<Oirginal article>

EFFECTS OF UREA NITROGEN ON THE METABOLISM OF PLANTS (1)

Studies on Nitrogen Absorption and Metabolism in Sunflower Leavessprayed with Urea Solution.

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金俊鎬: 植物의 代謝에 미치는 尿素窒素의 影響. (I)
해바라기에 葉面散布했을 때의 窒素의 吸收와 代謝.

ABSTRACT

KIM, Joon Ho (Dept. of Biology, Kongju Teachers College, Kongju, Korea) Effects of urea nitrogen on the metabolism of plants. I. studies on nitrogen absorption and metabolism by sunflower leaves sprayed with urea solution.

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In order to detect the way of absorption and metabolism of the urea it is sprayed on the surface of the leaves of sunflower. The sunflowers used in this study are grown in different conditions such that the one in nittogen aboundant and the other in nitrogen deficient soil, respectively. The urea-N, ammonia-N, amide-N, and 80% alcohol soluble-N in the leaves were quantitatively determined. All of the nitrogenous components measured are generally tended to increased with rising the concentration of urea except only amide-N at 24 hours after sprayed, and these were highly significances. It seemed that hydrolizing of urea into ammonia and carbon dixide and the assimilation of ammonia into other organic nitrogenous constituents were rapid in the young leaves than in the mature. It is interest that the amide content, in the young leaves and nitrogen defieient one were enhanced with the increasing concentration of urea, although in the mature leaves it did not show any change in the urea treatment. It is presumed that the assimilation rate of ammonia and the urease activity were lower in the matture leaves than in the young and nitrogen deficient leaves.

No significance at 5% level showed all of the nitrogenous components except total nitrogen between nitrogen abundant and deficent leaves. Urea content was a high peak at first 12 hours, ammonia at 48 hours, and amide and alcohol soluble nitrogen at 96 hours, whence decreased the content of these constituents gradually. The total nitrogen content is not increased obviously by only one time of urea spray in this study. When the concentration of urea was relatively high there appeared the wilting spots on the edge of leaves. As a whole, it seemd that sprayed urea was rapidly absorbed and taken part in nitrogen metabolism within relatively short period.

INTRODUCTION

The nitrogen fertilization to the green plants by spraying dilute urea solution has became practice. The usefulness of this practice was originally suggested by Hamilton [10] *et al* in 1943, and subsequently confirmed by the field experiments of many workers [4, 6, 7, 8, 13, 19, 20, 21, 23, 24]. On the other hand, to reveal the absorption mechanism of urea on the leaf surface, radioactive isotopes such as C^{14} labeled urea [9, 29, 11] and N^{15} labeled urea [2, 28] were used. According to these reports urea was taken up by urease in certain leaves [14, 29], but in others didn't [11]. After urea was taken up it

was followed by a rapid disappearance then accumulation of amino acids and amide occur[3, 15, 22]. When urea was absorbed by leaf, it was taken more rapidly in the lower surface of leaf than the upper surface, although at the end of a week the total taken up by upper and lower surface of leaves was not differ greatly[5]. Furthermore, Boynton *et al*[1] and Oland[17] repopted an interesting information about some principal nitrogenous components which appeared in the procese from urea to protein in the leaves of apple.

Author has also been interested in that how the foliar sprayed urea take part in the nitrogen metabolism. The results reported herein have been undertaken to ascertain the absorption and metabolism of the urea nitrogen after the urea solution sprayed on the sunflower leaves.

MATERIAL AND METHOD

Material: Sunflower seedlings were used as the material in these experiments.

Cultivation: After these grown up about 60 cm. high in the green house, ten seedlings were transplanted into sand boxes which were $12 \times 12 \times 45$ cm. The cultural boxes were distingguished the two series The one series was fertilized as much as three times with two liters of Knop's solution once in a week for nitrogen abundant series, the other was fertilized with the same procedure except nitrogen element from the cultural solution for nitrogen deficient series. Each box was placed on the stand of 60 cm. high on the ground of outdoors during the cultivation. Care was taken to expose the same intensity of light, furthermore, each box was arranged according to Latin square design. All of the apray treatments of urea were made with hand-controlled sprayer on the lower surfaces of the leaves. Spraying was done in the evening. The concentrations of the urea solution were 0.3 mol. and 0.5 mol.

