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Effects of NH_4-N, NO_3-N and Urea-N on the Growth of Soybean Plants Different in Phosphorus Sensitivity.

Hoon Park* and Charles A. Stutte**
Institute of Agricultural Science, Suwon, 170 Korea.

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燐酸感受性이 다른 大豆의 生育에 對한 암모늄態 硝酸態 및 尿素態窒素의 影響

朴 薰*·찰스, 에이, 스뚜디**

Summary

Effects of nitrogen sources oi phosphorus sensitivity in soybeans were investigated using three nitrogen source (NH_4-N, NO_3-N and urea) and six cultivars (Lee, Hill, R56-49, Harosoy, Clark-63 and Chippewa), differing in phosphorus sensitivity. Growth inhibition with ammonium or urea medium increased with phosphorus sensitivity indicating that the phosphorus sensitive cultivars are also sentitive to ammonium. The sensitive Clark-63 was inhibited two folds more than the tolerant Lee in ammonium medium. These results strongly suggest that phosphorus sensitivity is closely linked to nitrogen metabolism and ammonium toxicity.

Nitrogen sources gave special morphorogical growth patterns (short internode-many branchs in NO_3-N, long internode-no branches in NH_4-N and a low top to root ratio in urea) by affecting differently the growth of plant organs.

Introduction

The phosphorus level for maximum growth of the soybean is approximately 5μM, which is relatively low, compared to 60μM in the potato and as low as 1μM in silver grass[2]. The behavior of a number of species in nutrient solution culture was found to be similar to their behavior in the field. An application of phosphorus increases the population of brome grass (5μM) and decreases silver grass (1μM)[20]. Such population change of a plant comunity is attributed to different phosphorus susceptibility among different plants.

Phosphorus toxicity due to phosphorus-excess in soybean was reported in 1918 by Shive[21], who observed dark brown spot discoloration and decreased yield. However, phosphorus-excess did

* Former FAO fellowship graduate student at University of Arkansas, USA.
** Associate professor, University of Arkansas, Fayetteville, Arkansas 72701 USA.

not receive much attention in soybean cultivation until Howell[21] observed varietal differences in phosphorus susceptibility in 1954[9]. When phosphorus was increased five to ten fold, the tolerant variety, Chief, responded by increased vegetative growth and seed production. The susceptible variety, Lincoln, showed a reddish brown leaf discoloration, a reduced growth rate, and produced very few seeds. Howell and his coworkers[11] grouped 44 varieties into 5 categories according to their responses and found that susceptibility was closely related to genetic origin.

Phosphorus toxicity of a sensitive soybean variety was virtually eliminated by the addition of sufficient supplemental nitrates as potassium nitrate while other salts without nitrate were not effective[10]. It indicates nitrogen involves in phosphorus toxicity. It is not clear how nitrogen affects phosphorus toxicity, through either uptake, translocation, or metabolism.

Further it could be postulated that nitrogen metabolism itself attributes to phosphorus toxicity rather than phosphorus alone. Little information is available on nitrogen-phosphorus interaction in relation to phosphorus sensitivity since phosphorus excess has been considered as a function of one variable, phosphorus. This study was carried out as a first step in an attempt to ascertain the possible interrelationship of nitrogen sources and phosphorus sensitivity in soybean plants.

Materials and methods.

Cultural method

Six soybean varieties or cultivars(Glycine max) were used for the experiments as shown in Table 1. The seeds were germinated in vermiculite, and when the first true leaves(unifoliate leaf) unfolded, the plants were removed, and thoroughly rinsed in running tap water followed by distilled water. All adhering vermiculite particles were removed from the roots. The plants were then transfered into an aerated nutrient solution held in one gallon painted glass containers, with each container supporting two plants of different varieties. The three treatments using nitrate, ammonium, and urea were replicated three to six times depending on necessity for the plant sample.

