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900 VETERAN AVENUE

UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024

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EFFECT OF NITROGEN FERTILIZER AND NODULATION ON LIME-INDUCED CHLOROSIS IN SOYBEANS

Arthur Wallace

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KEY WORDS: Cation:anion balance, iron chlorosis, pH, nitrate-N.

Arthur Wallace
Laboratory of Biomedical and Environmental Sciences
University of California, Los Angeles 90024

ABSTRACT

Previous studies have indicated that addition of nitrogen to Fe-inefficient PI54619-5-1 soybeans, when grown in calcareous Hacienda loam soil, intensified iron chlorosis. It was reasoned that when large amounts of nitrate were taken up, more hydroxyl ions were expelled by roots with resultant less availability of soil Fe. It was further reasoned that if N were fixed symbiotically, cation (K, Ca, Mg) uptake would considerably exceed anion ($H_2PO_4^-$, $SO_4^=$, Cl^-) uptake with no need for uptake of NO_3^- with resultant increase in protons excreted. Iron availability then would be increased and there would be less Fe deficiency. An experiment was conducted with and without innoculation with the PI54619-5-1 soybeans in the calcareous soil to test the ability of nodules to prevent Fe chlorosis. The only plants in the experiment with nodules were those with innoculum added and these plants were most free of Fe chlorosis. Iron analyses indicated that the hypothesis may be correct. It can be concluded that cation-anion uptake balance has much to do with the onset of lime-induced chlorosis. The relative uptake of NH_4^+ and NO_3^- species of nitrogen can be important considerations in the cation-anion balance.

INTRODUCTION

Nitrate uptake by plants has been shown to be very much inhibited by Fe deficiency (Wallace et al. 1971). Less NO_3^- (or any anion) uptake in relationship to cation uptake would result in H^+ excretion by roots. Iron deficient roots are known to make the external solution more acid (Landsberg 1981, Wallace et al. 1968). An interesting corollary to this is that plants with a symbiotic N-fixation relationship would have great resistance to Fe deficiency because of the large potential for release of H^+ from cation uptake which in turn would help to mobilize Fe from soil. Is this a reason why soybeans are often Fe deficient during their first two or three weeks and then become green after the symbiosis starts? Another interesting relationship is that high HCO_3^- levels in soil or irrigation water are very conducive to the onset of Fe deficiency because HCO_3^- would neutralize the H^+ produced and circumvent the plants' ability to use that mechanism for overcoming its Fe deficiency.

Organic acids are synthesized in plants to maintain electrochemical balance. A model which explains proton release with Fe deficiency and simultaneously increased organic acids in plants follows:

> Enhanced cation uptake (K^+ for example) with equivalent proton release (the cation uptake exceeds anion uptake and the difference is equal to the amount of protons released).
>
> The metabolic release of the H^+ must result from the electron transport separation of water with the OH^- remaining in the plant resulting in KOH in plants as result of the K^+ uptake.
>
> The OH^- is buffered by CO_2 present and becomes HCO_3^-. The HCO_3^- becomes substrate for the PEP (phosphoenolpyruvate) carboxylase reaction.

$$PEP + HCO_3^- \rightarrow OAA^-$$

$$K^+ + OAA^- \rightarrow KOAA$$

$$KOAA \rightarrow K\ malate$$

This model results in H^+ release to the external medium and organic acid formation inside the plant.

Two types of NO_3^- uptake and assimilation lead to the result of no proton release to the external medium and one of these involves organic acid synthesis and one does not (Wallace et al. 1976, 1979, 1980).

<u>1st case.</u> Both K^+ and NO_3^- are taken up

$$KNO_3 \rightarrow K^+ + NO_3^-$$

$$HOH \rightarrow H^+ + OH^-$$

$$H^+ + NO_3^- \rightarrow HNO_3$$

$$HNO_3 \rightarrow \text{amino acid}$$

$$K^+ + OH^- + CO_2 \rightarrow KHCO_3$$

$$KHCO_3 \rightarrow K^+ + HCO_3^-$$

HCO_3^- is used by PEP carboxylase as above to yield OAA^- or $Malate^-$
$K^+ + malate^- \rightarrow K\ malate$

The net result of KNO_3 uptake is the production of both an amino acid and of the K salt of an organic acid.

