

Effects of Nitrogen Nutrient on the Yield, Protein, Amino acid, Chlorophyll, Carotene, RNA, and DNA Contents in Rye-Grasses

Chang, Nam Kee

(Dept. of Biology, College of Education, Seoul National University)

Rye-grass 類의 物質生産, 蛋白質, amino 酸, 葉綠素, Carotene, RNA 및 DNA 의 含量에 미치는 窒素의 影響

張 楠 基

(서울大學校 師範大學 生物學科)

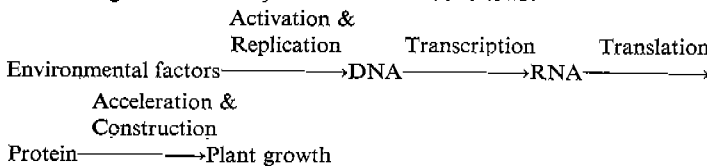
ABSTRACT

To study the response to plant growth by the environmental factors, the effects of application of nitrogen on changes in the yield, crude protein, amino acids, chlorophyll, carotene, total phosphorus, acid-soluble phosphorus, phospholipids, RNA, and DNA were investigated with westerworlds (*Lolium sublatum*) and perennial rye-grasses (*Lolium perenne*).

The amounts of dry weight, crude protein, amino acids, chlorophyll, carotene, total phosphorus, acid-soluble phosphorus, phospholipids, RNA and DNA of both rye-grasses increased with adequately increasing nitrogen, and reached a maximum with an adequate application of nitrogen.

The relationships between yields and crude protein contents, crude protein and RNA contents, and yields and RNA contents of westerworlds and perennial rye-grasses were found to be positively correlated, respectively.

Therefore, in general, the response to plant growth by the environmental factors such as nitrogen nutrient may be summarized as follows:



INTRODUCTION

The effect of application of fertilizer phosphorus on the content of DNA, RNA and other phosphorus fractions in an early cutting stage of growth of alfalfa and orchard-grass has been described by Chang (1972). From the molecular biological point of view, the most important result is the direct relationship between the nucleic acid content and plant growth. It suggests that nucleic acids in plant cells are subject to changes caused by external environmental factors. Ohlrogge et al.

(1957) demonstrated the unique effect of nitrogen to enhance plant uptake of fertilizer phosphorus. No data are available, however, in which any attempt is made to relate nucleic acid contents to the conditions of soil nitrogen. The present work attempts to do this for the field experiment including a range of application of fertilizer nitrogen which have subsequently been used for a study of the yield, protein, amino acid, chlorophyll, carotene, RNA, and DNA responses by introduced rye-grasses.

MATERIALS AND METHODS

This research was made possible by the Obihiro Zootechnical University, Obihiro. The Obihiro district is situated almost in the south-east center of Hokkaido, Japan (N 42°9', E 143°2'). The climate is characterized by short, cool and moist summer, a frost-free season of 120–140 days, and long cold winter. Snow cover may exceed 120 days and annual precipitation ranges between 1000–1200mm. Soils were derived from volcanic ash, contained about 10% organic matter, had soil pH of 5.75–6.55 in the surface and 6.00–6.75 in the subsoil, and were deficient in amounts of available nitrogen, phosphorus, potassium and magnesium.

Westerworlds rye-grass and perennial rye-grass were chosen for this experiment. On 10, May 1970, westerworlds (3kg/10a) and perennial (3kg/10a) rye-grasses were sowed directly above precision bands of 0–20–8 NPK supplying from 0 to 22.5 kg/10a of nitrogen and then were disked. Plot size was 1.65 × 9.144m (5 × 30ft) with 3 replication. After westerworlds and perennial rye-grasses had grown for 68 days from planting and for 56 days from the first cutting, these forage crops were harvested, dried at 60°C, weighed, and ground for analyses, respectively.

Determinations of yields of rye-grasses were made of dry weight of the above-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 contents of chlorophyll and carotene were determined by spectrophotometry. Rye-grasses from the main experiment were analysed for amino acids by Amino Acid Analyzer with sequential sampler (Hitachi Model KLA-5).