Table 1. The origin and other characteristics of the soybean cultivars investigated

Cultivars	Parantage	Phosphorus sensitivity	Maturity group	Growth type
Lee	S-100×Clemson non shatter	Tolerant	Ⅵ	D
Hill	D632-15×D49-2525	Slightly sensitive	Ⅴ	D
Harosoy	Mandarin×(Mand.×Ak)	Intermediate	Ⅱ	ID
Clark-63	Lincoln×(Lin.×Richland)	Sensitive	Ⅳ	ID
Chippewa	Lincoln×(Lin.×RL)	Very sensitive	Ⅰ	ID
R56-49	Offtype in Lee	Unknown	Ⅴ	D

The nutrient solutions were consisted of KH_2PO_4 (0.2772 g/l) $CaSO_4$(0.3404 g/l) $MgSO_4 \cdot 7H_2O$ (0.2465g/l) Fe-EDTA(Na) (32.8 μg/ml), $MnCl_2 \cdot 4H_2O$(1.8μ g/ml) $CuSO_4$ (0.05μ g/ml) H_3BO_3 (2.8μ g/ml) $H_2MoO_4 \cdot H_2O$ (0.017μ g/ml) Zn. EDTA (Na_2)(1.602μg/ml), and Ca $(NO_3)_2 \cdot H_2O$ (0.4553 g/l for nitrate, $(NH_4)_2SO_4$ (0.3304 g/l) for ammonium, $CO(NH_2)_2$ (0.1500 g/l) for urea treatment respectively based on the reports of others[1,13]. In both ammonium and urea solutions, an equivalent amount of calcium sulfate for calcium was added so that sulfate was the only variable. The initial pH of the solutions was adjusted to 6.7 and readjusted, as needed, to

approximately 6.5, using a saturated calcium hydroxide solution or dilute hydrochloric acid. During the first three days, the plants were supp lied with half-strength nutrient solution. The nutrient solutions were completely renewed every three to four days.

In order to find the nitrogen source effect on growth, nitrogen untake and pH change from and of solution, plants (var, Lee) were transplanted when the leaves began to emerge, and then grown in one liter glass containers holding one plant each. The nutrient solution was renewed every three days.

Plants were grown in a controlled environment chambers under Grolux light with some incandes cent lamps at 2,000 foot candles with 14 hour daylength. Temperature was 27C during the light cycle and 17C during the dark. After 21 days, plants were harvested and separated into leaves, stems and roots. The tissues were then stored at -18C prior to analysis.

Nitrogen uptake and pH change in the nutrient solution

Nitrogen uptake from the nutrient solution was determined by using nitrate specific ion electrode [18], while Nessler reagent was used for the ammonium determination, and urea was determined according to the method of Hunninghake and Crisolia [12]. pH was measured by using glass electrode attached to Orion ion meter.

Results

pH change and nitrogen uptake from the nutrient media

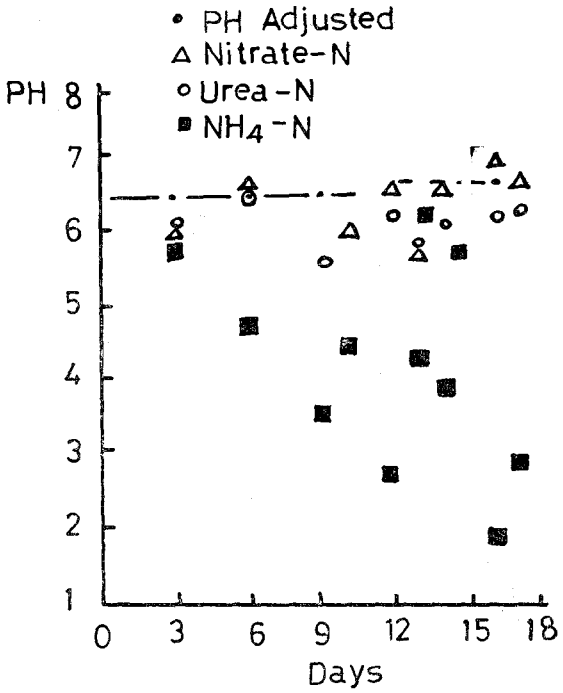

Fig. 1. pH change in soybean nutrient media as influenced by nitrogen source.

As shown in Figure 1, the pH decreased in the ammonium medium while it increased in the nitrate medium. The change in urea medium was intermediate.

This phenomena are similar to those reported in other plants [5,13]. Even in the nitrate medium there is a slight PH decreasing period after adjusting the pH to 6.5, at least until 15 days of gronwth. After this period nitrate uptake seems to affect the pH in the medium more. In the ammonium medium, pH change is much more

Table 2. Effect of nitrogen source on the fresh weight yield of different portions of a soybean plant (var. Lee).

