ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XXXII, 7 SECTIO C 1977

Instytut Biologii UMCS
Zakład Fizjologii Roślin

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The Effect of Extra Nitrogen Fertilizer on some Aspects of Photosynthesis in Maize Leaves

Wpływ intensywnego nawożenia azotem na pewne aspekty fotosyntezy w liściach kukurydzy

Влияние интенсивности азотного удобрения на некоторые аспекты фотосинтеза в листьях кукурузы

INTRODUCTION

The rate of photosynthesis in many higher plants depends on nitrogen supply. Low nitrogen supplies inhibit the assimilation of CO_2 in leaves (1, 2, 6, 8, 15, 16, 17).

Extra nitrogen fertilizer does not increase the rate of CO_2 assimilation as much as could be expected from the amount of nitrogen added (quoted by 21). Changes in the intensity of photosynthesis may be limited, among others, by the level and activity of carboxylating enzymes or by the activity of photosynthetic electron flow. In recent years a number of papers have been published on the correlation between the activity of ribulose diphosphate carboxylase and the rate of photosynthesis (1, 7, 21, 23).

No attention has been paid so far to electron transport in the chloroplast of leaves growing on medium supplied with extra nitrogen fertilizer. Only a few studies deal with photosynthetic electron transport in plants growing on mineral-deficient medium (5, 6, 19). The high Photosystem II activity in nitrogen-deficient plants found by Baszyński et al. (5) was correlated with the increase in grana stack membranes observed by

Hall et al. (11). More recently, detailed studies on this problem have shown that high activity of Photosystem II in nitrogen deficiencies may be independent of high grana stacking (6).

The photosynthesis in plants treated with extra nitrogen supplies was examined fragmentarily (1).

In this paper we present some data concerning the effect of excessive nitrogen supply on CO_2 assimilation in maize leaves and on the photosynthetic activities of their chloroplasts. The level of pigments and of lipoquinones in chloroplast membranes was also examined.

MATERIAL AND METHODS

Seeds of *Zea mays* L. var. Hybrid SM 259 were obtained from the Experimental Station at Ożańsk. The seeds were soaked in darkness for 24 hrs and were then put out on wet lignin and placed in a thermostat at 23°C. After three days of germination the plants were planted in Mitscherlich's pots filled with vermiculite and placed in a greenhouse where they were exposed to light for 18 hrs (4500 lx). The plants were watered daily with Knop nutrient solution as a complete medium, or with another solution containing 2, 5 or 10 doses of nitrogen (1 dose=118 mg N/l contained in full-nutrient solution). Hoagland's medium was used as the source of microelements, whereas iron was given in the form of 1% citrate in the amount of 2.5 ml/l of basic medium. After eight weeks leaf samples were taken for assays.

Photosynthetic oxygen evolution was measured microrespirometrically according to Zurzycki (26) using a light intensity of 3.4×10^4 ergs $\cdot$ cm$^{-2}\cdot$ s^{-1}. To reduce light intensities Baltzer's filters were used.

Carotenoids were chromatographed and estimated by the method of Hager and Bertenrath (10).

The plastid quinones were separated on silica gel layers in mixtures of light petroleum and diethyl ether (7:1, v/v) according to Lichtenthaler (13). The amounts of lipoquinones present in leaf tissues were determined spectrophotometrically after Lichtenthaler (12), and vitamin K_1 after Lichtenthaler and Tevini (14).

Chlorophyll determination and chlorophyll a/b ratio were carried out as described by Arnon (3).

Mesophyll cell maize chloroplasts were preparated by brief homogenization of the leaves in the medium described by Sane et al. (18). This homogenization breaks the minimum of bundle sheath cells. The slurry was filtered through eight layers of gauze and one layer of miracloth. The filtrate was centrifuged for 5 min at 200×g to remove nuclei and cell débris. Chloroplasts were pelleted from the supernatant by centrifugation at 1000×g for 10 min.

Photosystem I activity was studied by measuring the light dependent oxygen uptake of the chloroplasts in a system containing TMPD/ascorbate as electron donor couple and methyl viologen as electron acceptor. The reaction chamber was illuminated by red light at an incident intensity of 2.5×10^5 ergs $\cdot$ cm$^{-2}\cdot$ s^{-1}.

Photosystem II electron transport was assayed by the following ferricyanide reduction spectrophotometrically (at 600 nm) using water as reductant. The energy incident on a sample was 1.5×10^5 ergs $\cdot$ cm$^{-2}\cdot$ s^{-1}.