Sampling: Sampling was done in the evening. The leaves were separated into two subsamples. The one was for dry weight and total nitrogen determination, the other for the content of the some soluble nitrogenous components.

The fresh weight of first sample was obtained as soon as sampling, and the leaves were washed with acidulated distilled water to remove all of urea which was not taken up by the leaf. After washing the first sample it was subjected dry in a oven at 70°C. to obtain even weight for determination of water content and total nitrogen analysis.

Second sample was reweighed, and immersed in proper amount of 95per cent alcohol to get a final concentration of 80 per cent by volume after allowing for the rinse water remaining on the leaves and estimating original water content of the sample by a previous test. These procedures were rapidly done within an hour from the time of picking to arrest enzyme actions. The samples were stored in the refrigerator below zero degree C. till it was analyzed.

Analysis: Total nitrogen was determined according to the modification of the Kjeldahl procedure. Second sample which immersed in alcohol was ground in a motor adding 80 per cent alcohol. After grinding it was filtrated by vacuum pump adding a portion of 80 per cent alcohol and then this procedure repeated until no green color was visible in the yellow filtrate, whereas the insoluble residue became yellow color. This alcohol extract was made aliquot volume with 80 per cent alcohol, and a portion of this extract was utilized to determine the 80 per cent alcohol soluble nitrogenous component by the kjeldahl method.

The other portion of alcohol extract thereafter kept to evaporate all of the alcohol in vacuum state at 40 degree C. The resulting material was repeatedly extracted with chloroform to remove the water insoluble residue containing chlorophyll and lipids etc. Thus both water insoluble and soluble components were fractionated carefully. The water soluble components was diluted to a given aliquot volume, and then it was added two or three drops of toluene and then stored in a refrigerator. This aliquot was used for the determination of ammonia, amide, alpha-amino acid and urea by Schlenker method [27]. For measure of the urea some of water saluble fraction was hydrolyzed, at PH 7.2, by enzyme urease prepared from soybean seeds after ammonia component made to be removed with sodium permutid. Urease activity used in this study is shown in Fig. 1.

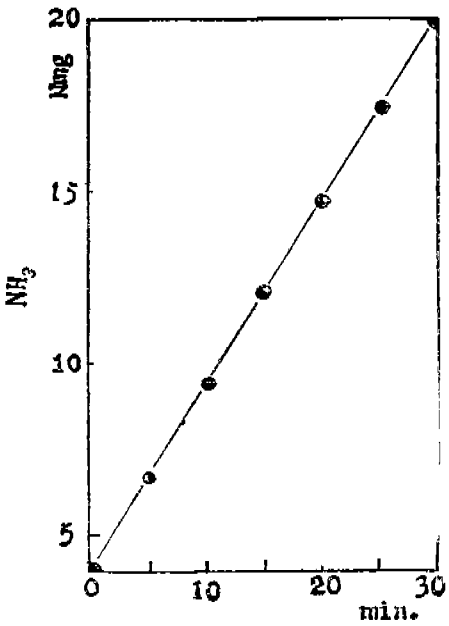

Fig. 1 Urease activity
0.2mol. urea.....10cc.
Phosphate buffer (Ph 7.2).....18cc.
Urease solution (coarse).....2cc.

RESULTS

A. *Absorotion and Metabolism of Nitrogen form the Urea Solution sprayed on the Mature leaves of Sunflower cultivated under Nitrogen Abundant Condition.*

The mature leaves in this experimet were treated as follows; 1) Control; O hour no spray 2) 24 hours water spray 3) 24 hours spray with 0.3 mole and 4) 24 hours spray with 0.5 mole urea solution. The nitrogen content in the leaves was expressed as milligram per gram of fresh weight for water soluble nitrogne and 80 per cent alcohol soluble, and as milligram of dry weight for total nitrogen.

The untreated leaves sampled at the end of the experimental period did not change the amount of the water soluble components and 80 per cent alcohol soluble, but increased somewhat total nitrogenous compounds (Table 1).

The urea sprayed leaves were increased in all of the constituents, especially ammonia, amino acid, urea, 80 per cent alcohol soluble and total nitrogenous components compared with untreated leaves. According to Boynton *et al* [1] who studied with young apple leaves, the amide nitrogen was enhanced by urea treatment, although in this study the amide content was high even in the cotroll and it did not show any change in the mature leaves of sunflower by treatment of urea. On the basis of the differences between 0.3 mol. and 0.5 mol. urea concentration used, each components except total nitrogen in 0.5 mol. increased as much two or three times as more than in 0.3 mol. This result may agree with the data by Boynton. *et al* [1].