	NO_3-N		NH_4-N		Urea-N		Mean	
	gm	%	gm	%	gm	%	gm	%
Who e plant	*21.10[a]	100	10.48[b]	100	11.15[b]	100	14.24	100
Laminae	8.60[a]	40.8	4.75[b]	45.3	4.25[b]	38.1	5.87	41.22
Petio l	1.70[a]	8.0	0.78[b]	7.6	0.85[b]	7.6	1.11	7.80
Stem	6.15[a]	29.2	3.05[b]	28.7	3.20[b]	28.7	4.13	29.00
Root	4.65[a]	22.0	1.90[b]	25.6	2.85[c]	25.6	3.13	21.98
T/R	3.54		4.52		2.92		3.55	

* Mean of four replicates, any means which followed by the same letters are not significantly different at P= 0.05 in the same organ.

rapid than in nitrate medium and a sharp decrease was observed in plant growth. The pH decreased below 2.0 in ammonium medium two days after adjusting the medium to 6.7 while it increased to 7.0 in nitrate medium.

The percent nitrogen uptake to initial nitrogen from the nutrient media is shown in Figure 2. Nitrogen uptake was highest in the nitrate treatment and lowest in the urea treatment. In contrast to the nitrogen uptake the higher fresh weight yield was obtained in the urea treatment than the ammonium treatment (Table 2).

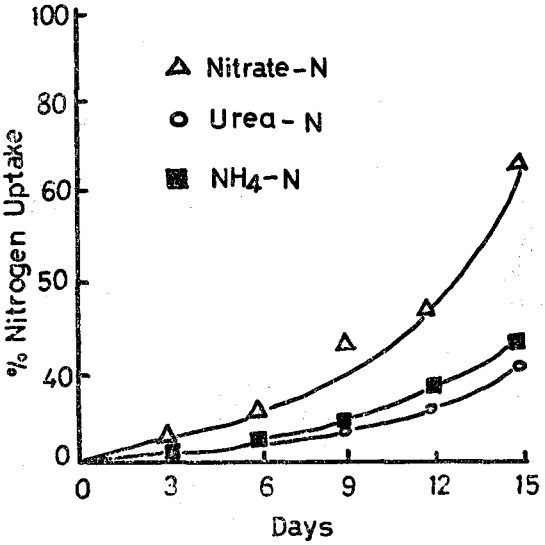

Fig. 2. Nitrogen uptake by soybean from nutrient media. ($100 \times N_f/N_i$ where N_f and N_i incicate final N amount and initial N amount in culture solution).

It indicates that plant yield per unit nitrogen is greatest from urea treatment. Such results would suggest different nitrogen metabolism in urea and ammonium treated plants. High yield per unit nitrogen in urea may be due to more efficient use of nitrogen, such as direct incorporation into protein, via carbamylphosphate[6]. The high efficiency of urea-N utilization has also been shown in tomato plants[13].

Nitrogen up take greatly affects the pH change of the nutrient media (Fig. 1 and 2.) especially in the ammonium medium. This suggests that nitrogen assimilations in the plant differ according to nitrogen source and the pH changes in the media are the result of these different assimilation processes. Thus, the more nitrogen that is taken up, the greater the pH change in the medium.

Plant growth and morphological response

The fresh weight yield of nitrate fed Lee variety was twice as much as that of the ammonium or the urea fed ones. Urea fed plants were slightly higher than the ammonium supplied plants (Table 2), even though nitrogen uptake was less than in the ammonium supplied plants. (Fig. 2). In comparison with the nitrate plants, urea or ammonium fed soybeans showed small and crumpled leaves. Crumpled symptoms may be due to urea or ammonium toxicity. Urea or ammonium fed plants had a yellowish color with red or brown spots in the lower leaf margin and the older leaves changed to a grayish-brown color and die-back was similar to phosphorus-excess[21]. The young leaves of ammonium fed plants were darker green than in the nitratefed plants. urea fed plants showed pale green color, similar to nitrogen deficiency.

As indicated in Table 2 and Table 3, nitrogen sources affect the growth of plant organs. When the organs are considered as sub plots, the analysis of variance on the effect of nitrogen source in plant growth shows not only significant nitrogen effect on total growth, but also significant interaction between nitrogen source and organ.

