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DNA, RNA, Protein and Yield of the Soybean Plant, *Glycine max* Merr., as Affected by Phosphorus Nutrition

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大豆의 核酸, 蛋白質 및 物質生產에 미치는 燐酸肥料의 効果

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ABSTRACT

The effect of phosphorus nutrition on the contents of deoxyribonucleic acid (DNA), ribonucleic acid(RNA), crude protein and plant growth of soybean plant(*Glycine max.* Merr.) was studied. Yields of the above- and under-ground parts of the soybean plant in terms of dry weight, the amounts of crude protein, RNA and DNA continued to increase with increasing phosphorus supply. The amounts of RNA and crude protein were highest in the leaf tissues where most intensive growth was taking place. The relationships among DNA, RNA, crude protein and plant growth appeared to consist of the central dogma which has immortalized, while DNA in plant tissue was subject to charges caused by external environmental facters such as phosphorus nutrition.

INTRODUCTION

In investigations of the mechanism for the response to plant growth by phosphorus nutrition, the relationships among DNA, RNA and protein in plant tissue have so far been the major issues which have attracted attention. However, phosphorus compounds themselves play a critical role in plant metabolism. Among these compounds are phosphate esters, phospholipids, nucleic acids and phosphoproteins. RNA plays an important role in cell division, protein synthesis, cell-wall formation and tissue differentiation. Therefore, the RNA content is high at the sites of intensive synthesis and growth. DNA is present in chromosomes, and in responsible for the transmission of genetic characteristics. RNA in plant tissue or cell is subject to charges caused by external environmental factors(Ali-Zade, 1959; Bobrysheva & Oknina, 1962; West, 1962; Williams, 1948; Rhee, 1972).

There were several reports that the direct relationship between growth and the RNA content was shown by Ali-Zade(1959) for tea, Bobrysheva and Oknina(1962) for currant and Chang(1973) for rye-grasses. However, a detailed description of the correlation among phosphorus nutrition, DNA, RNA, protein and yield is not much available.

The aim of this experiment was to determine the effects of phosphorus treatment on the DNA, RNA and crude protein contents, and growth in terms of dry weight of the soybean plant.

MATERIALS and METHODS

The soybean plant, *Glycine max* Merr. was chosen for the study. The plant was grown in a growth chamber using a split-medium technique (Tiffin et, al., 1960). After good and uniform growth of roots, about one week, plants were transferred to nutrient solutions. Eleven levels of phosphorus concentrations were applied to these seedlings. The levels employed were 0.0, 0.3, 0.6, 0.9, 1.2, 1.5, 2.0, 3.0, 4.5, 6.5 and 18.0 ppm as $NaH_2PO_4 \cdot H_2O$. New solutions were provided every other day to maintain phosphorus concentrations as constant as possible.

After soybean plants had grown for five weeks from planting, roots, stems and leaves were harvested, dried at 60°C, weighed, and ground for analyses, respectively. Determinations of yields of soybean plants were made of dry weight of the above- and under-ground parts. Total nitrogen was determined by the micro-Kjeldahl method and the amount of crude protein was calculated by multiplying nitrogen by 6.25.

The individual parts, such as roots, stems and separate leaves, were frozen immediately with liquid nitrogen, lyophilized, and analyzed by a modified procedure, combining an alkaline hydrolysis method with a subsequent cold perchloric acid extraction(Chang, 1973), for the estimation of DNA, RNA, phosphoprotein and other phosphorus fractions.

RESULTS and DISCUSSION

1. Yield, crude protein, RNA, DNA and other phosphorus fractions.

The effect of phosphorus nutrition on dry matter yield of the soybean plant is shown in Table 1. Dry weights of the above- and under-ground parts of the soybean plant continued to increase with increasing phosphorus concentration. This result suggests that the negative response of the soybean plant to high phosphorus, as determined by yield, may occur at higher concentrations of phosphorus than 18.0 ppm. Therefore, as compared with *Glycine max* var. *lincoln* reported by Howell and Bernard (1961), *Glycine max* Merr. seems to be tolerant to the high phosphorus application.