Table 1 is shown that the content of each 80 per cent alcohol fractions was generally higher than the total content of the water soluble expressed as Sum. It is presumably that 80 per cent alcohol fraction might contain considerable amount of the water insoluble nitrogenous constituents such as chlorophyll, lipids and polypeptide etc.

To make comprehensive idea about individual value obtained in this study the data is treated statistically

Table 1. Effect of foliar sprayed urea on the absorption and metabolism in the mature leaves of sunflower cultivated under nitrogen abundant condition.

	Urea untreated		Urea treated			
	control		0.3 mol		0.5 mol	
	0 hour	24 hours	24 hours	Gain*	24 hours	Gain**
Water content (%)	83.28	86.11	86.18	—	85.85	—
Urea-N(mg./g. fr. wt.)	0.014	0.013	0.333	0.320	0.858	0.845
Free ammonia-N (〃)	0.072	0.065	0.089	0.024	0.148	0.083
Amide-N (〃)	0.319	0.323	0.323	0.000	0.345	0.022
alpha Amino acid-N(〃)	0.480	0.413	0.561	0.148	0.635	0.222
Sum(Watur soluble)	0.885	0.814	1.306	0.491	2.016	1.203
80% acohol solub. (〃)	0.852	0.852	1.994	1.142	2.936	2.084
Total-N(mg./g. dry wt.)	44.872	46.710	50.778	4.068	49.132	2.422

t-values at 5% level are: urea-N; 0.633, free ammonia-N; 0.062, amide-N; 0.021, amino acid-N; 0.204, 80% alcohol soluble-N; 1.602, total-N; 3.717,

* Gain : 0.3 mol urea-24 hours minus no urea-24 hours.

**Gain : 0.5 mol urea-24 hours minus no urea-24 hours.

in Table 1.

B. *Absorption and Metabolism of Urea sprayed on the Young leaves of Sunflower cultivated under the Nitrogen Abundant Conditon.*

These young leaves were also treated as in experiment A. The control series did not show any changes in nitrogen components during this experiment as shown in Table 2. The treated leaves tended

Table 2. Effect of foliar sprayed urea on the absorptian and metabolism in the young leaves of sunflower cultivated under nitrogen abundant condition.

	Urea unteated		Urea treated			
	control		0.3 mol		0.5 mol	
	0 hour	24 hours	24 hours	Gain*	24 hours	Gain**
Water Content (%)	84.14	84.37	84.33	—	83.67	—
Urea-N (mg./g. fr. wt)	0.016	0.017	0.223	0.206	0.308	0.291
Free ammonia-N (〃)	0.049	0.040	0.068	0.028	0.075	0.035
Amide-N (〃)	0.227	0.225	0.314	0.090	0.376	0.151
alpha Amino acid-N(〃)	—	0.617	0.703	0.086	0.767	0.150
Sum (Water soluble)	—	0.899	1.308	0.410	1.526	0.597
80% alcohol solub. (〃)	0.962	1.136	2.030	0.894	2.440	1.304
Total-N(mg/g. dry wt.)	43.537	43.537	48.280	4.743	48.790	5.253

t-values at 6% level are: urea-N; 0.242, free ammonia-N; 0.001, amide-N; 0.117, amino acid-N; 0.138, 80% alcohol soluble-N; 1.126, total-N; 5.592.

* Gain : o.3 mol urea-24 hours minus no urea-24 hours.

**Gain : 0.5 mol urea-24 hours minus no urea-24 hours.

to be increased fairly in each nitrogenous fractions. The ammonia and amino acid were slightly increased by urea spray, and urea was greatly increased in the young leaves in spite of the fact that it did not change in the mature leaves. It was also observed that all of the nitrogenous constituents in 0.3 mol. samples were clearly lower than in 0.5 mol. ones. As shown Table 2 it is evedently found that the young leaves rapidly absorbed urea, then amino acid and amide were swiftly synthesized from urea within twenty four hours. According to evaluate of t-value significances at 5% level are difference in each nitrogen content between 0.5 mol-24 hour sample and untreated 24 hour, while no signiflcance at 5% level are between 0.3 mol-24 hour sample and untreated-24 hour.

