

## **Iron Nutrition of Soybean Plants in Relation to Nitrogen Form**

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**Abstract.** Fe-efficient Hawkeye and Fe-inefficient PI-54619-5-1 soybean plants were grown in perlite culture to study the effect of nitrogen form ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$  or  $\text{N}_2$ -atm.) on the growth response and iron uptake and translocation. Both genotypes showed the same response to N form.  $\text{NH}_4$ -fed plants did not grow well and exhibited chlorosis, restricted growth and necrotic spots throughout the leaves. The magnitude of plant growth was in the order of  $\text{NO}_3 > \text{N}_2 > \text{NH}_4$ .

Ammonium treatment resulted in greater plant iron content but the total uptake was reduced. This reduction was due mainly to the reduction of dry matter as a result of  $\text{NH}_4$  toxicity. The limitation of  $\text{NH}_4$  as a sole N form is discussed on the basis of its effect on the pH of the growing media and/or its toxicity per se.

### **Introduction**

Legumes growing under conditions of a minimal ionic N supply, clearly have some metabolic processes substantially different from those of other legumes and non-legumes receiving much of their N via entry of  $\text{NO}_3^-$  and/or  $\text{NH}_4^+$  into the root cells. First, effectively nodulated plants have a sequence of N assimilation and transport functions which differ in kind and location from non-nodulated plants. Secondly, a low  $\text{NO}_3^-$  or  $\text{NH}_4^+$  supply in the soil implies a much decreased influence of any moderating (antagonistic or synergistic) effect of these ions on the uptake and translocation of other essential elements [1].

Uptake and assimilation of the ionic N forms results in substantial cytoplasmic generation of H ions and their secretion to the ambient solution, depending on whether the dominant form is  $\text{NH}_4^+$  or  $\text{NO}_3^-$  [2, 3]. In addition, excess cation uptake causes substantial rhizosphere acidity generation [2].

Brown *et al.* [4] and Landsberg [5] showed that Fe-deficiency can result in increased acidification of the nutrient solution. This acidity may influence the availability of P, Fe, Al and probably other trace metals present in the growing media.

The objective of the present study was to examine the effects of different forms of nitrogen ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$  or  $\text{N}_2$ ) on the growth and iron uptake and translocation of soybean plants.

### Materials and Methods

#### Plant culture

Soybean seeds (*Glycine max* (L.) Merr.) of the Fe-efficient Hawkeye (HA) and the Fe-inefficient PI-54619-5-1 (PI) genotypes [6] were obtained from the U.S. Regional Soybean Laboratory, Urbana, Ill. The seeds were surface sterilized prior to planting by soaking into 75% (v/v) ethanol for three minutes followed by extensive rinsing with deionized water. The seeds were then inoculated with a commercial source (Agway, Inc. Syracuse, N.Y.) of *Rhizobium japonicum* and then planted in perlite in 20.5 cm diameter pots. One week after germination, seedlings were thinned to three per pot. All nutrient treatments were initiated after the primary leaves had begun to unfold. Nutrient solutions containing N (10 mM) were prepared by adding  $\text{KNO}_3$  or  $(\text{NH}_4)_2\text{SO}_4$  to a N-free nutrient solution composed of: 1.9 mM  $\text{CaSO}_4 \cdot 4\text{H}_2\text{O}$ , 4.7 mM  $\text{K}_2\text{SO}_4$ , 1.0 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25 mM  $\text{KH}_2\text{PO}_4$ , 0.25 mM  $\text{K}_2\text{HPO}_4$ , 18  $\mu\text{M}$  FeEDTA, and 1 ml of micronutrient stock solution containing (g/l): 3.72 KCl, 1.54  $\text{H}_3\text{BO}_3$ , 0.83  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.57  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.125  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and 0.12  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ . The K concentration of all solutions, including the N-free solution, was made equal to that of the 10 mM  $\text{KNO}_3$  solution by adding the appropriate amounts of  $\text{K}_2\text{SO}_4$ . The initial pH of the treatment solutions ranged from 5.8 to 6.0. Plants were cultured in an unshaded greenhouse. Illumination was provided by daylight, supplemented with incandescent lamps. During daylight hours, ambient temperatures were kept below 37°C with evaporative cooling. Night temperature was allowed to equilibrate with outdoor temperature by leaving vents open. The RH ranged from 40 to 50 % at midday and from 80 to 90 % at night.

#### Analytical procedure

At harvest (34 days from germination), plants were separated into leaves, stem plus petioles, and roots plus nodules. Root samples were rinsed twice in deionized water to remove surface contaminants. Plant material was dried in a forced air oven at 75°C for 48 hr. Dry weights were recorded and the dried materials were ground in a stainless steel Wiley mill using a 30 mesh screen. Ammonium nitrogen was determined by Kjeldahl analysis [7] and iron determination was made by atomic absorption spectrophotometry. The data were then statistically analysed by the method of Steel and Torrie [8].

