value of the dependent variable (5, 32). Non-linearities were not detected in the plot of residuals (16) and if present are not likely to be strong.

The best models obtained for GOT were:

$$(GOT) = 38.96 + 0.22\ Nase - 35.67\ \alpha\ KG + 0.69\ GDH \quad (1)$$

$$r^2 = 0.40,\ s = 18.48,\ \text{and}\ P = 0.0001***$$

$$(GOT) = 29.57 + 0.11\ Nase - 32.15\ \alpha\ KG + 5.69\ GS + 8.16\ GOGAT \quad (2)$$

$$r^2 = 0.61,\ s = 15.00,\ \text{and}\ P = 0.0001***$$

$$(GOT) = 27.54 + 0.11\ Nase - 32.77\ \alpha\ KD + 5.67\ GS + 7.03\ GOGAT + 0.20\ GDH \quad (3)$$

$$r^2 = 0.62,\ s = 15.00,\ \text{and}\ P = 0.0001***$$

None of the carbohydrates, and neither protein nor PLP's were able to cause any significant improvement of these models.

Because the GOT levels obtained in this experiment are extremely high and cannot be accounted for by any of the possible pathways for ammonia assimilation known today, great care must be taken during the interpretation of these models. However, they tend to indicate that larger variations on GOT activity can be explained by GS-GOGAT pathway when nitrogenase and α KG are the other companion independent variable in the model. The fact that in model (3) the GDH slope was only significant at P = 0.39 with no improvement being obtained for r^2 and s, indicates that GDH was an overfitting variable (16), thus, not making

significant contribution towards explanation of the variations on the dependent variable.

The best models obtained for GPT were:

$$(GPT) = 1.1169 + 0.0012\ Nase + 0.0564\ \alpha\ KG + 0.0364\ GDH \quad (1)$$

$$r^2 = 0.21,\ s = 0.74,\ \text{and}\ P = 0.0024^{**}$$

$$(GPT) = 0.8230 - 0.0028\ Nase + 0.2304\ \alpha\ KG + 0.3260\ GS + 0.2281\ GOGAT \quad (2)$$

$$r^2 = 0.54,\ s = 0.57,\ \text{and}\ P = 0.0001^{***}$$

The sharp increase in r^2 obtained when the GS-GOGAT pathway was tested, along with the decrease in s, indicates this enzyme couple can explain more of the variation in GPT activity levels than the GDH pathway.

Therefore, the fact that GDH was not a variable important in the account for variations in both GOT and GPT activity levels, when nitrogenase and α KG were fitted in these equations, gives support to the hypothesis that GDH and the couple GS-GOGAT were indeed separately comparmentalized within the nodule cytosol of the WB-21-8 Tinge plants and hence connected with different ammonia sources, namely the soil and the fixed N pools.

Similar equations were derived for the WB-12-11 Siempre variety and the best models obtained are listed below. As observed for the WB-21-8 Tinge plants, the fitting of PLP's, protein, and carbohydrates for the GOT models did not contribute toward their improvement.

$$(GOT) = 10.35 + 0.03\ Nase - 12.54\ \alpha\ KG - 0.50\ GDH \quad (1)$$

$$r^2 = \quad s = 5.24\ \text{and}\ P = 0.0294^{*}$$

$$(GOT) = 8.22 + 0.02\ Nase - 1.418\ \alpha\ KG + 1.94\ GS - 3.57\ GOGAT \quad (2)$$

$$r^2 = 0.33,\ s = 4.68,\ \text{and}\ P = 0.0001^{***}$$

Comparison of these two equations suggest that most of the variation in GOT activity was explained by the GS-GOGAT pathway.

However, the models for GPT:

$$(GPT) = 0.36624 - 0.00007\ Nase - 0.040933\ \alpha\ KG + 0.34492\ (GDH) \quad (1)$$

$r^2 = 0.54$, s = 0.33, and P = 0.0001***

$$(GPT) = -0.169 + 0.002\ Nase + 0.754\ \alpha\ KG + 0.86\ GS - 0.500\ GOGAT \quad (2)$$

$r^2 = 0.31$, s = 0.41, and P = 0.0002***

$$(GPT) = 0.17837 + 0.00003\ Nase - 0.50051\ \alpha\ KG + 0.09409\ GS + 0.09005\ GOGAT + 0.28560\ GDH - 0.00851\ PLP's + 0.05181\ sucrose \quad (3)$$

$r^2 = 0.61$, s = 0.32, and P = 0.0001***

indicate that larger variations in GPT activity are best explained when both GS-GOGAT and GDH pathways are fitted simultaneously in the linear model. This was also the only occasion in which PLP's and sucrose contributed toward improvement of the regression equation.

