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EFFECT OF UREA NITROGEN ON THE METABOLISM OF PLANTS (Ⅲ)

On the change of the chlorophyll content in sunflower
leaves after the urea foliar spray.

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金俊鎬：植物의 代謝에 미치는 尿素·窒素의 影響 (Ⅲ)

해바라기에 葉面散布한 後의 葉綠素含量的 變化에 對하여

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ABSTRACT

KIM, Joon Ho (Dept. of Biology, Kongju Teacher's College) Effect of urea nitrogen on the metabolism of plants (Ⅲ). On the change of the chlorophyll content in sunflower leaves after the urea foliar spray. *Kor. Jour. Bot.* V(4): 6-10, 1962.

Leaf samples, raised on the N-deficient and N-abundant sand and sprayed with varying concentration of urea, were analyzed for their total chlorophyll concentration.

It was observed that the depression periods of the chlorophyll content appeared at first by spraying with urea; it appeared on the 3rd day in the N-deficient plots and on the 6th day in the N-abundant plots. Causes of the depression of chlorophyll may be assumed to be in an excessive urea, an accumulation of ammonia from urea absorbed, and depression of water content owing to urea application.

The maximum content of the chlorophyll was shown on the 6th day in N-deficient and on the 12th day in the N-abundant plots.

The young leaves actively formed the chlorophyll by urea foliar spray, compared with the mature ones. This result was consistent with previous paper⁽⁶⁾.

INTRODUCTION

Since nitrogen is an essential component of the chlorophyll molecule, any appreciable lack of it in the nutrient supplying the plant should result in a suppression of chlorophyll formation.

According to Schertz⁽⁹⁾, Ville in 1889 was probably the first to note a definite relationship between supply and chlorophyll formation. The color of the plants observed varied according to the amount of nitrogen applied. Many^(3,9,10,11) have studied the change of the chlorophyll content after nitrogen salts being applied to the plants. Other^(4,7) observed that foliar application increase the content of chlorophyll more rapidly than the soil application, but they did not determine the chlorophyll content. In the previous paper the author studied with sunflower leaves after urea foliar spray, it was

determined that 80% of alcohol soluble nitrogen contained partly the chlorophyll and partly other nitrogenous compounds, and it was very interesting to observe the contents vary. The author has never seen any known references dealing with determining of the variation of the pure chlorophyll content when the urea spray being applied on the green leaves.

The purpose of this paper is to show the relationships between the chlorophyll content and various concentrations of urea nitrogen existing in leaves of sunflower plants after urea nitrogen is sprayed on the surface of leaves.

MATERIAL AND METHOD

Total chlorophyll determination was made on sunflower leaf, it was cultivated essentially the same as in the previous paper ⁽⁵⁾ except the following: The concentrations of urea sprayed were 0.2 mol. and 0.3 mol, and spraying was repeated three times every evening. The material leaves were picked in the evening on three day or six day intervals. The sampling was made before the first spray. The sample leaves were eripped with 4 square centimeter's punch and gathered. Accordingly, 100 cm² of 4 cm² leaf pieces (about 20 gm. fresh weight) were used to determine the chlorophyll. On the other hand, 100 cm² of those pieces were used to weigh the fresh weight and dried at 105°C. until they became constant dry matter, reweighed dry weight.

The method used in the extraction and separation of chlorophyll from other plant pigments was that of Willstätter and Stoll as modified by Schertz ⁽⁶⁾. Care was taken to grind the sample for a long period of time. Subsequent to extraction, saponification, and separation, the chlorophyllins were run into a 50 ml. volumetric flask and made to volume with distilled water. Determtinaions were made by comparison, in a Duboucq colorimeter, with the artificial color standard developed by Guthrie.

RESULTS

A. Effect of urea foliar application of the chlorophyll content in the nitrogenous deficient leaves. The chlorophyll contents of sunflower leaves shown in Table 1 were the results from the samples raised in the N-deficient sand. The samples used here were not divided into the mature leaves and

Table 1. The effect of urea foliar spray on the chlorophyll content in sunflower leaves which were raised on the N-deficient sand.