ATP synthesis coupled to cyclic electron transport with phenazine metosulphate as the cofactor was performed as described by Avron (4). Illumination for 5 min was provided by light at an incident intensity of 1.2×10^5 ergs · $cm^{-2} \cdot s^{-1}$.

The method and conditions used for measurement of non-cyclic photophosphorylation are identical with those used in the study of cyclic photophosphorylation with the exception of the composition of the reaction mixture.

Other experimental details for measurements of photochemical activities are described in the legend of table 1.

Table 1. Effect of extra nitrogen supplies on electron transport activities and photophosphorylation of maize mesophyll chloroplasts

Doses of nitrogen in medium	Ratio chl a/b	Photosystem I TMPD → meviol. μmoles O_2 uptake per mg chl · h	Photosystem II H_2O → Ferricyanide μmoles acceptor red. per mg chl · h	Photophosphorylation	
				cyclic μmoles P_i	non-cyclic per mg chl · h
control					
1	3.3	791	70	60	70
2	3.1	702	59	49	55
5	3.2	591	54	37	44
10	2.7	446	46	31	40

The assays for Photosystem I activity were carried out in a solution (3 ml) containing, in μmoles: Tricine-NaOH (*pH* 8.0), 150; DCMU, 0.03; sodium ascorbate, 50; TMPD, 0.2; methyl viologen, 0.4; chloroplast equivalent to 15 μg of chlorophyll. The standard reaction mixture for measuring of Photosystem II activity contained the following components, in μmoles: Tricine-NaOH (*pH* 7.0), 150; potassium ferricyanide, 1; chloroplasts containing 30 μg of chlorophyll in final volume 3 ml. The reaction mixtures for cyclic photophosphorylation measurements contained the following components, in μmoles: Tricine-NaOH (*pH* 8.0), 50; KCl, 50; $MgCl_2$, 10; Na_3PO_4, 10; ATP, 4; phenazine metosulphate, 0.15; sodium ascorbate, 20; chloroplast equivalent to 50 μg of chlorophyll in a final volume of 3 ml. For measuring of non-cyclic photophosphorylation 3 μmoles of potassium ferricyanide were used instead of phenazine metosulphate.

Abbreviations: DCIP, 2,6-dichlorophenolinodophenol; DCMU, 3,4-dichlorophenyl-1,1-dimethylurea; TMPD, N,N,N′,N′-tetramethyl-p-phenylene diamine; Tricine, N-tris hydroxymethyl-methylglycine.

RESULTS

The data in Fig. 1 show the photosynthetic O_2 evolution of the leaf sections. Photosynthesis increase in the leaves of plants given extra nitrogen is not in proportion to the amount of nitrogen added. Five times higher amounts of nitrogen in comparison with control plants produced an increase in photosynthesis of about 17 per cent. A tenfold nitrogen dose, on the other hand, resulted in a decrease in photosynthesis by about 10 per cent.

On the basis of chlorophyll content the rates of O_2 evolution decrease in relation to all doses of nitrogen added.

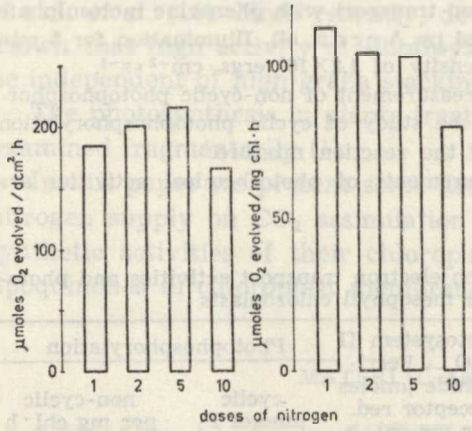

Fig. 1. Effect of extra nitrogen doses on photosynthetic activity in maize leaves (1 dose equal to 118 mg N/l contained in full-nutrient solution)

In the previous paper (6) it was shown that mesophyll chloroplasts of maize leaves demonstrated a higher Photosystem II activity, as measured by DCIP or ferricyanide photoreduction, according to the decreasing amounts of nitrogen in the medium. Table 1 shows that in the case of excessive nitrogen supplies all measured photosynthetic activities decrease in accordance with the nitrogen added. This refers both to Photosystems activity and photophosphorylations.