## Results and Discussion

### Dry matter and nitrogen

The overall growth of  $N_2$ -dependent plants was restricted, relative to plants supplied with  $NO_3$ -N, due to N stress (evidenced by yellow leaves during the first week of growth). At harvest, however, the leaves of plants dependent on  $NO_3$  or  $N_2$ -N were of normal green color and it was evident that nodules became capable of supplying adequate N for growth. The latter was further supported by similarities among treatments in the N percentage of the leaves (Tables 1 and 2). At the N concentration used in this study (10 mM),  $NO_3^-$  and  $NH_4^+$  forms inhibited nodulation and, therefore these plants were almost exclusively dependent upon  $NO_3$  or  $NH_4$  for their N supply.

The greatest production of dry matter took place in  $NO_3$ -dependent plants followed by  $N_2$  and  $NH_4$ -dependent plants, respectively (Table 1). The total accumulation of N (Table 3) followed a similar trend as the production of dry matter in relation to N form. Plants deriving varying amounts of N from the different N forms followed about the same proportions of the total dry matter to the different plant parts. About 50-60 % of the total dry matter accumulated by plants was allocated to the leaves irrespective of N form. The relative distribution among plant parts varied with the source of N (Table 1). The growth of the stem was proportionally the same, in relation to the rest of the plant in all the three N forms (21-25 % of the total). Thus, the morphological appearance of the plant was altered in terms of size but not in proportion. These observations are in agreement with those reported by DeJong and Phillips [9] for Alaska peas.

The poorest dry matter production in  $NH_4$ -fed plants (Table 1) may be, in part, attributed to a possible release of H ions associated with  $NH_4$  uptake which may have led to acidification of the growing media to a point that limited somewhat root growth, or due to an accumulation of  $NH_4$  ions in the cytoplasm which become toxic to the plant [10 and 11].

Ammonium fed plants exhibited physiological and morphological disorders in comparison to those dependent on  $NO_3$  or  $N_2$  for their N supply. These disorders were observed as chlorosis of the leaves, restricted growth, necrotic spots, and, in some cases, death of the leaves.

The mechanism of  $NH_4^+$  toxicity is not known. It has been suggested that  $NH_4$  ions substitute for K ions and prevent the latter from fulfilling their role in protein structure [12]. There is also evidence that  $NH_4^+$  effectively inhibits respiration [13]. Photosynthesis may also be restricted by  $NH_4$  ions through the uncoupling of noncyclic photo-phosphorylation [14]. This may explain why the total root mass (dry weight) was significantly lower in  $NH_4$ -fed plants as compared to  $NO_3$  or  $N_2$ -depend-

**Table 1. Dry matter yield of HA and PI soybean genotypes as affected by nitrogen form in the nutrient solution.**

Nitrogen form	Genotype	Dry matter			
		Root	Stem	Leaves	Total
		g/plant			
NO <sub>3</sub>	HA	0.57a*	0.75a	1.75a	3.07a
	PI	0.50b	0.77a	1.73b	3.00b
NH <sub>4</sub>	HA	0.16e	0.14c	0.35d	0.65e
	PI	0.14f	0.13c	0.35d	0.62f
N <sub>2</sub>	HA	0.30d	0.44b	1.12c	1.86d
	PI	0.35c	0.45b	1.13c	1.93c

\* values are means of four replicates.

Means within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test.

dent plants (Table 1), and may also explain, to some degree, the reduction of dry matter produced by NH<sub>4</sub>-grown plants in spite of the presence of nearly equal (if not higher) amounts of N within the tissue as in plants grown with NO<sub>3</sub> or N<sub>2</sub>-N. The two soybean genotypes tested gave almost the same response regarding the N forms (Tables 1, 2 and 3).

**Table 2. Nitrogen and iron content of HA and PI soybean genotypes as affected by nitrogen form in the nutrient solution.**

Nitrogen form	Genotype	Nitrogen			Iron		
		Root	Stem	Leaves	Root	Stem	Leaves
		%			μg/g dry matter		
NO <sub>3</sub>	HA	2.97a	1.97c	3.96a	133.75d*	48.75b	132.50ab
	PI	2.89b	1.88d	3.84b	91.25e	43.75b	62.00d
NH <sub>4</sub>	HA	3.01a	3.02a	3.94a	356.25a	110.00a	138.75a
	PI	2.95ab	2.86b	3.94a	197.50b	38.75b	138.75a
N <sub>2</sub>	HA	1.46c	1.70e	3.77b	140.00d	48.00b	121.25b
	PI	1.47c	1.66e	3.76b	172.50c	42.50b	105.00c

\* values are means of four replicates.