C. *Absorption and Metabolism of Urea sprayed on the leaves of Sunflower cultivated under Nitrogen Deficient Condition.*

It is assumed that in these material the initial nitrogen content would be low in the leaves before urea treatment. In sampling of this plot the materials were not distingguished the mature leaves from the yonng one, and the data from the O hour sample could not be obtained in this study. Evəry nitrogenous constituents were apparently increased by urea foliar sprayed (Table 3). Increasing the concentration of urea was generally proportional to the amount of each nitrogenous components except total nitrogen. In spite of the fact that the amount o' total nitrogen in the untreated leaves under nitrogen deficient condition was clearly lower than those of the mature and young leaves under nitrogen abundant condition, it was greatly high regardless of the concentration of urea, however, it was not increased so high as nitrogen abundant conditon until 24 hours after urea apolied. From the fact that the amount of nitrogen could be absorbed from the urea sprayed within 24 hours, it might have a certain limit. The data indicated by Oland [17] and Boxnton [1] studying with apple leaves were not so conspicuously high as the result in this study with sunflower leaves under nitrogen deficient condition. These results also showed the similar trend in the young leaves under niterogen abuntant.

Table 3. Effect of foliar sprayed urea on the absorption and metabolism in the leaves of sunflower cultivated under nitrogen deficient condition.

	urea untreated	urea treated			
	control	0.3 mol		0.5 mol	
	24 hours	24 hours	Gain*	24 hours	Gain**s
Water content (%)	84.58	85.57	—	84.00	—
Urea-N (mg./g. fr. wt.)	0.016	0.342	0.326	0.475	0.459
Free ammonia-N (〃)	0.038	0.065	0.027	0.075	0.037
Amide-N (〃)	0.227	0.328	0.110	0.368	0.141
alpha Amino acid-N (〃)	0.450	0.525	0.075	0.565	0.115
Sum (Witer soluble)	0.731	1.260	0.538	1.483	0.752
80% alcohol solub. (〃)	0.824	1.619	0 795	2.272	1.448
Total-N (mg./g. dry wt.)	32.319	45.070	13.751	46.579	19.442

t-values at 5% level are: urea-N; 0.580, free ammonia-N: 0.001, amide-N; 0.227, amino acid-N; 0.144, 80% alcohol soluble-N; 1.843, total-N; 19.442.

* Gain : 0.3 mol urea-24 hours minus no urea-24 hours.

**Gain : 0.5 mol urea-24 hours minus no urea-24 hours.

However, in the sample of 0.5 mol-24 hours there was no significance at 5% level by t-test of each components except ammonia content.

D. *The Course of the Metabolism of Principal Nitrogenous Comoonents in the leaves of Sunflower during Six Days after Urea sprayed.*

In order to compare the effect of the urea applied on the leaves which initially contained much nitrogen with that of deficient in nitrogen content, the plants were cultivated unter two different conditions; nitrogen abundant and deficient for three weeks before urea treatment. The leaves of such two series were treated as follow; 1) untreated-o hour, 2) treated-12 hours, 3)-24 hours, 4)-48 hours, 5)-96 hours, and 6)-144 hours (without N-deficient series) respectively. The concentration of urea was prepared only 0.3 mol. solution in this study.

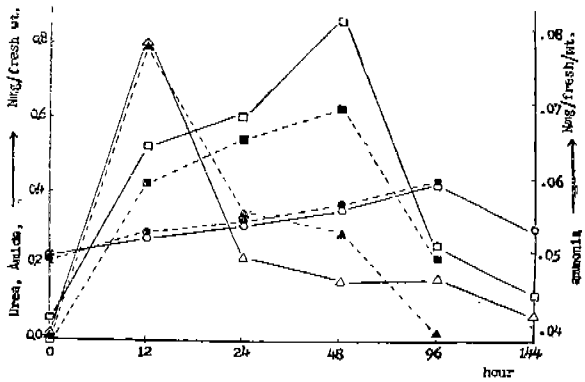

Fig. 2 Effects of sprayed urea on the nitrogen metabolism of sunflower leaves which is initally abundant and deficient in nitrogen.
△—Urea, N-abundant □—anmania, N-abundant
▲···Urea, N-deficient ■···ammania, N-deficient
○—amide, N-abundant
●···amide, N-deficient