The above-ground parts of westerworlds and perennial rye-grasses were frozen immediately with liquid nitrogen, lyophilized, and analysed for DNA, RNA and other phosphorus fractions. Since there was not a standard methods available for determining nucleic acids in plant tissue, a workable procedure for estimating RNA and DNA

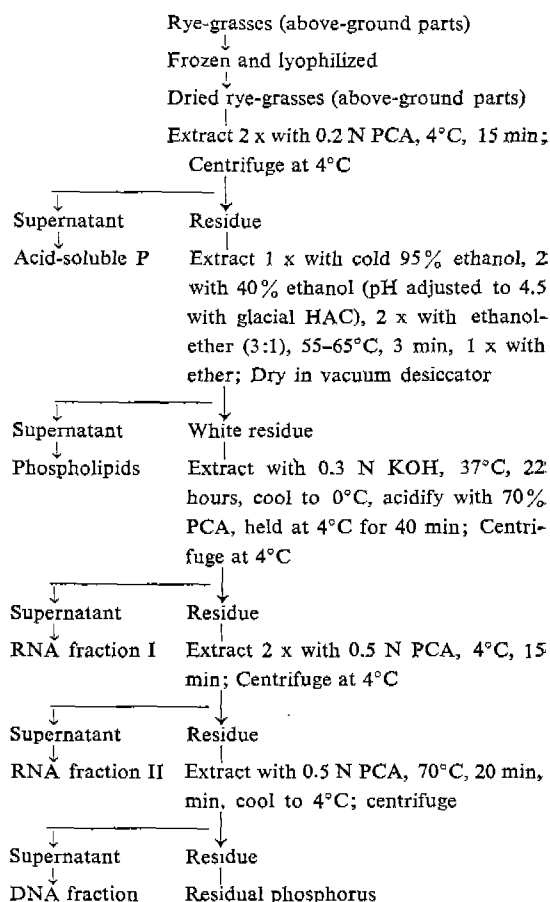


Fig. 1. Procedure for the extraction of the nucleic acids from the above-ground parts of rye-grasses.

in rye-grasses was developed from previous methods (Markham, 1955; Nieman & Poulsen, 1963; Ogur & Rosen, 1950; Schmidt & Thannhauser, 1945; Smillie & Krotker, 1960; Spirin, 1958). Procedure adopted is shown in Fig. 1. For complete extraction of RNA from rye-grasses, a modification of Schmidt and Thannhauser's alkaline hydrolysis (1945) using potassium hydroxide and subsequent cold perchloric acid (PCA) extraction was necessary. The two RNA fractions were combined before analysis. For removal of the DNA fraction, Ogur and Rosen's procedure (1950) proved to be satisfactory. RNA and DNA were determined at 260 and 268 m μ , respectively, using a DU spectrophotometer. Phosphorus content of the above-ground parts of rye-grasses was determined for acid-soluble phosphorus (phosphate esters) and

phospholipids using the phosphomolybdate-acetone complex method modified by Hirada & Appleman(1959). This method made it possible to determine phosphorus in the range of 1 to 10 μ g with a high degree of accuracy and precision. Total phosphorus concentration in rye-grasses was measured by the conventional vanadomolybdophosphoric acid(Kitson & Mellon, 1944) procedure.

RESULTS AND DISCUSSION

The effect of nitrogen on dry matter yields of westerworlds and perennial rye-grasses in the first and second cuttings is shown in Table 1. For westerworlds rye-grass in the first cutting, yields in terms of dry weight increased to a maximum with an application of 20 kg N/10a, but decreased with 22.5 kg N/10a. As application of fertilizer nitrogen increased to 12.5 kg N/10a, yields of

westerworlds rye-grass in the second cutting were markedly increased and showed a maximum value at 12.5 kg N/10a. Dry weight of perennial rye-grass in the first cutting continued to increase with increasing nitrogen application, while as application of nitrogen went up to 20 kg N/10a, yields of perennial rye-grass in the second cutting reached a maximum with 20 kg N/10a but decreased with 22.5 kg N/10a. Total yields of rye-grasses in the first and second cuttings were markedly increased with increasing nitrogen application but reduced with excessive nitrogen supply. These results suggest that generally, nitrogen has a high positive response to the yield of rye-grasses but high nitrogen levels in soils may have depressed organic matter production and the regrowth. Fukunaga (1967) reported that in westerworlds rye-grass, 1 ton/10a or more as dry matter was produced by