Urea concentration	Sampling days				
	0	3	6	9	10
	mg. per g. dry weight basis				
control	18.77	15.47	14.00	13.24	12.13
0.2 mol	—	15.60	16.49	15.23	13.03
0.3 mol	—	15.92	18.02	15.55	14.33
	mg. per g. fresh weight basis				
control	2.82	2.35	2.10	1.99	1.82
0.2 mol	—	2.39	2.47	2.28	2.10
0.3 mol	—	2.43	2.70	2.33	2.21
	mg. per 100 square cm.				
control	5.79	4.54	4.29	4.00	3.51
0.2 mol	—	4.36	4.77	4.60	3.78
0.3 mol	—	4.42	5.02	4.33	4.11

the young ones. The chlorophyll concentration was again calculated in three ways; on the fresh, dry weight and on the 100 cm² basis, because the leaf of each sample was different in its thickness and water content.

Table 1 shows that the chlorophyll contents of the 3rd day samples were decreased by the treatment of urea rather than that of the zero day same of the control. Both 0.2 and 0.3 mol urea treated samples reached the maximum content of chlorophyll on the 6th day. The former was increased as much as 17.7% and the latter 28.5% compared with the control plot on the 6th day. Thereafter the chlorophyll content of both samples was diminished. With the increase of the urea concentration the chlorophyll contents were proportionate to it until these reached the maximum content of chlorophyll, on the 6th day.

B. Effect of urea foliar application on the chlorophyll content of the mature and the young leaves. The materials in this experiment were cultivated in the N-deficient sand. The mature and the young leaves were separated according to the previous paper⁽⁵⁾.

Table 2. The effect of urea foliar spray on the chlorophyll content in the young leaves and the mature ones of sunflower. These materials were made on the N-deficient sand.

Basis of expression days	mg./g. dry wt.			mg./g. fresh wt.			mg./100 cm ² .		
	0	6	12	0	6	12	0	6	12
	mature leaves								
control	17.17	15.10	12.93	2.49	2.27	1.94	5.33	4.11	3.58
0.2 mol	—	18.39	13.57	—	2.76	2.24	—	5.32	4.72
0.3 mol	—	17.17	15.33	—	2.58	2.30	—	5.52	4.46
	young leaves								
control	16.83	16.86	15.89	2.53	2.53	2.28	5.05	4.80	4.52
0.2 mol	—	20.83	17.40	—	3.12	2.61	—	6.07	5.11
0.3 mol	—	18.56	17.47	—	2.78	2.62	—	5.25	5.13

The results of the determination of chlorophyll concentration on dry, fresh weight and area basis are shown in Table 1. As shown on the data of the experiment A, the mature and the young leaves reached the maximum content of chlorophyll on the 6th day of the spray of urea. The mature leaves on the dry basis contained 21.7% more of the chlorophyll in 0.2 mol, 13.7% more in 0.3 mol and the young leaves 23.6% more of the chlorophyll in 0.2 mol, 10% more in 0.3 mol than those of control plots.

It was shown that the chlorophyll content, both in the mature and the young, increased by spraying 0.2 mol of urea rather than by 0.3 mol on the 6th day when the maximum content of it reached except in the mature leaves on area basis, while those mentioned above were reciprocal on the 12th day (Table 2).

C. Effect of urea foliar application of the chlorophyll content in the nitrogenous abundant plants. Samples used here were raised on the nitrogenous abundant soil according to the previous paper⁽⁵⁾, but were not divided into 2 groups of the mature and the young leaves.

As shown in Table 3, the chlorophyll contents of urea treated samples, unlike those of Experiment A, showed a decrease on the 6th day samples. On this day, having the minimum content of it, the 0.2 mol sample was decreased as low as 7.6% and the 0.3 mol as low as 9.4% compared with those of the control. While on the 12th day the content of chlorophyll of both samples increased much more

Table 3. The effect of urea foliar spray on the chlorophyll content in sunflower leaves which were raised on the N-abundant sand.