The urea content showed a high peak in the 12 hours sample and swiftly reduced after 12 hours, though indicated a slight variety in both nitrogen abundant and deficient series (Fig. 2). The free ammonia concentration remarkably rise until the first 12 hours, and reached at a pack at 48 hours and then dropped in the both series. It is also noteworthy that there was always higher amounts of ammonia in the nitrogan abundant series than in the nitrogen deficient. The amide continuously increased slowly and it ran parallel with both series up to 96 hours, however, it decreased in the nitrogen abundant series after that time. The distribution of 80 per cent alcohol soluble constituent rapidly increaed with elapsed time by 96 hours in both series, furthermore, its amount in the nitrogen abundant series were conspicusly enhanced more than that of the deficient series, but the former reduced rapidly after 96 hours (Fig. 3). The content of total nitrogen continuously increased in the nitrogen abundant series with elapsed time, it did not show, however, that there was an actual changes in the nitrogen deficient series after 24 hours.

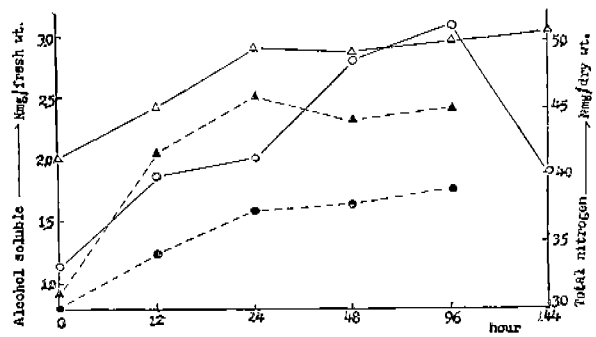

Fig. 3 Effect of sqrayed urea on the nitrogen metabolism of sunflower leaves which are inidially abundant and deficien in nitrogen.
△——Total-Nitrogen, N-abundont
▲·····Total-Nitrogen, N-deficient
○——Alcohol soluble, N-abundant
●·····Alcahol soluble, N-deficient

DISCUSSION

The foregoing data show that the nitrogen is rapidly taken up from the urea foliar sprayed and it is actively metabolized in leaves which have the different ages and different initial nitrogen contents.

Furthermore, in order to compare in detail each results of sampling at 24 hours, discussion has been made about Table 1-3 and Fig. 4. The urea content was almost negligible in the untreated leaves, however, it shows highest amount in the mature leaves and lowest in the young leaves treated with 0.5 mol. urea (Fig. 4, a). This results agree well with the data by Burr *et al* [2], they have shown that the N^{15}-labeled urea was absorbed and extensively distributed throughout sugar cane within 24 hours, Volk and McAuliffe [28] have suggested that it was same in tobacco plant, and Boynton *et al* [1] have also observed to take up the most amount of urea at 8 hours after spray in the apple leaves. Besides, it is probably considered that the activity of urease in the mature leaves was lower than in the young leaves as shown by Freiberg and Payne[9] who reported that, by the study with banara leaf, hydrolysis of the C^{14} labeled urea occurred in the growing points where urease activity was detected. Moreover, if above results were associated with the data by Webster *et al* [29], who stated that urea was absorbed rapidly than it was hydrolized by bean leaves, it plausibly assumes that the urea slowly hydrolized in the mature leaves than in the young one Therefore, it should have to detect urease activity in the leaves of different ages of sunflower, since Hinsvark *at al* [11], on the one hand, have shown that urea hydrolizing into CO_2 and NH_3 by the enzyme urease was a rate-limiting reaction in the absorption and utilization of nitrogen, on the other hand, since Kuykendall and Wallace [14], who studied with detached citrus leaves, concluded that urease activity was so great that the rate of urea hydrolysis should not be a limiting factor in the assimilation of foliar applied urea.

The ammonia content, as a whole, was slightly low in all of the samples but it was remarkably higher in the mature leaves than in the others (Fig. 4. b). It should seem that the mature leaves had no ability to assimilate so much the organic nitrogen compounds from ammonia as in the young leaves or in the nitrogen deficient. This also seems to be connected with the synthesis of amide as well as other soluble organic nitrogenous constituents.