2nd case.

NO_3^- only is taken up.

An OH^- expelled by pump, H^+ remains in root.

$H^+ + NO_3^- \rightarrow HNO_3$.

$HNO_3 \rightarrow$ amino acid.

No organic acid is involved in case two. In neither of these two cases is a H^+ excreted to the external medium. In case two, an OH^- is expelled which can add to the Fe chlorosis problem. This important effect of nitrate on the onset of Fe deficiency does commonly occur when plants are grown in soil (Wallace et al. 1981).

In the special case of monocots, Landsberg found that Fe deficiency did not result in proton release to the external medium at least in amounts sufficient to cause significant acidification of solution. A major difference between monocots and dicots in their effects on Fe uptake may be that monocots take up fewer cations than do dicots so that there is less likelihood of an excess cation over anion uptake in monocots with little chance of H^+ production.

Monocots would be more likely than dicots to exclusively use the two nitrate assimilation mechanisms shown which do not result in H^+ excretion. One of these does lead to organic acid accumulation and would fit results obtained by Landsberg. Dicots would be less likely to use the NO_3^- mechanism in case two (without a cation) but use case one as well as the cation uptake mechanism without NO_3^- uptake which would excrete H^+. With Fe deficiency there would be a shift to that mechanism from that of case one.

The balance of electrons in the two equations

$$(HNO_3 + 2H_2O \rightarrow NH_4OH + 2O_2)$$

$$(NH_4OH + RC(-OH)\ COOH \rightarrow RC(-NH_2)\ COOH + 2H_2O)$$

is such that the whole molecule of HNO_3 or of NH_4OH participates in the reaction which does not result in a residual electrical charge (Wallace et al. 1976, 1979). This is the basis for many of the above conclusions.

MATERIALS AND METHODS

PI54619-5-1 soybeans were grown in 500 g. quantities of calcareous Hacienda loam soil (fine-loamy, mixed, thermic aquic Natrargid). For one set seeds were innoculated and in another set seeds were not innoculated. For both innoculated seeds and noninnoculated seeds, N was applied at a rate of 100 μg N/g soil as NH_4NO_3 and no N was applied to other plants. When seven days old seedlings were transferred from sand to the soil. Soil moisture was maintained near -1/3 bar and plants were grown for 28 days. Plants were separated to plant parts, acid and deionized water washed, dried, weighed and prepared for analysis by optical emission spectrometry (Alexander and McAnulty, 1981).

RESULTS AND DISCUSSION

The soybeans grown with innoculum and without added N were tallest and greenest at various stages of growth and at harvest time these were the only plants with nodules. The worst looking plants and with the most Fe deficiency were those with added N without innoculum. This effect of N has been reported (Wallace et al. 1981). The terminal growing points of these plants died of Fe deficiency. Plants without innoculum and without N were almost as green as those with innoculum and without N, but some leaves were yellow.

It would appear that these results indicate that elimination of the need for ionic N uptake because of innoculation does minimize the effect of induced Fe chlorosis. When there is need for NO_3^- uptake, Fe chlorosis can follow the NO_3^- uptake, but not when an Fe chelate source is used.

The lack of chlorosis for the no-N innoculum treatment was most pronounced in the new trifoliate leaves. Those leaves of this treatment were lowest in P, lowest in K, highest in Ca (all typical of freedom from chlorosis), highest in Mg, lowest in Cu, highest in Fe, highest in Mn, lowest in B, lowest in Al, highest in Sr. Not all these differences were significant (Table 1). The differences may be related to Fe deficiency in a causal way. The Fe chlorosis encountered in soybean fields early in the year could be related to the phenomenon. Later in the year the chlorosis often disappears. Implied is that cation-anion uptake balance is crucial to whether or not plants can be Fe chlorotic.

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TABLE 1

Effects of Innoculation and N on PI54619-5-1 Soybeans Grown in Calcareous Soil.