Table 1. Yields of westerworlds and perennial rye-grasses in terms of dry weight in the first and second cuttings (kg/10a)

Amount of N applied (kg N/10a)	Westerworlds rye-grass			Perennial rye-grass		
	1st cutting	2nd cutting	Total yield	1st cutting	2nd cutting	Total yield
0	173	148	322	146	115	216
2.5	267	231	498	219	141	360
5	385	298	683	250	209	459
7.5	489	413	902	286	327	613
10	592	564	1156	325	413	738
12.5	610	576	1186	327	434	761
15	618	573	1191	343	437	780
17.5	631	571	1202	345	441	786
20	639	570	1209	347	445	792
22.5	635	550	1185	359	438	797

Table 2. Crude protein contents of westerworlds and perennial rye-grasses in the first and second cuttings (%)

Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	7.91	7.00	8.36	9.20
2.5	8.97	8.13	9.01	9.94
5	11.85	10.67	11.98	11.99
7.5	12.89	11.21	12.44	12.57
10	12.99	13.69	13.09	14.51
12.5	13.75	13.86	14.11	14.66
15	14.30	14.65	15.55	15.93
17.5	14.82	14.98	15.82	16.89
20	15.00	15.16	16.67	16.96
22.5	14.93	15.12	15.63	16.56

Table 3. Changes in amino acid contents of westerworlds rye-grass in the first and second cuttings(%)

Amount of N applied (kg N/10a)	0		5		10		15	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Crude protein	7.91	7.00	11.85	10.67	12.99	13.69	14.30	14.65
Lysine	0.39	0.35	0.67	0.54	0.77	0.79	0.92	0.90
Histidine	0.22	0.20	0.31	0.30	0.32	0.31	0.33	0.34
Arginine	0.30	0.27	0.62	0.57	0.63	0.64	0.60	0.65
Aspartic acid	0.55	0.51	0.93	0.83	1.17	1.19	1.18	1.20
Threonine	0.25	0.26	0.47	0.44	0.50	0.51	0.57	0.56
Serine	0.27	0.23	0.56	0.51	0.58	0.59	0.62	0.63
Glutamic acid	0.54	0.49	1.07	0.99	1.19	1.17	1.46	1.45
Proline	0.40	0.35	0.68	0.67	0.70	0.73	0.83	0.85
Glycine	0.28	0.24	0.53	0.52	0.59	0.58	0.71	0.72
Alanine	0.33	0.32	0.60	0.58	0.69	0.71	0.86	0.88
Valine	0.20	0.16	0.59	0.55	0.61	0.58	0.69	0.67
Methionine	0.02	0.01	0.04	0.03	0.05	0.06	0.08	0.08
Isoleucine	0.19	0.16	0.33	0.30	0.34	0.37	0.42	0.43
Leucine	0.48	0.40	1.02	0.98	1.03	1.04	1.15	1.17
Tyrosine	0.17	0.15	0.32	0.31	0.33	0.35	0.37	0.38
Phenylalanine	0.36	0.33	0.70	0.68	0.75	0.73	0.62	0.62
Total	4.95	4.43	9.44	10.25	10.35	10.35	11.41	11.60

Table 4. Changes in amino acid contents of perennial rye-grass in the first and second cuttings(%)