The crude protein content in the above- and under-ground parts of the soybean plant is given in Table 2. These data show that the above-ground parts of this plant contain more crude protein than the under-ground parts. There is a significant difference at the 1% level between the amounts of crude protein in the above- and under-ground parts of the soybean plant. As the phosphorus concentration in solution rises up to 18.0 ppm, the crude protein content in the above- and under-ground parts of the soybean plant increases from 13.47% to 18.50%, and from 6.92% to 12.23%, respectively.

Total phosphorus content in soybean roots is given in Table 3. These data show that phosphorus

Table 1. Yields of the above- and under-ground parts of the soybean plant in terms of dry weight (g/plant)

P-concentration (ppm)	Above-ground parts	Under-ground parts	Total
0.0	3.23 ± 0.548	0.57 ± 0.113	3.80 ± 0.560
0.3	3.80 ± 0.590	0.65 ± 0.095	4.44 ± 0.598
0.6	4.30 ± 0.603	0.70 ± 0.165	50.0 ± 0.625
0.9	4.86 ± 0.615	0.76 ± 0.189	5.62 ± 0.643
1.2	5.51 ± 0.575	0.89 ± 0.144	6.40 ± 0.593
1.5	5.91 ± 0.617	0.91 ± 0.165	6.82 ± 0.639
2.0	5.99 ± 0.636	0.96 ± 0.220	6.95 ± 0.673
3.0	6.02 ± 0.595	0.98 ± 0.213	7.00 ± 0.632
4.5	6.25 ± 0.744	0.99 ± 0.180	7.24 ± 0.765
6.5	6.29 ± 0.537	1.02 ± 0.188	7.31 ± 0.570
18.0	6.30 ± 0.792	1.08 ± 0.253	7.38 ± 0.691

accumulation in soybean roots increases with increasing phosphorus concentration. The ability of the soybean plant to take up increasing phosphorus from solutions of increasing phosphorus supply may be the reason for its susceptibility to a high phosphorus supply. The phosphorus fractions of lower molecular weight found in soybean leaves are also shown in Table 3. The acid-soluble phosphorus fractions extracted with 0.2 N perchloric acid (PCA) at 4°C include inorganic phosphate, sugar phosphates, various free nucleotides, phosphoglyceric acid, thiamine pyrophosphate and phosphoryl choline. Phospholipids are the fractions soluble in lipid-solvents (alcohol and ether), and include phosphatides. The phosphorus fractions of higher molecular weight found in soy-

Table 2. The content of crude protein of the soybean plant (%)

P-cocentration in solution (ppm)	Above-ground parts	Under-ground parts
0.0	13.47 ± 2.301	6.92 ± 1.496
0.3	14.38 ± 2.259	7.45 ± 1.591
0.6	15.66 ± 2.332	7.90 ± 1.228
0.9	16.74 ± 2.606	8.49 ± 2.081
1.2	16.95 ± 2.121	9.49 ± 2.081
1.5	17.79 ± 2.395	10.55 ± 1.705
2.0	17.90 ± 3.182	11.03 ± 2.186
3.0	18.14 ± 2.861	11.45 ± 2.159
4.5	18.37 ± 3.688	11.78 ± 1.968
6.5	18.47 ± 3.101	12.01 ± 2.961
18.0	18.50 ± 3.356	12.23 ± 2.201

Table 3. Phosphorus content of selected parts of the soybean plant (μg/mg)

P-concentration in solution (ppm)	Total p*	Acid soluble p**	Phospholipid p**	Protein p**
0.0	2.20 ± 0.389	0.31 ± 0.046	0.53 ± 0.079	0.43 ± 0.054
0.3	2.83 ± 0.398	0.42 ± 0.052	0.82 ± 0.068	0.52 ± 0.046
0.6	3.07 ± 0.372	0.54 ± 0.057	0.96 ± 0.085	0.65 ± 0.042
0.9	3.29 ± 0.326	0.65 ± 0.071	1,03 ± 0.163	0.76 ± 0.068
1.2	3.91 ± 0.418	0.83 ± 0.088	1.19 ± 0.159	0.97 ± 0.067
1.5	4.56 ± 0.474	1.06 ± 0.090	1.35 ± 0.107	1.15 ± 0.083
2.0	5.64 ± 0.445	1.22 ± 0.092	1.51 ± 0.179	1.24 ± 0.091
3.0	7.55 ± 0.546	1.47 ± 0.107	1.63 ± 0.155	1.37 ± 0.109
4.5	8.84 ± 0.549	1.62 ± 0.152	1.76 ± 0.193	1.48 ± 0.112
6.5	10.74 ± 0.618	1.85 ± 0.123	1.88 ± 0.188	1.56 ± 0.118
18.0	11.85 ± 0.611	1.62 ± 0.201	1.79 ± 0.222	1.55 ± 0.113