Basis of expression days	mg./g. dry wt.			mg./g. fresh wt.			mg./100 cm ² .		
	0	6	12	0	6	12	0	6	12
control	21.25	19.53	15.22	3.19	2.93	2.54	7.06	6.30	5.70
0.2 mol	—	18.05	19.60	—	2.71	2.94	—	5.84	6.30
0.3 mol	—	17.69	20.73	—	2.65	3.11	—	5.95	6.54

than that of the control: increase as much as 28.7% in 0.2 mol; 36.2% in 0.3 mol sample, as compared with the control. With the increase of the concentration of urea treated, the chlorophyll contents were inversely proportionate to it on the 6th day when those reached the minimum content. On the other hand, the chlorophyll contents were proportionate to it on the 12th day when these reached the maximum content of chlorophyll.

DISCUSSION

The foregoing data have shown that the chlorophyll content of sunflower leaves spraying urea decreased at first (depression period) and increased later (impression period).

Unlike Oland⁽⁷⁾ and many others have indicated with much attention, the unexpected temporary decrease of the chlorophyll content was noticed after foliar absorption. In the N-deficient plots (Experiment A), the content of chlorophyll showed a decrease on the 3rd day, while in the N-abundant plots (Experiment C) it decreased on the 6th day, and the young and the mature sample were also the same as that of the N-deficient plots.

Boynton et al⁽¹⁾, studying with apple leaves, stated that chlorophyll-lipid fraction would not or hardly increase from 8 hours to 4 days after urea foliar application. It is assumed that the result by Boynton et al mentioned above is related to the depression of the chlorophyll content of the present data, although they did not determine the pure chlorophyll.

About the reasons for the depression period of chlorophyll, the followings are presumable: (1) injury to the leaves from high concentration of urea, (2) injury by accumulation of ammonia from hydrolysis of urea, and (3) injury by the depression of water content in the leaves by urea foliar application etc. In the previous paper⁽⁸⁾ the followings were observed; the wilting necrotic spots appeared on the leaf blade; a considerable amount of ammonia remained even after the 6th day; and water content of the leaves decreased when higher concentration of urea sprayed on the leaves (see Table 1, 2 and 3 in the previous paper⁽⁸⁾). These should be an important reason for the decrease in the chlorophyll content at the depression period.

The chlorophyll formation, by urea foliar application, occurred 6 days later in the N-abundant plots than in the N-deficient (Table 1 and 3).

According to previous paper⁽⁸⁾ in the N-deficient plots the urea content in the leaves after urea foliar absorption already disappeared on the 4th day, while in the N-abundant plots a considerable amount of urea remained even after 6 days. With this result it is assumed that the N-abundant plants may slowly assimilate urea absorbed through leaf. Therefore the impression period in the N-abundant plots may occur slowly compared with the N-deficient plots.

In the mature and the young leaves, the chlorophyll content in the latter is formed much more than

that of the former with the same concentration of urea. As indicated in the previous paper ⁽⁵⁾, the young leaves may utilize the urea more actively to be absorbed through leaf surface. It is worthwhile to discuss that increasing amount of chlorophyll, due to urea foliar absorption in the N-deficient plots, is not kept unexpectedly long.

摘 要

前報(5)의 方法에 따라서 少窒素 及 多窒素狀態에서 砂耕栽培한 해바라기의 잎에 尿素를 葉面撒布하여 葉綠素含量的 變化를 實驗하였다. 少窒素區에 있어서는 幼葉과 老葉을 區別한 實驗도 하였다.

期待한 것과는 달라서 尿素葉面撒布后 葉綠素含量은 일단 減少期가 나타난 다음 增加되었다. 減少期는 少窒素區에서는 3 日, 多窒素區는 6 日째에 나타났고, 增加期는 少窒素區는 6 日에, 多窒素區는 12 日后에 나타났다. 增加期에 있어서의 增加量은 少窒素區는 17.7~28.5%, 多窒素區는 28.7~36.2% 만큼 增加되었다. 減少期가 出現하는 것은 前報(5)의 結果에 의해서 Ammonia의 蓄積, 水分含量의 減少 등에 原因이 있다고 생각된다. 幼葉은 老葉보다 撒布된 尿素에 의하여 많은 葉綠素를 合成하였는데 이것은 前報(5)의 結果와 一致하며, 그것을 굳게 뒷받침 한다고 생각된다.

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