Most of the work carried out to determine which enzymes are involved in the N assimilation mechanism in the roots were conducted using media in which only a single source of N was present (22, 25, 70). Apparently, little, if any, work has been conducted to determine the effect of soil N fertility treatments on nodule enzymatic activity levels using N levels which do not inhibit nodulation.

Before the discovery of GOGAT (66), Fottrell and Mooney (19) had already demonstrated that high amounts of GDH, GOT and GPT were induced in Rhizobium japonicum when NH_4Cl + AKG were added to the growing medium and at that time GDH was considered to be the most important enzyme in the assimilation of NH_3. However, after the discovery of GOGAT, Miflin and Lea (47) concluded that the GS-GOGAT is the mostly likely route to NH_3 assimilation because its higher activity and lower K_m for ammonia.

They pointed out that GDH is not apparently in contact with high NH_3 levels in the roots because although nitrate reductase is probably found in the cytosol, nitrite reductase is associated with root plastids, whereas GDH has been determined in the cytosol and mitochondria. Furthermore, GDH activity in the cytosol has been attributed to leaching from the mitochondria (8), and it is usually considered to be a marker for the mitochondria matrix.

On the other hand, McParland and co-workers (44) have determined that after centrifugation, at 40,000 g for 30 minutes, 90% of GS activity was determined in the nodule cytosol of soybean nodules. What the authors called cytosol after centrifugation very likely did not contain the mitochondria since this organelle can be removed from rat liver cells (38) after centrifugation at 15,000 g for 5 minutes and these authors in their procedure centrifuge the nodule material at 40,000 g for 30 minutes.

Thus, it is concluded from these experiments that during different phases of the winged bean plant development, a switch in N assimilation pathways can occur due to the presence of different ammonia pools, and priorities in the distribution of available photosynthate. It is suggested that during growth, prior to flowering, the plant N requirements were met by the rhizobial nitrogenase activity and that in this case NH_3 was assimilated by the GS-GOGAT pathway. However, when a large reduction in available nodule carbohydrates occurred during flowering, high GDH activities were induced, probably in response to the present soil ammonia pool, and independent of nitrate reductase regulations (25).

It is further suggested that these pathways are compartmentalized, and probably the GS-GOGAT enzymes are found in the cytosol and GDH in the

mitochondria. In these experiments, GOT activity accounts for the utilization of all glutamate formed by both NH_3 pathways studied in these experiments.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two greenhouse experiments were conducted with a Dark Red Latosol (Typic Eutrustox) from Brazil to determine the effects of soil fertility treatments on the growth, development, nodulation, and some selected nodule cytosol enzymes and carbohydrates related to N fixation in two varieties of Psophocarpus tetragonolobus L (DC). The varieties WB-12-11 Siempre and WB-21-8 Tinge were obtained from the Mayaguez Institute of Tropical Agriculture, Puerto Rico.

In both experiments, the fertility treatments consisted of a P, S, Ca, Mg and K 2^5 complete factorial arrangement in a randomized complete block design. Each treatment was replicated three times.

The fertility levels and nutrient sources for the WB-21-8 Tinge variety were: 50 ppm of $NH_4H_2PO_4$, 50 ppm of Na_2SO_4, 6 meq/100 g of soil of $CaCO_3$, and 2 meq/100 g of soil of both $MgSO_4.7H_2O$ and KCl. The base cation ratio, thus, was equal to one. These nutrient levels were doubled for the WB-12-11 Siempre experiment.

To balance for the presence of NH_4 in the P source, an equivalent of NH_3 as ammonium acetate was applied to all pot cultures that did not receive the P nutrient.

The pot cultures in these experiments consisted of 100 grams of the actual soil, diluted in 400 grams of acid (0.1 N HCl) washed white quartz sand. Fertility treatments were applied on a soil basis.

Seeds of both winged bean varieties were germinated in vermiculite for 5 days and then transplanted to the pot cultures. The germinated seedlings were inoculated with 3 ml of Rhizobium leguminosarum culture containing more than 10^8 viable cells per ml, obtained from a selection of Strophostyles sp. nodules.

The WB-21-8 Tinge cultivar was grown during a short day period (from 1-31-79 to 4-16-79), and the plants were flowering when harvested at 75 day-age. The WB-12-11 Siempre cultivar was grown for 52 days during a long day period (from 5-4-79 to 6-25-79), and did not flower.

The two varieties produced increased shoot growth when the soil was fertilized with P; whereas K and Ca improved the top growth of the WB-21-8 Tinge and WB-12-11 Siempre, respectively. In contrast, P effect on root growth was favorable for the WB-21-8 Tinge, but had negative effect on the WB-12-11 Siempre plants.