Amount of N applied(kg N/10a)	0		5		10		15	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Crude protein	8.36	9.20	11.98	11.99	13.09	14.51	15.55	15.93
Lysine	0.56	0.58	0.52	0.50	0.58	0.60	0.98	0.97
Histidine	0.18	0.20	0.19	0.18	0.20	0.21	0.39	0.40
Arginine	0.42	0.43	0.52	0.53	0.56	0.57	0.60	0.61
Aspartic acid	0.60	0.61	1.11	1.14	1.26	1.28	1.74	1.76
Threonine	0.19	0.18	0.35	0.33	0.52	0.54	0.51	0.55
Serine	0.36	0.38	0.41	0.41	0.58	0.61	0.57	0.57
Glutamic acid	0.55	0.56	1.20	1.17	1.23	1.25	1.29	1.30
Proline	0.26	0.25	0.68	0.65	0.87	0.89	0.89	0.91
Glycine	0.27	0.30	0.48	0.48	0.64	0.65	0.70	0.72
Alanine	0.29	0.30	0.73	0.74	0.90	0.93	1.02	1.04
Valine	0.34	0.35	0.51	0.50	0.56	0.55	0.80	0.80
Methionine	0.01	0.02	0.05	0.05	0.17	0.18	0.12	0.19
Isoleucine	0.20	0.23	0.41	0.40	0.43	0.45	0.49	0.50
Leucine	0.41	0.42	0.90	0.85	1.03	1.07	1.24	1.26
Tyrosine	0.08	0.10	0.18	0.17	0.31	0.32	0.22	0.33
Phenylalanine	0.42	0.40	0.44	0.42	0.56	0.58	0.59	0.60
Total	5.14	5.31	8.68	8.52	10.40	10.68	12.15	2.51

the application of nitrogen amounting to 10 kg/10a or more. Maeno and Ehara(1970) showed that there was high correlation between the amount of

nitrogen and total available carbohydrate in the stubble at the time of defoliation, and the regrowth of herbage plants. Thus when less than the opti-

Table 5. Chlorophyll contents of westerworlds and perennial rye-grasses in the first and second cuttings(%*ee*)

Amount of N applied (kg N/10a)	Westerworlds rye-grass				Perennial rye-grass			
	1st cutting		2nd cutting		1st cutting		2nd cutting	
	Chl. a	Chl. b	Chl. a	Chl. b	Chl. a	Chl. b	Chl. a	Chl. b
0	3.41	1.48	3.39	1.45	3.60	1.53	3.62	1.54
2.5	3.54	1.50	3.51	1.48	3.75	1.59	3.73	1.60
5	3.63	1.53	3.64	1.52	3.81	1.62	3.80	1.64
7.5	3.78	1.61	3.91	1.60	3.97	1.75	3.99	1.78
10	3.90	1.65	4.00	1.65	4.08	1.89	4.10	1.90
12.5	3.97	1.82	4.08	1.73	4.12	1.90	4.15	1.89
15	4.08	1.91	4.12	1.79	4.43	2.01	4.39	2.05
17.5	4.11	1.94	4.17	1.83	4.59	2.04	4.60	2.08
20	4.15	1.99	4.20	1.87	4.68	2.17	4.71	2.13
22.5	4.21	2.00	4.22	1.90	4.72	2.19	4.76	2.21

imum amount of nitrogen was applied, the regrowth of forage crops increased as the yield response to applied nitrogen. According to the result of "t" test, yields of westerworlds rye-grass had highly significant difference between the first and second cuttings. It is obvious that dry matter yield of westerworlds rye-grass in the first cutting was higher than that in the second cutting. However, application of 0-5 kg N/10a brought a large decrease in the regrowth of perennial rye-grass. As compared with the first cutting, increasing yields of perennial rye-grass in the second cutting were obtained from nitrogen supply of 7.5-22.5 kg/10a.

As shown in Table 2, increases in nitrogen application increased the crude protein content in both rye-grasses in the first and second cuttings except for the highest nitrogen level. The content of crude protein was higher with perennial rye-grass at 5% significant level than with westerworlds rye-grass. This is similar to the total nitrogen content which was higher with perennial rye-grass than with westerworlds rye-grass. The crude protein contents of westerworlds rye-grass in the first cutting was similar to the second cutting, while perennial rye-grass in the second cutting contained higher amounts of crude protein in comparison with the first cutting. The positive response of the crude protein content in rye grasses to applied nitrogen was all plots of 2.5-22.5 kg N/10a but a maximum content of crude

protein in rye-grasses was obtained from 20 kg N/10a. This is in agreement with the results of Fukunaga(1967).