Table 3. Effect of nitrogen source on soybean vegetative growth (var. Lee).

	Top height		Root length		Axil branch	
	Mean, cm	Range, cm	Mean, cm	Range, am	Number	Length, cm
NO_3-N	42.6	38.45.5	32.8	21-46	1.3	22
NH_4-N	45.5	45-46.5	23.6	19.5-29	0.0	0
Urea-N	41.2	38.3-45.0	27.1	25-29	0.5	10

Thus, the percentage contribution of each organ to total plant weight is different, primarily more due to root and laminae than by stem and petiole (Table 2). The poor root system and a high percentage of laminae are characteristic in ammonium fed plants and the reverse is true in urea supplied plants.

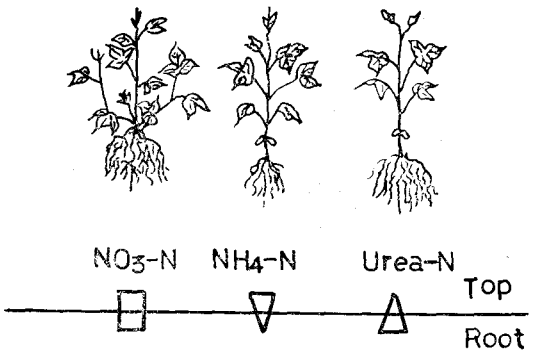

Fig. 3. Schematic drawing of morphological change in soybean due to nitrogen source. (Square, wedge and pyramid shape respectively).

Nitrogen assimilation rather than pH is likely to be more responsible for the growth of organs. From these results it appears that nitrogen source influences whole plant growth by affecting organ growth differently. The morphological change occurs simultaneously with the change of fresh weight yield by nitrogen source, as shown in Table 2 and Figure 3. The length of internodes in the ammonium supplied plants were much longer than that of the nitrate supplied ones. The lateral branch formation was also affected by nitrogen source. There was no branching in the ammonium supplied plant but many branches with good growth were observed in the nitrate supplied plant. Urea fed plant was also intermediate in branch formation. The top/root ratio was heighest in the ammonium fed plant and lowest in the urea fed plant. The pattern of morphological response in the different media can be schematically expressed as in Figure 3, square shape fore the balanced growth in nitrate, pyramid shape for urea and wedge shape for the ammonium fed plant.

The early leaf senescence in the ammonim medium was shown by the early die-back of lower leaves. In the nitrate fed plant even the unifoliate leaves remained green to the harvesting stage. Similar results to those observed in the Lee variety as mentioned above, were also shown in all other varieties. The symptoms in leaves, as described above, appeared in all varieties with consistent increases in severity parallel to the increase of phosphorus sensitivity. From the consistency of symptoms, ammonium excess seems to have a close relation to phosphorus sensitivity.

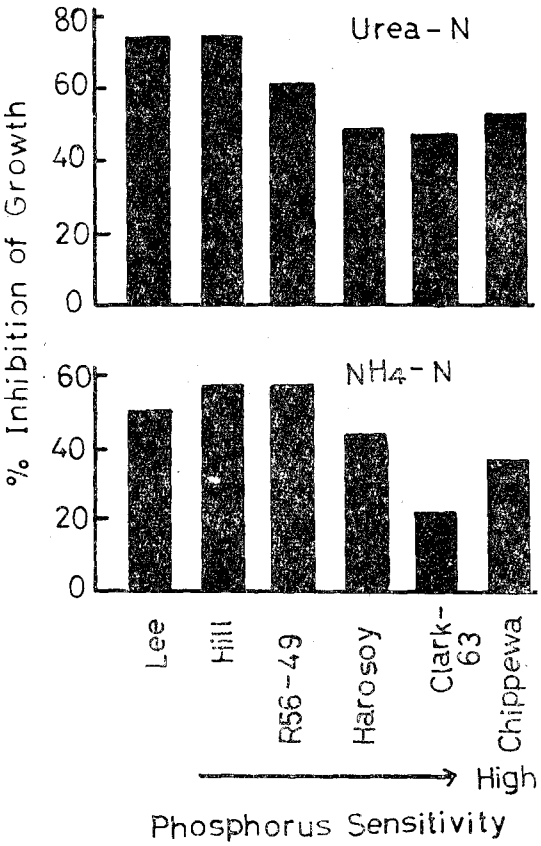

Fig. 4. Soybean growth inhibition by urea and ammonium nitrogen compared to nitrate nitrogen. (for 3 weeks)

The growth inhibition, in ammonium and urea media, considering nitrates as control, is shown in Figure 4. Phosphorus sensitive varieties are inhibited more in dry matter yield in the ammonium medium than are the tolerant varieties.The five varieties, Lee, Hill, Harosoy, Clark-63 and

Table 4. Nitrogen source effect on the dry matter yield of different soybean varieties that differ in phosphorus sensitivity.