Means within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test.

**Table 3.** Total N and Fe uptake of HA and PI soybean genotypes as affected by nitrogen form

Nitrogen form	Plant part	N		Fe	
		HA	PI	HA	PI
		mg/plant		μ/plant	
NO <sub>3</sub>	root	16.13*	14.45	76.24	45.62
	stem	14.77	14.48	36.56	33.69
	leaves	69.30	66.43	231.87	107.26
	total	101.00	95.36	344.67	186.57
NH <sub>4</sub>	root	4.82	4.13	57.00	27.50
	stem	4.23	3.72	15.40	5.04
	leaves	13.79	13.79	48.56	48.56
	total	22.84	21.64	120.96	81.10
N <sub>2</sub>	root	4.38	5.14	42.00	60.37
	stem	7.48	7.47	19.80	19.12
	leaves	42.22	42.49	135.80	118.65
	total	54.10	55.10	197.60	198.14

\* values are means of four replications.

### Iron

Data presented in Table 2, show clearly that the Fe-efficient genotype HA was more efficient in taking up Fe than the Fe-inefficient genotype PI. The Fe content was also influenced by the N source with the greatest content being produced with the NH<sub>4</sub> source. The total Fe uptake was higher for HA than for the PI soybean plants under all N treatments but the PI plants translocated the same amount of Fe to the leaves as did the HA in the NH<sub>4</sub> treatment. These differences in Fe uptake among the two soybean genotypes are mainly due to the genetic control of Fe uptake [4].

The Fe-efficient genotype HA produces more H ions and reductant than does the Fe-inefficient genotype PI. Acidic as well as reducing conditions are known to increase Fe availability and plant uptake (Table 2). The effect of N source may be due to a release of H<sup>+</sup> ions to the root medium, as a result of NH<sub>4</sub><sup>+</sup> intake or N<sub>2</sub> fixation. The lack of such H ions release by the NO<sub>3</sub> supplied plants resulted in less Fe uptake. These results agree with those obtained by Israel and Jackson [2]. The total Fe uptake was higher for HA plants than for PI plants at any N treatment (Table 3). The reduction in total Fe uptake in both genotypes when plants were supplied with NH<sub>4</sub> is due mainly to the reduction in dry matter production as a result of NH<sub>4</sub> toxicity as mentioned earlier.

In conclusion, one may speculate about the results obtained, with much of this speculation based on a greater production of H ions and therefore a lower root surface pH for the HA than for the PI genotypes. First, Fe uptake is related to the production of H ions as indicated by the greater uptake leads mainly to an accumulation of Fe in the roots. Therefore, there must be a second Fe control mechanism that controls Fe translocation to the plant tops. This second mechanism would be either the demand for Fe by the plant tops or the translocation mechanism itself. It has been suggested that Fe translocation may be limited by the amount of organic anion such as citrate available to form a negatively charged species for Fe translocation [15].

Last, if the production of H<sup>+</sup> ions in the presence of NH<sub>4</sub><sup>+</sup> is the cause of reduced growth, there should be greater reduction in growth for the HA than for the PI genotype due to greater H<sup>+</sup> ion production. Since the growth reduction was very similar, the NH<sub>4</sub><sup>+</sup> toxicity mechanism may be NH<sub>4</sub><sup>+</sup> accumulation in the plant rather than acidity.

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## العلاقة بين صور النيتروجين وامتصاص الحديد بواسطة نبات فول الصويا

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ملخص البحث . أجريت التجربة تحت ظروف الصوبة الزجاجية لدراسة تأثير صور النيتروجين المختلفة (نترات، أمونيا وأزوت جوى) على النمو وامتصاص الحديد بواسطة صنفين من نبات فول الصويا -Haw. keye (HA), PI54619-5-1 (PI)

وقد أوضحت الدراسة أن النباتات المعاملة بالأمونيوم كمصدر للنيتروجين كانت محدودة النمو، قزمية مع ظهور الاصفرار وكان السلوك العام للنبات من حيث المادة الجافة المتحصل عليها حسب المعاملات بالترتيب  $\text{NH}_4 < \text{N}_2 < \text{NO}_3$ . وبالنسبة لامتصاص الحديد، كان تركيز عنصر الحديد عالياً في معاملة الأمونيوم، بينما كان الحديد الكلي منخفضاً في المعاملة نفسها ويرجع ذلك أساساً إلى الانخفاض في المادة الجافة بسبب سمية الأمونيوم.

وبالنسبة للأصناف، أوضحت النتائج أن هناك اختلافات معنوية بين الصنفين HA, PI من حيث امتصاص عنصر الحديد، حيث يعتبر الصنف HA أكثر كفاءة في امتصاص ونقل الحديد من الصنف PI.