It was found that the amide content in the young and N-deficient leaves were conspicuously heigh as increasing urea concentration within 24 hours as studied by Boynton *et al* [1]. Whereas, there was very interesting behavior in the amide content that it was contained much in the mature leaves than the other samples before urea treatment, but it was not changed by the urea applied in the mature leaves. These may be consistent with the finding of Steward *et al* [25] that the amide content was higher in the non-growing tissues than in the actively proliferating tissues. From earlier Prianischnikow's study of the nitrogen metabolism of plants about fifty years ago, it has been known that the amides of many plants occupy a special position among the metabolic componnents from absorbed ammonis. Generally it is accepted that the amide acts as a *detoxicator* of ammonia and is the intermediates in amino acid synthesis. From the data it suggested that the mature leves of sunflower had slightly or hardly any ability to synthesis amide from excessive ammonia. Besides alpha-amino acid was initially contained highest amount in the young leaves but the synthetic rate of amino acid by urea is similar in all series. The content of ammonia, amino acid (Fig. 4, c), 80 per cent alcohol soluble(e) and amide (without in the mature) (d) were actually enhanced in parallele with the concentration of urea applied

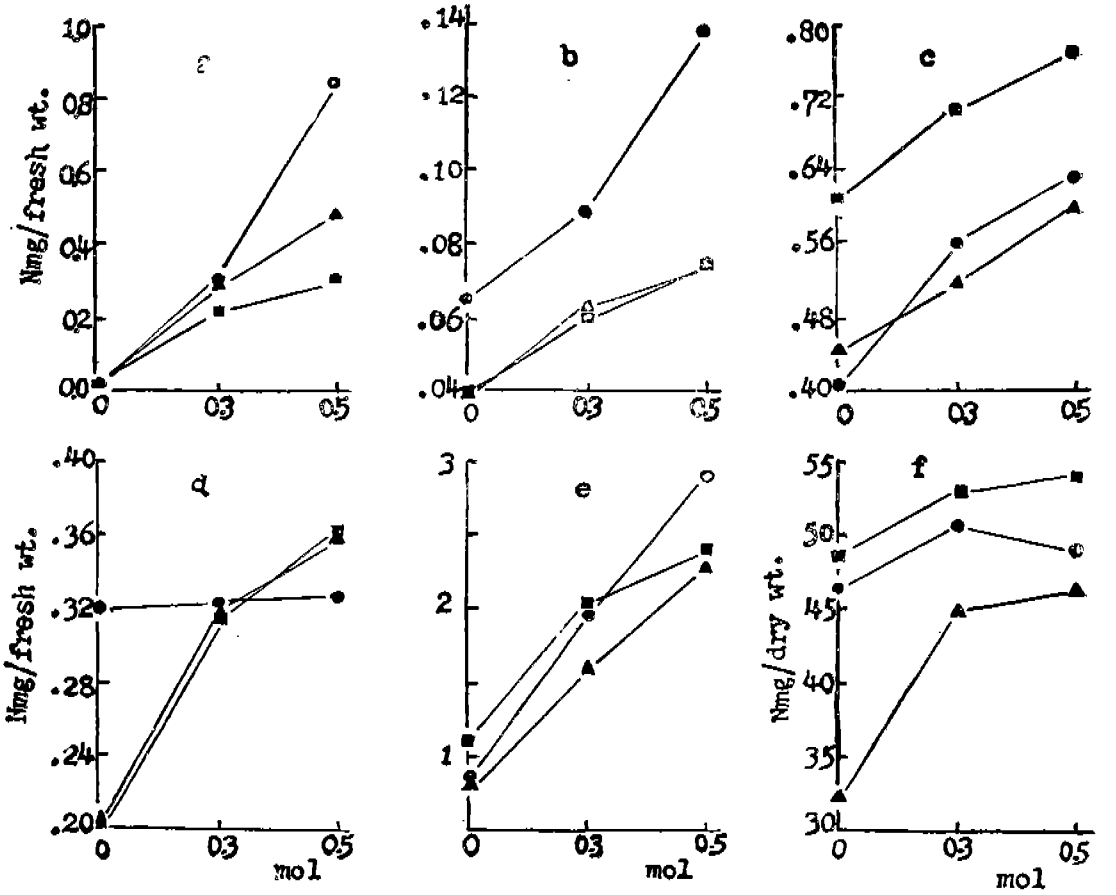

Fig. 4 Effects of sprayed urea on the principal nitrogenous fraction of sunfower leaves which are initially mature, young, and N-defient condition.

a, Urea-N, b, ammonia-N c, amino acid-N, d, amide-N, e, alcohol soluble-N, f. total-N.

●——mature leaves, ■——young leaves, ▲——N-deficient.

within 24 hours.