Rye-grass plots of 0, 5, 10 and 15 kg N/10a were chosen for analysis of amino acids because of their distinctive differences in response to increasing nitrogen application (Tables 3 and 4). In the case of westerworlds rye-grass, contents of aspartic acid, glutamic acid, leucine and lysine were higher than those of other amino acids, while perennial rye-grass contained greater amounts of aspartic acid, lysine, glutamic acid, arginine, phenylalanine and leucine in comparison with other amino acids. Amino acid contents of westerworlds and perennial rye-grasses increased with increasing nitrogen application. This result suggests that the increasing amounts of amino acids in the cell of rye-grasses promote protein synthesis. In addition, it is clear that increase of amino acids such as glycine, glutamic acid and aspartic acid leads to formation of the increasing nitrogenous bases which will be utilized for nucleotides and deoxynucleotide formations, and enhances the synthesis of porphobilinogen to form chlorophyll.

As given in Tables 5 and 6, the amount of chlorophyll a and b was higher with perennial rye-grass than with westerworlds rye-grass, while carotene contents have no significant difference between both rye-grasses. There are also no significant differences of the contents of chlorophyll and carotene in both rye-grasses between the first

Table 9. RNA contents of westerworlds and perennial rye-grasses in the first and second cuttings (ug N/mg)

Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	1.85	1.72	1.90	1.87
2.5	2.57	2.41	3.09	2.96
5	4.20	4.15	5.12	4.95
7.5	6.99	6.80	8.79	8.53
10	8.53	8.30	10.58	10.60
12.5	10.86	10.53	11.14	11.21
15	11.35	11.09	12.95	13.00
17.5	13.11	13.01	13.98	14.01
20	14.04	13.18	14.17	14.16
22.5	12.72	12.22	14.20	14.13

soluble phosphorus and phospholipid fractions contain highly active metabolic intermediates, the high content present may be associated with more active metabolism and growth. The decrease with 22.5 kg N/10a could be due to excessive nitrogen. As shown in Table 7, according to the tendency for these phosphorus fractions, total phosphorus contents of both rye-grasses increased to a maximum with 20 kg N/10a except for the plot of 22.5 kg N/10a of westerworlds rye-grass in the first cutting but decreased with 22.5 kg N/10a. It suggests that the unique effect of nitrogen nutrient enhances plant uptake of fertilizer phosphorus. This agrees with the results of Ohlogge et al. (1957).

As given in Table 10, the amounts of RNA of perennial rye-grass at all levels of nitrogen applications were higher than those of westerworlds rye-grass. As compared with the cutting stages, the content of RNA was higher at the plots of westerworlds rye-grass in the first cutting, but for the RNA contents of perennial rye-grass, there was no significant difference between the first and second cuttings. For westerworlds rye-grass in the first and second cuttings, RNA increased to a maximum with 20 kg N/10a, but decreased with 22.5 kg N/10a. The RNA contents of perennial rye-grass in the first cutting continued to increase with increased nitrogen application,

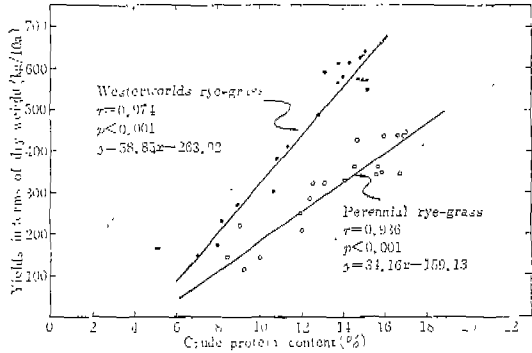


Fig. 2. Relationship between the yields in terms of dry weight and the contents of crude protein in the above-ground parts of rye grasses.