* In root, single freeze-dried sample.
** In 3rd trifoliate leaf from terminal bud, single freeze-dried sample.

Table 4. Changes in the RNA content of the soybean plant (μg RNA/mg)

P-concentration in solution (ppm)	Above-ground parts	Terminal bud	Trifoliate leaf 1*	Trifoliate leaf 3**
0.0	3.5 ± 0.29	14.2 ± 1.36	17.2 ± 1.62	5.4 ± 0.61
0.3	4.2 ± 0.43	16.7 ± 1.90	19.9 ± 1.73	6.5 ± 0.75
0.6	5.3 ± 0.47	18.0 ± 1.58	21.3 ± 1.87	7.7 ± 0.82
0.9	6.1 ± 0.52	19.6 ± 1.50	23.5 ± 2.02	9.8 ± 0.76
1.2	7.4 ± 0.58	20.5 ± 2.11	24.2 ± 2.14	12.9 ± 1.25
1.5	8.6 ± 0.61	21.9 ± 1.73	24.8 ± 2.17	15.6 ± 1.53
2.0	9.3 ± 0.69	22.3 ± 2.08	25.2 ± 2.43	16.0 ± 1.38
3.0	9.8 ± 0.66	22.7 ± 2.19	25.6 ± 2.50	16.5 ± 1.16
4.5	10.0 ± 0.94	22.9 ± 2.15	25.8 ± 2.14	16.9 ± 1.73
6.5	10.3 ± 1.15	23.2 ± 2.35	26.0 ± 2.07	17.2 ± 1.87
18.0	10.5 ± 1.27	23.3 ± 2.30	26.8 ± 2.26	17.4 ± 1.79

* Trifoliate leaf next to terminal bud.
** Third trifoliate leaf from terminal bud.

Table 5. Changes in the DNA content of ehe soybean leaves (μg DNA/mg)

P-concentration in solution (ppm)	Above-ground parts	Terminal bud	Trifoliate leaf 1*	Trifoliate leaf 3**
0.0	0.91 ± 0.101	2.3 ± 0.167	3.3 ± 0.192	1.2 ± 0.107
0.3	0.97 ± 0.092	2.7 ± 0.139	3.8 ± 0.165	1.3 ± 0.112
0.6	1.04 ± 0.098	3.0 ± 0.108	4.0 ± 0.299	1.4 ± 0.120
0.9	1.13 ± 0.091	3.2 ± 0.126	4.2 ± 0.284	1.5 ± 0.137
1.2	1.16 ± 0.144	3.3 ± 0.191	4.5 ± 0.273	1.5 ± 0.144
1.5	1.25 ± 0.095	3.4 ± 0.168	4.7 ± 0.282	1.6 ± 0.143
2.0	1.29 ± 0.084	3.5 ± 0.172	5.0 ± 0.290	1.6 ± 0.115
3.0	1.33 ± 0.099	3.6 ± 0.183	5.1 ± 0.326	1.7 ± 0.138
4.5	1.35 ± 0.127	3.7 ± 0.249	5.2 ± 0.410	1.7 ± 0.151
6.5	1.37 ± 0.121	3.8 ± 0.191	5.4 ± 0.391	1.8 ± 0.164
18.0	1.38 ± 0.115	3.8 ± 0.232	5.6 ± 0.349	1.8 ± 0.153

* Trifoliate leaf next to terminal bud.
** Third trifoliate leaf from terminal bud.

bean leaves are also given in Table 3. Phosphoprotein is the fraction of the final residue extracted with 0.5 N PCA at 70°C. Increases in solution phosphorus increased the phosphorus contents of the acid-soluble phosphorus, phospholipid and phosphoprotein fractions except at the highest phosphorus level. The soybean plant appears to accumulate the greater amounts of phosphorus in these fractions at lower concentrations and to approach a greater maximum content. Since both the phospholipid and acid-soluble phosphorus fractions contain highly active metabolic intermediates, the high content present may be associated with more active metabolism and growth. The decrease of the amounts of these phosphorus fractions with 18.0 ppm of phosphorus concentration in solution could be due to excessive level.