W a t a n a b e and Y o s h i d a (24) did not find any effect of nitrogen fertilization on ATP synthesis coupled to cyclic photophosphorylation, whereas T o m b e s i et al. (22) are of the opinion that also ATP synthesis coupled to non cyclic photophosphorylation is independent of nitrogen supplies. These authors, however, used a lesser excess of nitrogen than in the present investigation.

Figure 2 summarizes light saturation rates for photosynthetic O_2 evolution by leaf sections of maize growing in a full-nutrient or extra nitrogen

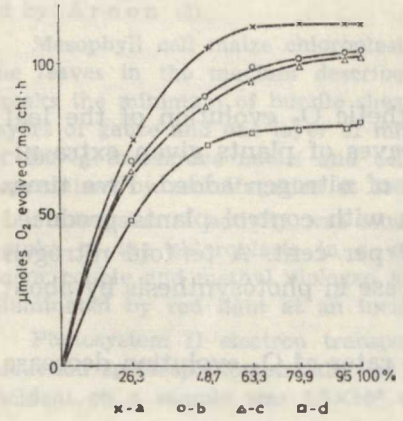

Fig. 2. Photosynthetic light intensity response curves of maize leaves grown in Knop nutrient solution with: a — 1, b — 2, c — 5 and d — 10 nitrogen doses (100% equal to 3.4×10^4 ergs · $cm^{-2} \cdot s^{-1}$)

medium. These data show a lower point of light saturation for leaves growing at a higher level of nitrogen. These differences especially for tenfold nitrogen doses are very distinct.

Table 2 presents the content of chloroplast pigments and lipoquinones in maize leaves growing at a higher than normal nitrogen level. Chlorophyll content increases with the addition of nitrogen. Over 2 doses of nitrogen this increase is not high. The content of carotenoids with the exception of neoxanthin does not undergo greater fluctuations.

Table 2. Effect of extra nitrogen doses on plastid pigments and lipoquinones in maize leaves (in μg per g of fresh weight)

	Doses of nitrogen			
	1	2	5	10
Chlorophyll a	1257.0	1433.0	1581.0	1600.0
Chlorophyll b	382.0	499.0	502.0	510.0
Carotenoids	271.9	250.5	248.4	233.6
β-carotene	78.2	65.3	72.0	60.8
lutein	143.4	148.0	146.4	120.0
neoxanthin	26.0	15.2	15.0	24.0
violaxanthin	24.3	22.0	15.0	28.8
Benzoquinones	309.3	197.3	190.6	124.3
plastoquinone A	193.3	128.7	102.2	70.7
plastohydroquinone A	42.1	24.4	36.6	11.8
α-tocopherol	60.9	36 6	35.5	34.3
α-tocoperylquinone	12.0	7.6	16.3	7.5
Vitamin K_1	13.7	18.8	13.8	9.3

Particularly the synthesis of benzoquinones was strongly inhibited during nitrogen increase in the medium.

Table 3 shows lipoquinones and pigments in relation to chlorophyll content in the membranes. The amount of carotenoids in relation to chlorophyll was decreased, especially that of neo- and violaxanthin.

Table 3. Plastid pigments and lipoquinones in maize leaves, growing on extra nitrogen fertilizer (in μg per 100 μg of chlorophyll)

	Doses of nitrogen			
	1	2	5	10
Chlorophyll a	100.0	100.0	100.0	100.0
Chlorophyll b	30.4	34.8	31.8	31.9
Carotenoids	21.6	17.5	15.7	14.6
β-carotene	6.2	4.6	4.6	3.8
lutein	11.4	10.3	9.3	7.5
neoxanthin	2.1	1.1	0.9	1.5
violaxanthin	1.9	1.5	0.9	1.8
Benzoquinones	24.6	13.8	12.1	7.8
plastoquinone A	15.4	9.0	6.5	4.4
plastohydroquinone A	3.4	1.7	2.3	0.7
α-tocopherol	4.8	2.6	2.2	2.1
α-tocopherylquinone	1.0	0.5	1.0	0.5
Vitamin K_1	1.1	1.3	0.9	0.6

The amounts of benzoquinones per unit of chlorophyll decreased considerably. In leaves growing in all extra nitogen doses the total amount of benzoquinones was about 2 times lower than that in control leaves. The changes in vitamin K_1 content were not too significant.