		Whole Plant,	Leaf		Stem		Root	
		gm	gm	%**	gm	%	gm	%
NO_3-N	Lee	4.96*'[a]	2.92[b]	58.9	1.23[b]	24.7	0.81[b]	16.4
	R56-49	5.29	3.25	61.4	1.42	26.9	0.62	11.7
	Clark-63	3.21	1.91	59.6	0.84	26.0	0.46	14.4
	mean	4.49	2.70	60.1	1.16	25.9	0.63	14.0
NH_4-N	Lee	2.49	1.56	62.7	0.63	25.4	0.30	11.9
	R56-49	2.99	1.80	60.2	0.85	28.3	0.34	11.5
	Clark-63	0.66	0.40	60.1	0.17	25.8	0.09	14.1
	mean	2.05	1.25	61.2	0.55	26.8	0.26	12.0
Urea-N	Lee	3.60	1.90	52.8	1.08	29.9	0.62	17.3
	R56-49	3.16	1.77	55.9	0.89	28.4	0.50	15.7
	Clark-63	1.48	0.91	61.6	0.36	24.0	0.21	14.4
	mean	2.75	1.53	55.5	0.78	28.3	0.44	16.2
Grand mean		3.10	1.83	59.0	0.83	26.8	0.44	4.2

[a]LSD (0.01)=0.091 [b]LSD (0.01)=0.116 between any two numbers in the same column.
*Mean of 3 replicates. **% of the whole plant.

Table 5. Analysis of variance for the effect of nitrogen source on the organ growth of various soybean varieties.

Source of Variation	df	SS	MS	F
Replication	2	0.4187	0.2093	32.70**
Nitrogen(N)	2	9.4735	4.7367	740.10**
Error (a)	4	0.0256	0.0064	
Variety (V)	2	7.7574	3.8787	315.34**
V.N	4	0.2773	0.0693	5.63**
Error (b)	12	0.1477	0.0123	
Organ (O)	2	27.5355	13.7677	1720.96**
O.N	4	3.4805	0.8701	180.76**
O.V	4	2.3749	0.5937	74.21**
O.V.N	8	0.2734	0.0342	4.27**
Error (c)	36	0.2883	0.0080	
Total	80	52.0528		

** Significant at P=.0.01

Chippewa belong to the different phosphorus sensitivity groups(Table 1)[11]. According to their growth and symptoms, phosphorus tolerant variety is tolerant to ammonium. Thus, the nitrogen source has a different effect on variety and on organs as shown in Table 4. There are also highly significant second and third order interactions among nitrogen source, variety, and plant organ growth (Table 5). The disturbance of organ growth by nitrogen source may have a certain relation to ammonium toxicity. In comparison with other varieties, the most ammonium tolerant variety, Lee, was primarily affected in root growth. This indicates that ammonium toxicity is not related totally to root growth alone.

Discussion

Plant uptake of nitrogen in the form of NH_4^+ and NO_3^- depends on the ionic uptake whereas urea depends on molecular uptake. The molecular uptake of urea has been shown by the measurement of urea in the plant, however, its uptake process, either active or passive, is still questionable [15,19].