The RNA content of separate soybean leaves is given in Table 4. RNA continued to increase with increasing phosphorus in solution, or appears to have approached a maximum with no evidence of a decrease when subjected to 18.0 ppm phosphorus. This observation may be a possible factor with non-susceptibility of the soybean plant to high phosphorus treatment. The content of RNA in leaves of the soybean plant at all concentrations of solution phosphorus is highest in the first trifoliate leaf. The RNA content is only slightly lower in the terminal bud, but much lower in the third trifoliate leaf. These analyses bear out the general idea that the RNA content is high at sites of intensive synthesis and growth. That is, RNA is high in meristematic and intensively dividing cells and in elongating tissue.

The DNA content of soybean leaves is shown in Table 5. Thc DNA fractions increased with increasing phosphorus concentration in solution. Verietal differences in the DNA content were not apparent, and there was no indication of a decrease in the soybean plant with high phosphorus. From these observations, the DNA content seems more or less independent of growth and not affected by high-phosphorus supply. Since most of DNA is present in chromosomes, and every somatic cell, regardless of its type, has the same amount of DNA in its nucleus, a slight increase in the DNA content with increasing phosphorus in solution might be due to an increase in the number of cells per unit weight.

2. The relationships among DNA, RNA, protein, plant growth and phosphorus nutrition.

The positive response of soybean yields to phosphorus up to 18.0 ppm in solution was shown in Fig. 1. As given in Fig. 1, the yield curve for phosphorus concentration in solution is in agreement with the equation of Mitsherich (1909). Oohara et al. (1968) reported that large yields of forage were produced over a 5 year period by alfalfa-orchardgrass and by ladino clover-orchardgrass mixtures where adequate phosphorus fertilizer was

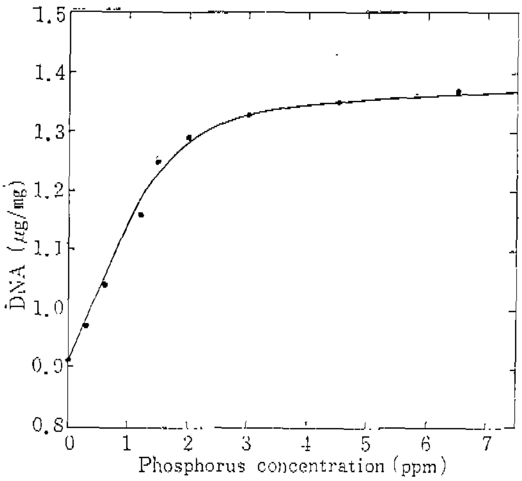

Fig. 1. The relationship between yields of the above-ground parts of the soybean plant in terms of dry weight and phosphorus concentration in solution.

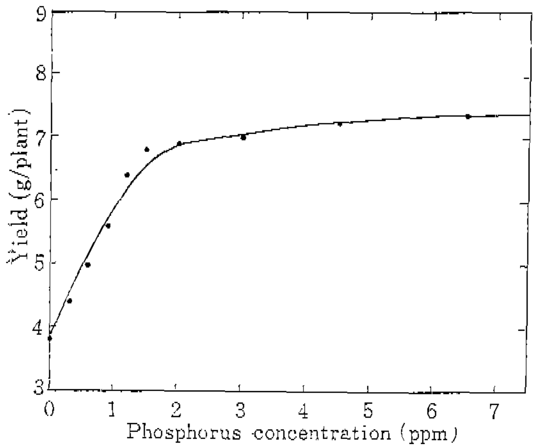

Fig. 2. The relationship between the DNA content in the above-ground parts of the soybean plant and phosphorus concentration in solution.