Though Steward *et al* [26] have reported that in the rapidly dividing tissues of plant the alcohol soluble nitrogen was markedly reduced compared to its alcohol insoluble in the non-dividing, alcohol soluble nitrogen content was relatively high, furthermore, it was detcted to increase in high urea concentration in each series in this experiment(ig. 4, e). The alcohol soluble nitrogen may mainly consist of free amino acid, amide, chlorophyll, lipids and polypeptids. The two former compounds must be available for protein synthesis, of coures, it is indespensable for the satisfactory plant growth. It was shown that the total nitrogen content was rapidly increased until 24 hours but thereafter it was slightly or hardly enhanced. Oland [18] has found that, however, in the apple leaves total nitrogen was incresed by 48 hours and swiftly dropped after that time. The periodical differences obtained by Oland and present author may be depened on the time of urea spray.

In order to evaluate quantitatively the interrelationships prevailing in the data obtained, they were

Table 4. The results of analysis of variance among the mature, young and nitrogen deficient leaves in the 24 hour samples, and t-test between nitrogen abundant and deficient series.

Dependent variable	Proportion among three different conditions(%) (1)	Proportion of different urea concentration(%) (2)	error (%) (3)	t (9) (4)
Urea-N	12. 65	74. 51*	12. 84	0. 2655
Ammonia-N	39. 31	47. 94*	12. 73	0. 1819
Amide-N	5. 38	72. 29	2. 37	0. 0694
Amino acid-N	57. 60**	39. 20*	3. 20	0. 9441
Alcohol soluble-N	5. 47	92. 87**	1. 97	2. 4412*
Total-N	43. 77*	50. 20*	6. 09	

The statistical analysis was made from the data, exclusive o mol--0 hour sample.
* Significant at the 5% level.
** Significant at the 2% level.

statistically reduced by an analysis of variance and t-test (Table 4) to gain the summary values [25]. In developing table 4, the total sums of squares for the variability among the nine observations take the three different levels of treatment, i. e. O. mol, 0. 3 mol, and 0. 5 mol. in three different physsiological conditions, i. e. the mature, young and N-deficienent, are designed as 100 per cent of the variability observed for a given criterion, and indicated t-values to calculate from originally the two different nitrogen content series.

In the table 4, column 1 shows the percentage of total sums of squares due to two degree of freedom from mean levels of the three conditions, such as two different leaf age and nitrogen deficient condition; column 2 shows the proportion of total sums of squares attributable to two degree of freedom arising from different concentration of urea solution; column 3, error, indicates the proportion of total sums of squares assigned to the eight degrees of freedom for error; column 4, or t(9), presents t-values from which the comparison with the average values of two different samples, which are nitrogen abundant series and deficient under the process of nitrogen metabolism during six days (data from Fig. 2-3). Significance of a given component of variance is designated by asterisks according to the usual convention.

The statistics shows that the variations of all the components, with the minor exception, tended to be directly related to the concentration of urea solution with relatively high degree, althongh no significances are in general among the different ages and nitrogen deficient except in amino acid and total nitrogen content.

摘 要

成熟葉, 幼葉, 窒素含量의 多少등 生理學的 條件이 다른 狀態에 있는 해바라기의 葉에 尿素를 葉面散布 한 後, 그 吸收와 代謝에 對하여 硏究하여 다음과 같은 結果를 얻었다.

1) 葉面散布한 尿素의 吸收는 大部分은 12 時間 內에 이루어 졌고, 다음에 Amino 酸 Amide의 合成에 참여 하는 것 같았다.

2) 모든 窒素 成分, 卽 Ammonia, Amide, Amino 酸, 80% alcohol-可溶性 窒素 及 總 窒素는 大體로 尿素 濃度가 짙음에 따라 增加 되었다. 그러나 尿素의 比較的 높은 濃度는 葉에 해를 이르켰다.

3) 尿素의 吸收 能力은 成熟葉 보다 幼葉이 旺盛 했고, 더욱 成熟葉은 剩餘 Ammonia 에서 Amide를 合成 하는 活性이 낮은 것 같았다.

4) 몇가지 例外는 있었지만 6日間의 窒素 代謝에서 有窒素區 及 窒素 缺乏區 사이에는 有意差를 發見하지 못했다.

5) 尿素 含量은 처음 12 時間에 가장 많았고 Ammonia는 48時間, Amide와 alcohal 可溶窒素는 96 時間까지 徐徐히 增加 되었다.

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