Whether the ionic uptake of NH_4^+ and $NO_3^-$is through direct ionic exchange or accompanied with counter ions, is also obscure. The change of pH with the increase of nitrogen uptake(Figure 1 and 2) indicates the close relationship between these two phenomena. Kirkby and Mengel[13]

Nitrogen source	Assimilation in plant	Exchange in medium
NO_3-N	$N^{5+}O_3+8H^++8e \leftarrow\cdots\cdots$	$\cdots\cdots NO_3^-$
	$\rightarrow N^{3-}H_3+2H_2O+OH^-\cdots\cdots$	$\cdots\cdots\rightarrow OH^-$
	$OH^-+CO_2\rightarrow HCO_3\ \cdots\cdots$	$\cdots\cdots\rightarrow HCO_3^-$
NH_4-N	$NH_4^+ \leftarrow\cdots\cdots$	$\cdots\cdots NH_4^+$
	$\rightarrow NH_3+H^+\ \cdots\cdots$	$\cdots\cdots\rightarrow H^+$
Urea	Urea+phosphate $\leftarrow\cdots\cdots$	$\cdots\cdots\rightarrow CO(NH_2)_2$
	$\rightarrow$Carbamylphosphate+NH_3	
	$CO(NH_2)_2+H_2O\cdots 2NH_3+CO_2$	
	$CO_2+H_2O\rightarrow H^+\cdots\cdots$	$\cdots\cdots\rightarrow H^+$
	$+H^+CO_3\cdots\cdots$	$\cdots\cdots\rightarrow HCO_3^-$

Fig. 5. Nitrogen assimilation in plant and its effect on culture media.

suggested that H^+ produced during ammonium and urea assimilation would diffuse out, lowering the pH of the nutrient media while OH^- or HCO_3^- produced in nitrate assimilation would diffuse out, increasing the pH. The rapid pH change in ammonium medium, in spite of the lower uptake of nitrogen than in the nitrate medium, suggests that nitrogen assimilation rather than nitrogen uptake is more related to pH change. Furthermore, nitrogen uptake itself is probably affected by nitrogen assimilation. The interrelation between nitrogen assimilation, its uptake and pH change may be expressed as Figure 5.

The relative amounts of OH^- and HCO_3^- in the nitrate medium and that of H^+ and HCO_3^- in the urea medium will determine the pH. The bicarbonate formation from OH^- in the nitrate assimilation is unknown in quantity. But the slow increase of pH of the nitrate medium in spite of the great uptake of nitrate indicates that the diffused OH^- or HCO_3^- is likely a very small amount.

It is also expected that the quantity of H^+ accumulated by NH_4^+ assimilation is much higher than OH^- accumulated by NO_3^- assimilation per unit nitrogen. Consequently, the quantity of diffused H^+ is greaterthan OH^-. This would be a probable explanation of the rapid decrease of pH and the greater effect of nitrogen uptake on pH in the ammonium medium. The small variation of pH in the urea medium suggests the above scheme (Fig. 5) in which H^+ and HCO_3^- are diffused out. The direct exhange of OH^- and H^+ by NO_3^- and NH_4^+ has been suggested as a primary process for maintaining the cation-anion balance both in the plant and in the nutrient media[13]. This is, however, questionable in this experiment, due to the lack of precise quantitative investigation of H^+ and nitrogen uptake, even though assimilation effects are assumed as suggested above. The assimilation schemes and the experimental results support the direct ionic exchange of NH_4^+ or NO_3^-, if not totally, at least to a considerable extent. The cyclic relation of assimilation and uptake in which assimilation of a nitrogen form stimulates the uptake of the same form, occurs in ammonium and nitrate assimilation. The cyclic relation in ammonium aggravates the physiological condition internally and externally, due to the ammonium uptake-dependent pH decrease which leads to a certain point that retards assimilation, uptake, and consequently, plant growth, while the cyclic relation in nitrate continuously increases assimilation and uptake, resulting in the best growth. The nitrogen uptake-dependent pH change may have the important role in the nutritional availability in the rhizosphere. The lowest uptake of nitrogen in urea may suggest that molecular uptake is slower than ionic uptake and not fast enough to meet the demand for nitrogen. The pale green color and the low free amine acid

content in the leaves (unpublished) strongly indicates nitrogen deficiency. This might be due to the lack of urease in the soybean leaves[3]. In the urease-free soybean leaves, urea assimilation may be primarily through carbamyl phosphate. However, the decrease of pH in the medium suggests the existence of urea hydrolysis.