supplied at planting in bands below the seed. According to Howell and Bernard (1961), yields of *Glycine max* var. *lincoln* tissue in terms of dry weight were markedly decreased in high-phosphorus solutions, while for *Glycine max* var. *chief*, dry weights continued to increase with increasing phosphorus concentration. Olson, et al. (1962) showed that growth and eventually yields of some plants were greatly reduced with high phosphorus application. This negative response, referred to as phosphorus toxicity, was noted particularly with young plants. Certain species, such as soybean

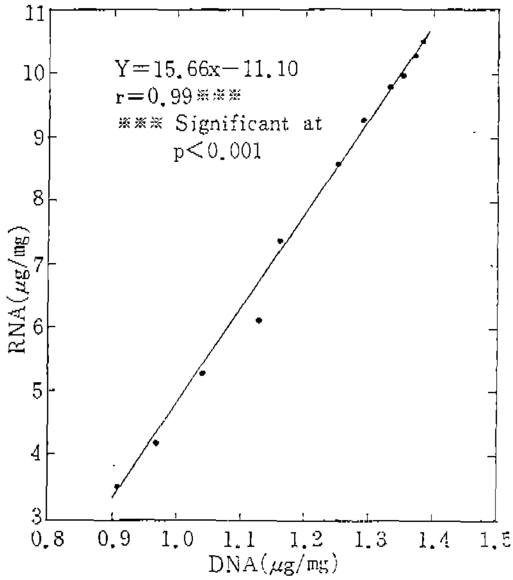

Fig. 3. The correlation between the DNA and RNA contents in the above-ground parts of the soybean plant.

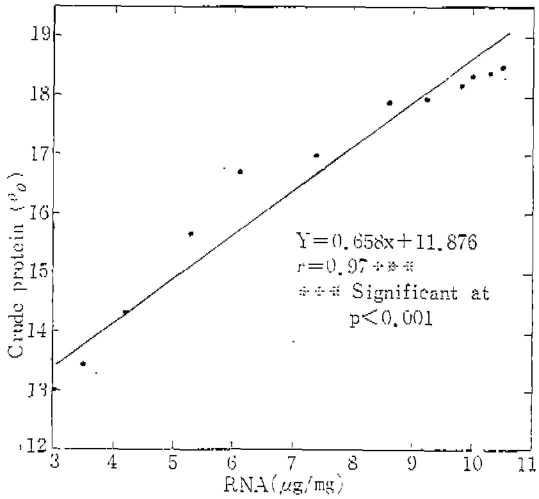

Fig. 4. The correlation between the crude protein and RNA contents in the above-ground parts of the soybean plant.

plants, showed marked varietal differences. To elucidate some phase of the mechanism by which phosphorus as one of the environmental factors affects plant growth, changes in total phosphorus, acid-soluble phosphorus, phospholipids, RNA, DNA, crude protein, and yields were considered with the soybean plant from the molecular biological view-point.

The relationship between the DNA content in

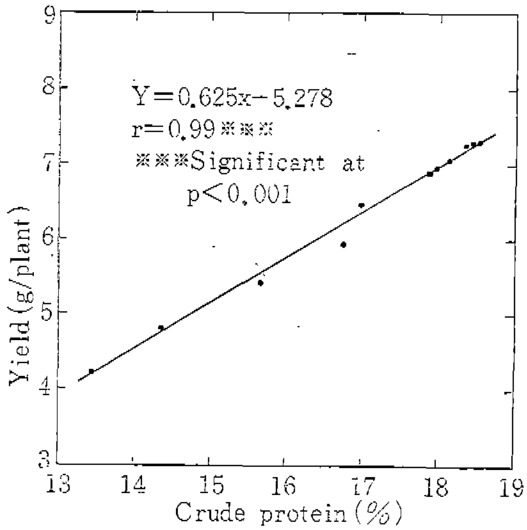

Fig. 5. The correlation between the crude protein content and dry matter yield of the above-ground parts of the soybean plant.