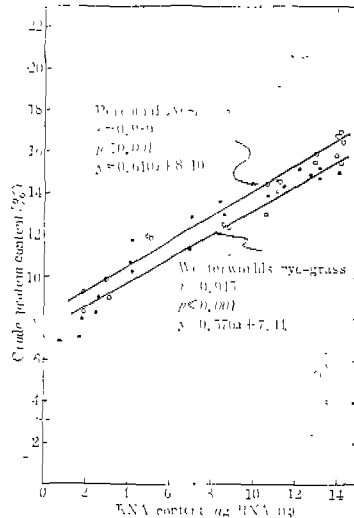


Fig. 3. Relationship between the crude protein and RNA contents in the above-ground parts of rye-grasses.

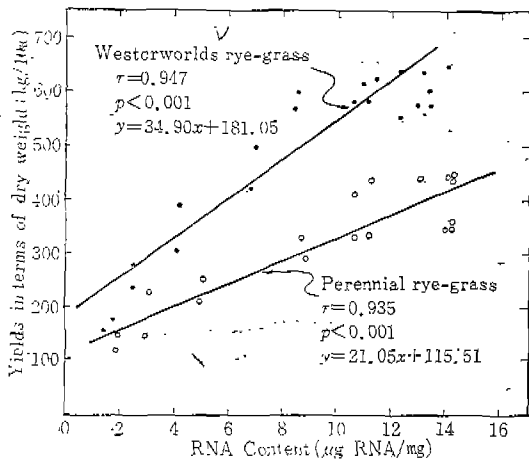


Fig. 4. Relationship between yields in terms of dry weight and RNA contents in the above-ground parts of rye-grasses.

Table 6. Carotene contents of westerworlds and perennial rye-grasses in the first and second cuttings (%)

Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	0.355	0.334	0.365	0.371
2.5	0.410	0.406	0.437	0.443
5	0.525	0.503	0.550	0.537
7.5	0.682	0.623	0.679	0.689
10	0.633	0.735	0.767	0.750
12.5	0.797	0.788	0.784	0.797
15	0.844	0.853	0.821	0.846
17.5	0.896	0.893	0.883	0.899
20	0.958	0.964	0.936	0.943
22.5	0.969	0.970	0.981	0.959

and second cuttings. For both rye-grasses, chlorophyll and carotene continued to increase with increasing nitrogen in soils, or appears to have approached a maximum with no evidence of a decrease when subjected to 22.5 kg N/10a. Fukunaga(1967) reported that carotene content was higher with the top-dressing plot of nitrogen than with the non-topdressing plot, while carotene contents in rye-grasses were mostly parallel to crude protein contents. Lee and Lee(1968) reported that when *Chlorella* cells were grown in a Mg-free medium, the contents of phosphate in the DNA protein, RNA-polyphosphate complex, nucleotidic-labile P, and PCA-soluble fractions decreased

Table 7. Total phosphorus contents of westerworlds and perennial rye-grasses in the first and second cuttings ($\mu\text{gP}/\text{mg}$)

Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	2.24	2.20	2.34	2.33
2.5	2.81	2.79	3.10	3.07
5	3.40	3.35	3.85	3.80
7.5	3.99	3.76	4.49	4.51
10	4.55	4.27	4.99	5.04
12.5	4.87	4.65	5.41	5.48
15	5.12	5.02	5.90	5.91
17.5	6.03	5.39	6.18	6.26
20	6.14	5.91	6.25	6.30
22.5	6.15	5.80	6.21	6.24

as compared with those of the control.