For both cultivars, nodule fresh weight responded to P, S, and K fertilization, but only P and K increased the number of nodules in these plants. This data indicates that although S is required for nodule growth, it apparently had no effect on nodule setting.

Nitrogenase activity was determined as reduction of ethylene to acetylene and expressed as μ moles C_2H_4 produced g^{-1} fresh nodule hr^{-1} for both varieties. P and K had a beneficial effect on this enzyme activity for the WB-21-8 Tinge cultivar, but K had a depressive effect on the WB-12-11 Siempre plants. However, when activity was expressed as reduction of ethylene per pot culture, in terms of μ moles C_2H_4 pot^{-1} culture hr^{-1}, a beneficial effect was noted for P and S, as well as a negative one for Ca.

Correlation between nitrogenase, plant growth and development and

nodulation, indicated that a practical way to increase nitrogen fixation with the WB-21-8 Tinge variety was to fertilize this Dark Red Latosol with P, K and S in order to obtain plants with larger shoot and root, as well as increase nodule fresh weight and number. The same objective can be achieved by fertilizing the WB-12-11 Siempre plants with P, S, Ca and K. Results obtained in these experiments indicate the Mg fertilization is not beneficial either for plant growth and development or for nitrogenase activity in this Brazilian Oxisol.

After nitrogenase activity was determined, the nodules were picked from the root system, crushed in a buffer solution, sonicated for cell disruption and cell free cytosol attained with removal of the bacteroids by centrifugation at 12 x 10^3g for 10 minutes. The supernatant, referred to as nodule cytosol, contained the enzyme systems involved in the pathways of ammonia assimulation by the plants.

In the cytosol fraction, the enzymatic activity of glutamate dehydrogenase (GDH) glutamine synthetase (GS), glutamate synthase (GOGAT) glutamate-oxaloacetate transaminase (GOT), and glutamate-pyruvate transaminase (GPT) was determined and expressed as enzyme unites g^{-1} fresh nodule. One enzyme unit is defined as the amount of enzyme that can convert one μ mole of specific substrate min^{-1} at 27°C and 1 cm light path.

For the WB-21-8 Tinge plants, all enzymes assayed had their activity increased when the soil was fertilized with P. However, this nutrient only benefited the GS-GOGAT couple and the transaminase GOT in the WB-12-11 Siempre cultivar, with a negative effect on GPT activity. Sulphur depressed GOGAT and GPT activities in the former variety, and GOT in the latter.

With a few exceptions, the bases Ca, Mg and K depressed enzymatic activities. Thus, these results indicate that fertility treatments for optimum plant growth and development are not necessarily higher activity of enzymes involved in NH_3 assimilation within the nodule cytosol.

Concentrations of alfa ketoglutarate (αKG), soluble protein (prot), pyridoxyl phosphates (PLP's), and the carbohydrates glucose, sucrose and starch were also determined in the nodule cytosol fraction. As for the enzymes, P seems to be the nutrient with larger effect on these nodule components.

To determine possible routes for NH_3 assimilation in the P. tetragonolobus varieties, the specific activity of each enzyme was obtained by dividing the activity expressed in terms of g^{-1} fresh nodule per mg of protein g^{-1} fresh nodule. Thus, specific enzymatic activity is expressed as μ moles of substrate utilization mg^{-1} protein min^{-1}. These data indicated that enzyme specific activity can be affected by soil fertility treatments, and apparently P is a beneficial factor.

Specific nitrogenase activity for the WB-21-8 Tinge variety during flowering was 35.4% lower than for the non-flowering WB-12-11 Siempre. Apparently lower nitrogenase activities observed during flowering is due to lower availability of photosynthate for nodule protein. In these studies, flowering plants had a reduction of 18% in glucose, 76% in sucrose and 45% in starch when compared to the non-flowering plants.

Very high specific GDH activity was observed for the WB-21-8 Tinge plants glutamate-oxaloacetate transaminase specific activity in both varieties were extremely high, and could not be accounted for by either the GS-GOGAT pathway or by the GDH activity.

It is suggested that in the flowering plants, the high GDH specific activity obtained was induced by a present soil ammonia pool. However, apparently the fixed N was being assimilated by the GS-GOGAT pathway, thus indicating that both pathways were simultaneously active.

Data were presented pointing to the fact that during carbohydrate shortage in the nodule, the WB-21-8 Tinge plants have the potential to uptake NH_3 from the soil to satisfy its requirement, which in this case cannot be met by nitrogenase activity. Probably these mechanisms are compartimentalized within the nodule cytosol, with GDH being found in the mitochondria and the GS-GOGAT couple in the cytosol.

Apparently, there is a range of available soil N that do not harm nodule setting and development and nitrogenase activity, that can be utilized for leguminous plants when symbiotically fixed N is in adequate supply.

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