If NO_3^- and NH_4^+ uptake is not only one to one exchange with OH^- or H^+, the counter ions have to be accompanying them. Therefore, nitrates are accompanied with a large amount of cations while ammonium uptake causes the greater entry of anions to maintain an ionic balance in the tissue. It is expected that cation deficiency and anion excess could occur in the ammonium plant while anion deficiency and cation excess could occur in the nitrate plant partly because of the same kind of ion competition with NO_3^- and NH_4^+ at the absorption sites and partly due to the result of NO_3^- or NH_4^+ assimilation. Since the diffused H^+ can exchange with cations in the urea medium, the ionic unbalance is not as critical as in the ammonium medium, and this may be the reason for better growth in the urea plant than in the ammonium plant.

It is well expected that ammonium induces phosphorus uptake. The ammonium-induced anion uptake seems to be very favorable to phosphorus uptake in soybean[10], rice[22], corn[4], and even high bush blueberry plants[23]. The kinetic study with barley root revealed a higher rate of $H_2PO_4^-$ uptake by 10 times that of $HPO_4^=$ and the occurence of a different absorption site for the different phosphorus ions. It was also shown that OH^- competes with both ions at the absorption sites[8].

Thus, the lowering of pH and consequent high $H_2PO_4^-$ concentration in ammonium medium is likely to accelerate phosphorus uptake. In the ammonium and urea medium the phosphorus sensitive varieties, Harosoy, Clark-63 and Chippewa showed greater growth inhibition than the phosphorus tolerant varieties, Lee, Hill and R56-49(Fig. 4). Such growth inhibition could be interpreted as phosphorus sensitivity induced by ammonium as mentioned above.

If it is true the phosphorus tolerant varieties should uptake ammonium faster than the phosphorus sensitive varieties do. The varietal difference in nitrogen uptake and consequent pH change in the medium could not investigated in this experiment because two varieties were grown in the same container.

It is, however, expected that there is varietal difference in nitrogen uptake and consequently pH change is different. The pH reduction rate in ammonium medium in certain variety could be a measure of ammonium assimilatory power in that variety and the pH increasing rate, as a nitrate assimilation power.

Thus it is highly probable that phosphorus excess occurs simultaneously with ammonium excess in phosphorus sensitive varieties. Phosphorus sensitivity may depend on the ammonium uptake rate.

The inhibitory effect of ammonium nitrogen on cyclic phosphorylation was observed[16]. The unconpling of non-cyclic photophosphorylation by 10^{-3} M ammonium ion in spinach chloroplast was also reported[14].

The growth inhibition in ammonium medium (Fig. 4), thus, could be interpreted as ammonium toxicity and it could be said that the greatly inhibited three varieties are more sensitive to ammonium by introducing ammonium sensitivity concept. In seems that phosphorus excess could not occurs without ammonium excess. It may be concluded that phosphorus sensitivity should be considered in relation to ammonium sensitivity.

Such conclusion is supported by the fact eliminating phosphorus sensitivity by the addition of sufficient supplemental nitrates[10]. Metabolic mechanism of phosphorus sensitivity in the plant, however, may quite different from that of ammonium sensitivity since the morphological patterns are different among nitrogen sources(Fig 3).

Such morphological difference may be attributed to the difference of hormonal balance[17] which is affectet by nitrogen sources. The long internode

in ammonium fed soybean plant and short internode in nitrate plant seem to cosistent with the long filamentous mycelium of *Ustilago zeae* obseved in the ammonium medium and the short form in the nitrate medium[7]. It is may also relate to the fact that nitrate source increased the cell division but decresed the cell elongation[24].

要 摘

燐酸感受性이 서로다른 여섯개 大豆品種(Lee, Hill, R56-49, Harosoy, Clark-63, Chippewa)을 암모늄 態, 硝酸態 및 尿素態의 窒素源을 使用하여 燐酸感受性에 對한 窒素源의 影響을 調査하였다.

암모늄態나 尿素態 培養에서의 生育沮害는 燐酸感受性이 클수록 증가하여 燐酸感受性 의 大豆는 암모늄態 窒素에도 感受性인 것을 보였다.

感受性인 Clark-63은 耐性인 Lee보다 암모늄態 窒素培養에서 두배나 生育이 沮止되었다. 이들 結果는 燐酸感受性이 窒素代謝와 암모늄 毒作用에 密接히 關連되어 있음을 强力히 示唆하는 것이다.

窒素源은 各 器管의 發育에 달리 作用하여 특이한 形態的 生育樣相(硝酸態에서 短稈多枝, 암모늄態에서 長稈無枝, 尿素態에서 낮은 地上部根重比)을 보였다.

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