It is shown that perennial rye-grass absorbs more phosphorus than westerworlds rye-grass (Table 7). The lower molecular weight phosphorus fractions found in westerworlds and perennial rye-grasses are shown in Tables 8 and 9. The acid-soluble phosphorus fractions extracted with 0.2 N PCA at 4°C includes inorganic phosphate, sugar phosphates, various free nucleotides, phosphoglyceric acid, thiamine pyrophosphate, and phosphoryl choline. Increase in application of nitrogen increased the phosphorus contents of the acid-soluble fractions but application of 22.5 kg N/10a decreased these contents in perennial rye-grass in the second cutting. Phospholipids are the fractions soluble in lipid solvents(alcohol and ether), and include phosphatides. For rye-grasses in the first and second cuttings, the amounts of phospholipids increased to a maximum with an application of 20 kg N/10a, but decreased with 22.5 kg N/10a. The contents of the acid-soluble phosphorus and phospholipid fractions were higher with perennial rye-grass than with westerworlds rye-grass. Both rye-grasses in the first cutting appear to accumulate greater amounts of phosphorus in both fractions at low applications of nitrogen than those in the second cutting. However, these forage crops were more adversely affected by high nitrogen. Because both the acid-

Table 8. Phospholipid contents of westerworlds and perennial rye-grasses in the first and second cuttings($\mu\text{g P}/\text{mg}$)

Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	0.30	0.27	0.33	0.33
2.5	0.46	0.45	0.48	0.45
5	0.50	0.49	0.53	0.51
7.5	0.55	0.57	0.61	0.64
10	0.62	0.60	0.72	0.79
12.5	0.70	0.68	0.83	0.87
15	0.84	0.81	0.96	0.98
17.5	0.95	0.93	1.02	1.06
20	1.06	1.01	1.10	1.13
22.5	1.00	0.99	1.05	1.12

while in the second cutting, these contents increased to a maximum with 20 kg N/10a but

Table 10. DNA contents of westerworlds and perennial rye-grasses in the first and second cuttings ($\mu\text{g DNA/mg}$)

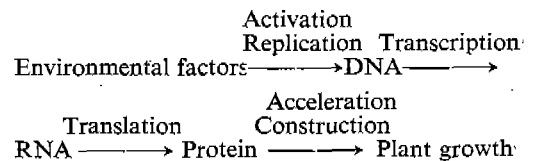
Amount of N applied (kg N/10a)	Westerworlds rye-grass		Perennial rye-grass	
	1st cutting	2nd cutting	1st cutting	2nd cutting
0	0.51	0.45	0.58	0.56
2.5	0.57	0.48	0.63	0.60
5	0.64	0.59	0.75	0.74
7.5	0.78	0.62	0.84	0.88
10	0.85	0.75	0.97	1.00
12.5	0.96	0.83	1.03	1.06
15	1.13	1.04	1.19	1.25
17.5	1.20	1.18	1.27	1.29
20	1.28	1.23	1.31	1.34
22.5	1.30	1.27	1.33	1.35

decreased with 22.5 kg N/10a. It suggests that there is the relationship between RNA contents and plant growth.

According to the result of this investigation, the author has found the correlation between RNA and plant growth. The correlation coefficients between yields, in terms of weighed growth, and crude protein contents, and crude protein and RNA contents in the above-ground parts of westerworlds and perennial rye-grasses were 0.974, 0.936, 0.989 and 0.945, respectively. This result shows that RNA plays an important role in protein synthesis, cell division, cell formation, tissue differentiation and plant growth. As shown in Fig. 4, the correlation coefficients between yields and RNA contents of westerworlds and perennial rye-grasses were 0.947 and 0.935, respectively. These simple regression equations were given in Figs 2, 3 and 4. However, West(1962) has suggested that RNA is accumulated under reduced growth conditions with corn. The direct relationship between growth and the RNA content shown by Alie-Zade(1959) for tea, Bobryshéva and Oknina (1962) for currant, and Chang(1972) for alfalfa and orchard-grass appears to hold also for westerworlds and perennial rye-grasses.