DISCUSSION

Assimilation of CO_2 after reaching a maximum did not show an increase proportional to the amount of nitrogen in the medium. CO_2 uptake on chlorophyll basis decreased with the increase in nitrogen content. Andreeva et al. (1) concluded from their experiments that changes in the synthesis and in the activity of ribulose diphosphate carboxylase may effectively regulate the rate of photosynthesis. Björkman (7) found that differences in the photosynthetic rate were correlated with changes in the activity of ribulose diphosphate carboxylase.

In this investigation it was found that the photosynthetic rate of extra nitrogen fertilized leaves was limited not only by the levels of carboxylating enzymes, which was shown by Andreeva et al. (1), but also by photosynthetic electron transport. A decrease in Photosystem I and II activities may result in a worse utilization of light harvesting-chlorophyll. The percentage of chlorophyll utilization decreased with the increase in its amount in the leaves due to nitrogen fertilization. On the other hand, we know that the effect of nitrogen on photosynthesis is really mediated by chlorophyll (24).

Light saturation curves (Fig. 2) indicate a correlation between extra nitrogen supply and photosynthetic unit size. The photosynthetic unit size may be regulated (besides light intensity and genetic factors) also by nutritional factors (9). A higher point of light saturation is a characteristic feature of chloroplasts with a smaller photosynthetic unit (25). In our previous paper it was shown that the saturation rates of O_2 evolution of nitrogen deficient chloroplasts were higher than those of control plants (6). We have therefore suggested that nitrogen deficient leaves possess more active centers of chlorophyll.

In our experiments leaves of maize given extra nitrogen represented a lower light saturation point following nitrogen amounts added in relation to control leaves. These leaves had probably a higher photosynthetic unit. This supposition is supported by lower concentration levels of benzoquinones in the experimental plants compared to control leaves on a chlorophyll basis. It is known from standard measurements of photosynthetic unit size that a lower unit size represents a higher rate of relative concentration of plastoquinone to chlorophyll (25). Our data confirm this

supposition and indicate an effect of a mineral nutrition on the saturation point of photosynthesis through an alteration of the size of the photosynthetic unit.

CONCLUSION

1. Extra nitrogen fertilizer resulted in reduction of photosynthesis in maize leaves on the basis of chlorophyll content. This decrease of photosynthetic O_2 evolution was limited by a lower rate of photosynthetic electron transport and photophosphorylation.

2. On the basis of a lower light saturation point and lower concentration ratio of plastoquinone to chlorophyll of leaves we can assume that an excess of nitrogen fertilizer increases the size of photosynthetic unit.

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STRESZCZENIE

Celem pracy było zbadanie wpływu intensywnego nawożenia azotem na fotosyntetyczne wydzielanie tlenu i aktywność fotosyntetyczną chloroplastów liści kukurydzy. Określono także zawartość barwników i lipochinonów w błonach chloroplastowych.

Stwierdzono, że wysokie dawki azotu obniżają fotosyntezę. Ten spadek fotosyntezy jest związany z niższą aktywnością I i II układu fotosyntezy i fotofosforylacji. Świetlne reakcje fotosyntezy stanowią zatem, obok wcześniej wykazanej przez innych autorów roli karboksylazy rybulozodwufosforanu, czynnik ograniczający fotosyntezę w tych warunkach. Stwierdzenie niższego punktu wysycenia światłem u roślin nadmiernie nawożonych azotem w porównaniu z kontrolą oraz niższego stosunku plastochinonu do chlorofilu pozwala na wysunięcie przypuszczenia, że nadmierne nawożenie azotem wpływa na powiększenie jednostki fotosyntetycznej.

РЕЗЮМЕ

Целью работы было изучение влияния интенсивного внесения азота на фотосинтетическое выделение кислорода и фотосинтетическую активность хлоропластов листьев кукурузы. Определялось также содержание пигментов и липохинонов в хлоропластовых оболочках.

Установлено, что высокие дозы азота снижают фотосинтез, что связано с низшей активностью I и II систем фотосинтеза и фотофосфорилляции. Следовательно, в этих условиях световые реакции наряду с карбоксилазой рибулозодифосфата (что было установлено другими авторами) являются факторами, организующими фотосинтез. Установленное низшее насыщение светом у растений, чрезмерно удобренных азотом, по сравнению с контрольными, низшее отношение пластохинона к хлорофиллу дают возможность выдвинуть предположение, что чрезмерное азотное удобрение влияет на увеличение фотосинтетической единицы.