Plant	Plant part	No N No. Innoc.	No N Innoc.	N No. Innoc.	N Innoc.	F Value for N	F value for Innoc.
			Yield, mg/plant				
Old trifoliate leaf		469	437	252	269	18.1	0.3
New trifoliate leaf		322	467	140	140	33.4	5.5
Primary leaf		203	167	190	234	1.3	3.7
Stem		739	717	520	509	21.7	0.1
			Iron, µg/g				
Old trifoliate leaf		62.6	56.5	59.4	52.0	1.2	0.0
New trifoliate leaf		66.6	70.3	50.4	43.0	40.9	2.6
Primary leaf		68.4	66.3	68.2	64.1	0.0	0.0
Stem		34.3	26.4	29.7	21.3	1.5	0.0
			P, µg/g				
Old trifoliate leaf		1373	2368	1922	1688	13.3	1.6
New trifoliate leaf		2843	1791	3676	3831	38.8	3.8
Primary leaf		1136	894	1471	1233	12.0	6.1
Stem		1043	897	1345	1191	24.6	6.3
			K, %				
Old trifoliate leaf		1.78	1.79	1.98	1.93	2.3	0.1
New trifoliate leaf		1.83	1.69	2.14	2.09	48.4	3.7
Primary leaf		1.84	1.65	1.64	1.54	4.4	3.8
Stem		1.17	1.08	1.04	0.95	6.6	3.0
			Ca, %				
Old trifoliate leaf		1.32	1.25	1.58	1.57	5.4	0.1
New trifoliate leaf		1.30	1.42	1.23	1.28	1.7	1.0
Primary leaf		1.38	1.46	1.58	1.73	9.3	2.1
Stem		0.67	0.65	0.90	0.93	25.5	0.0
			Mg, %				
Old trifoliate leaf		0.46	0.44	0.60	0.56	28.4	1.2
New trifoliate leaf		0.50	0.57	0.46	0.45	20.4	3.7
Primary leaf		0.41	0.46	0.51	0.50	4.9	0.3
Stem		0.46	0.43	0.47	0.45	0.6	2.1

TABLE 1. (Continued)

Effects of Innoculation and N on PI54619-5-1 Soybeans Grown in Calcareous Soil.

Plant	Plant part	No N No. Innoc.	No N Innoc.	N No. Innoc.	N Innoc.	F Value for N	F value for Innoc.
			Zn, μg/g				
Old trifoliate leaf		30	23	27	23	0.2	2.8
New trifoliate leaf		39	29	27	26	11.3	6.6
Primary leaf		25	20	25	19	0.0	6.8
Stem		20	15	22	17	2.7	13.1
			Cu, μg/g				
Old trifoliate leaf		7.1	5.7	8.1	7.1	2.7	2.5
New trifoliate leaf		13.3	10.0	17.4	15.3	11.2	3.9
Primary leaf		5.6	4.5	7.1	6.0	1.6	0.8
Stem		6.6	6.1	8.8	6.2	1.4	2.2
			Mn, μg/g				
Old trifoliate leaf		52	68	14	22	37.0	2.7
New trifoliate leaf		61	80	14	21	106.9	6.7
Primary leaf		45	77	15	22	92.2	18.7
Stem		7.6	7.9	2.1	2.7	46.6	0.4
			B, μg/g				
Old trifoliate leaf		85	73	89	83	1.0	1.6
New trifoliate leaf		85	61	107	98	21.3	6.6
Primary leaf		45	82	64	55	0.1	1.1
Stem		16	16	22	21	44.3	0.1
			Sr, μg/g				
Old trifoliate leaf		42	40	55	56	12.8	0.1
New trifoliate leaf		40	47	39	40	1.5	2.0
Primary leaf		43	50	57	57	11.7	1.5
Stem		46	47	49	54	5.2	1.4
			Al, μg/g				
Old trifoliate leaf		23	28	32	28	1.7	0.0
New trifoliate leaf		24	21	37	34	25.5	1.4
Primary leaf		39	40	34	34	1.2	0.0
Stem		15	15	20	16	2.6	2.0
			Ba, μg/g				
Old trifoliate leaf		39	36	54	54	23.9	0.1
New trifoliate leaf		39	43	44	42	0.2	0.1
Primary leaf		34	46	54	51	7.4	1.0
Stem		50	51	50	51	0.0	0.2

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