As shown in Table 11, DNA increased with

increasing nitrogen application. Varietal differences: the ni DNA contents of both rye grasses in the first and second cuttings were apparent, and there was no indication of a decrease in either rye-grass with high nitrogen application. From these observations, the DNA content seems more or less independent of growth, not affected by high nitrogen supply. Since 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 nitrogen in soils might be due to an increase in the number of cells per unit weight. However, it is sure that the DNA activity in the plant increases with the increasing nitrogen concentration in soils. Furthermore, RNA synthesis does not occur without the presence of template DNA. DNA exerts its control principally by specifying the synthesis of particular protein molecules. RNA plays a central role in this highly regulated process. The overall pattern of relationship between the environmental factors and plant growth may be schemalized as follows:



ACKNOWLEDGMENTS

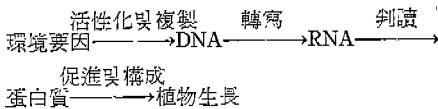
The author wishes to thank the following gentlemen for their encouragement and guidance in this work: Professor Hisatomo Oohara for his long-suffering supervision; Professor Norihito Yoshida for his helpful advice; the members of Dept. of Grassland Science, Obihiro Zootechnical University, who provided assistance and special facilities. This work was carried out whilst the author held a Japanese Government(Mombusho) Scholarship Student at Obihiro Zootechnical University, Hokkaido, Japan.

摘 要

窒素의 施用에 따른 植物의 生長効果에 대한 機構을 Westerworlds rye-grass 와 perennial rye-grass 를 材料로 物質의 生産, 粗蛋白質, amino酸, 葉綠素, carotene,

全磷, 酸可溶性磷, 磷脂質, RNA 및 DNA의 量的 變化를 研究한 結果를 要約하면 다음과 같다.

1. Westerworlds rye-grass의 一次刈取時에는 窒素의 施用量 增加에 따라 生産量도 增加하여 20kgN/10a의 施用區에서 最高에 이르고 二次刈取時에는 12.5kgN/10 a 區에서 最高에 달하였다.
한편 perennial rye-grass의 一次刈取時에는 窒素 施用量の 增加에 따라 生産量도 계속 增加하나 二次 刈取時에는 20kgN/10a의 施用區에서 最高에 달하고 22.5kgN/10a의 施用區에서 減少하기 始作하였다.
2. 粗蛋白質의 含量은 Westerworlds rye-grass 區에 比하여 perennial rye-grass 區에서 높았고 窒素 20kgN /10a 施用區에서 最高에 이르고 22.5kgN/10a 에서는 減少하였다.
3. Westerworlds rye-grass에 있어서는 amino산 중 aspartic acid, glutamic acid, leucine, proline 과 lysine의 含量이 높았고 perennial rye-grass에 있어서는 aspartic acid, lysine, glutamic acid, arginine, phenylalanine 및 leusine의 含量이 높았다.
4. 이들 rye-grass의 葉綠素와 carotene의 含量은 窒素의 施用量 增加에 따라 增加하였다.
5. 全磷含量은 窒素施用量에 따라 增加하나 22.5kgN/10a의 施用區에서는 減少하였다. 다만 Westerworlds rye-grass의 一次刈取時 增加를 계속하였다.
6. 酸可溶性磷의 含量은 窒素施用量에 따라 增加하였으나 perennial rye-grass의 二次刈取時에 한하여 20kgN/10a 區에서 最高에 이르렀다.
7. 이들 rye-grass에 含有된 磷脂質의 量은 窒素의 施用量 增加에 따라 增加하여 20kgN/10a의 施用區에서 最高에 달하고 22.5kgN/10a 區에서 減少하였다.
8. RNA의 含量은 增加하는 窒素施用量에 따라 增加하여 20kgN/10a 區에서 가장 높았고 22.5kgN/10a 區에서 減少하나 perennial rye-grass의 一次刈取時의 경우에는 減少하지 않았다.
9. DNA의 含量은 窒素의 施用量에 따라 감소함이 없이 增加하였다.
10. 이들 rye-grass의 物質生産과 粗蛋白質과 RNA의 含量, 物質生産과 RNA 含量間에는 各各 正相關係가 存在한다.
11. 따라서 環境要因으로서의 窒素施用量에 對한 植物의 生長効果는 다음과 같이 要約된다.



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(Received March 30, 1973)