the above-ground parts of the soybean plant and phosphorus concentration in nutrient solution are graphed in Fig. 2. DNA increased rapidly with the increasing phosphorus concentration at first, then slowly reached a steady state in the high phosphorus concentration. A curve fit of such estimates for this sequence has a general logarithmic shape. The effect of phosphorus nutrition on DNA fractions of the soybean plant was a positive response. Chang (1973) showed that the DNA content in rye-grass leaves increased slightly with increasing nitrogen in soil solution, and there was no indication of decrease with high phosphorus. It was interesting that all phosphorus fractions but DNA in *Scenedesmus* sp. and *Pseudomonas* sp. start to decline in a phosphorus-free medium when the concentrations are expressed on a per cell basis (Rhee 1972). A marked increase in DNA would be expected because of their importance in cellular mechanisms as genetic material. An increase in the DNA content with increasing phosphorus in solution might be due to an increase in the number of cells per unit weight. From these observations, the DNA replication was affected by the increasing phosphorus concentration in solution.

The correlation between the RNA and DNA contents in the above-ground parts of the soybean plant is given in Fig. 3. The correlation coefficient between the RNA and DNA contents was 0.99, and the simple regression equation was

$$Y = 15.66x - 11.10$$

where Y is the RNA content in the above-ground parts of the soybean plant as μg per mg, and x expresses the DNA content as μg per mg. From an inspection of this result, it is no surprise that the synthesis of all three types of RNA is DNA dependent.

The above-ground parts of the soybean plant were analyzed to explore the relationship between the RNA and crude protein contents. The correlation coefficient between the RNA and crude protein contents was 0.97 and the simple regression equation was

$$Y = 0.658x + 11.876$$

where Y is the crude protein content in the above-ground parts of the soybean plant as per cent of dry weight basis and x expresses the RNA content as μg per mg. This equation means that the crude protein content increases with the increasing amount of RNA in the soybean plant. However, it is necessary to check the significance of the slope of this regression equation by using the t-test. From the t-test it can be seen that the two variables are significantly related to the RNA content of the soybean plant. This relationship suggests that RNA plays a central role in the highly regulated process of protein synthesis. As discribed above, the relationships among the DNA, RNA and protein contents can thus be explained by the central dogma which Crick (1958) has established. Therefore, genetic information in DNA is expressed through messenger RNA (m-RNA) synthesis. This process, during which a strand of DNA dictates the synthesis of a complementary strand of RAN, is transcription. The genetic message encoded by transcription in the base sequence of m-RNA is translated into a particular protein by the cell's protein synthesizing machinery (Goldsby, 1969).

The increasing dry matter yields of the above-ground parts of the soybean plant relate to the crude protein contents. The correlation between the crude protein content and growth in terms of dry weight of the soybean plant is shown in Fig. 5. The correlation coefficient between the crude protein content and dry matter yield was 0.99 and the simple regression equation is

$$Y = 0.625x - 5.278$$

where Y is the dry matter yield of the soybean plant as g per plant and x expresses the crude protein content as per cent of dry weight basis. The direct relationship between growth and the RNA content shown by Ali-Zade (1956) for tea, Bobrysheva and Oknina (1962) for currant and Chang (1973) for rye-grasses appears to hold also for the soybean plant. This best evidence suggests that plant growth results in the increase of structural, enzymatic and special proteins.

DNA in plant tissue is subject to charges caused by external environmental factors and DNA exerts its control principally by specifying the synthesis of particular protein molecules. RNA plays an important role in this highly regulated process.

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摘 要

施用燐酸量이 植物의 生長, 粗蛋白質, RNA 및 DNA의 含量에미치는 影響을 밝히기 위하여 *Glycine max* Merr.를 材料로 實驗을 行한 結果 燐酸의 施用量이 增加할때 生産量, 粗蛋白質, RNA 및 DNA의 植物體含量이 增加하며 RNA와 粗蛋白質의 含量은 生長이 가장왕성한 잎에서 가장 높았다.

DNA RNA, 粗蛋白質 및 生長間의 關係는 Central dogma에 입각하며 DNA의 含量은 施用燐酸量이 增加함에 따라 增加하나 그 增加比는 漸減한다.

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