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Influence of light and mineral nitrogen forms on the activity of some enzymes in *Cucumis sativus* L. cotyledons*

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Abstract

It was demonstrated that when nitrogen was deficient in the medium, the activity of glutamine synthetase (GS), glutamate dehydrogenase (GDH), alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) in etiolated cucumber cotyledons was higher than in those of seedlings growing under light. When the plants grew on nitrate or ammonium medium, light stimulated GS activity and depressed that of GDH and GOT, without changing the activity of GPT. It was found that the influence of the form of mineral nitrogen on the activity of the studied enzymes was dependent on light.

On the basis of the results obtained, the contribution of the GS glutamate synthase system and GDH to the incorporation of the taken up nitrogen into the amino acids in light and in darkness is discussed.

INTRODUCTION

The investigations of Bassham and Kirk (1964), Kirk and Leech (1972) demonstrated that, during amino acid synthesis, the first product of amination is glutamic acid formed by way of reductive amination of α-ketoglutaric acid. These authors also found that the amino group of glutamic acid may be transferred by transamination to a number of α-ketoacids. The reaction of NH_4^+ ions attachment to α-ketoglutaric acid is catalysed by glutamate dehydrogenase (GDH). Leech and Kirk (1968) and Kretovich et al. (1970) localized this activity in the chloroplasts.

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In 1974 Lea and Miflin found that there occurs in chloroplasts synthesis induced by light of glutamic acid from glutamine and α-ketoglutaric acid. These authors suggest that in green plant tissues the first enzyme which causes ammonia addition to the amino groups of amino acid is glutamine synthetase (GS). They did not exclude the biosynthetic role of GDH, however, they believed it is limited to systems in which the concentration of ammonia ions is high. The presence of GS in the chloroplasts of higher plants has been confirmed by Haystead (1973), Mitchell and Stocking (1975). GS activity in chloroplasts, mitochondria and the cytoplasmic fraction of spinach leaves was found by O'Neal and Joy (1973a), being the highest in the cytoplasmic fraction of the cells.

Mecke and Holzer (1966), Weissman (1972), Ferguson and Sims (1974a, b), Mitchell and Stocking (1975), Rhodes et al. (1975), Marwaha and Juliano (1976), Glick and Weissman (1978) studied the influence of various mineral nitrogen sources on GS activity. The relation between GDH and the presence of NH_4^+ ions was established in the papers of Wakiuchi et al. (1971), Kanamori et al. (1972), Kretovich et al. (1972, 1973a, b) Barash et al. (1973), Caldas and Caldas (1976), Ehmke and Hartmann (1976). The stimulation of GDH and GS activity by NH_4^+ ions, demonstrated by these authors, was dependent on the plant organ examined.

The studies of Deitzer et al. (1974), Mitchell and Stocking (1975), Postius et al. (1976), Postius and Jacobi (1976), Duke et al. (1978) demonstrated that light is an important factor regulating primary amino acid biosynthesis. Nicklisch et al. (1976) believe that under light the pathway of glutamic acid biosynthesis is induced not only by GDH, but also by glutamate synthase (GOGAT). Associated with light is the formation of glutamine by isolated chloroplasts (Mitchell and Stocking, 1975) and so is the activity level of GDH and aminotransferase (Postius et al., 1976, Postius and Jacobi, 1976; Hedley and Stoddart, 1971). Moreover, Barash et al. (1976) noted a relation between light and the effect of NH_4^+ ions on GDH activity.

In the present work the influence of various mineral nitrogen forms on GDH, GS, GPT and GOT in etiolated and green cucumber cotyledons was investigated.

MATERIAL AND METHODS

Preparation of material. Cucumber seeds (variety 'Monastyrski') were washed with distilled water and placed on Petri dishes

on filter paper moistened with distilled water, and germinated in darkness for 2 days at 27°C. The seedlings were then transferred onto medium with pH 6.0 containing in 1000 ml the following components: KCl — 223 mg $CaCl_2 \cdot 6\ H_2O$ — 98 mg, KH_2PO_4 — 114 mg, $MgSO_4 \cdot 7H_2O$ — 182 mg, iron citrate — 7 mg, H_3BO_3 — 1·543 mg, $MnSO_4 \cdot 5\ H_2O$ — 1.18 mg, $ZnSO_4 \cdot 7\ H_2O$ — 0.571 mg, $CuSO_4 \cdot 5\ H_2O$ — 0.117 mg, H_2MoO_4 — 0·094 mg. Nitrate or ammonium salts were the source of nitrogen. They were added in the following amounts: $NaNO_3$ — 1213 mg (200 mg NO_3-N), $NaNO_3$ — 121 mg (20 mg NO_3-N), $(NH_4)_2SO_4$ — 943 mg (200 mg NH_4-N), $(NH_4)_2SO_4$ — 94 mg (20 mg NH_4-N). One combination of plants was left in darkness, the other was placed under continuous light of fluroscent tubes (light intensity 5500 lux at the plant level). After the time specified in the results, the cotyledons were cut from the seedlings and GS, GDH, GPT and GOT activity was determined in them.

Analytical methods. GS and GDH extraction was done according to Harper and Paulsen (1969) and GPT and GOT were extracted after Hedley and Stoddart (1971). The enzymatic extracts were centrifuged at 2°C for 15 min at 20 000 g. The supernatant served as source of the enzyme.

Glutamine synthetase. GS activity was determined after Elliot (1953). The incubation mixture contained: 0.35 ml 0.143 M $NH_2OH \times HCl$ and 0.143 M $MgSO_4$ in 0.57 M tris-HCl, pH 7.2, 0.25 ml 0.5 M L-sodium glutamate, pH 7.2, 0.25 ml 25 mM ATP, 0.25 ml enzymatic extract. The samples were incubated for 15 min at 30°C. The reaction was interrupted with 1.1 ml of iron reagent prepared according to O'Neal and Joy (1973b), consisting of 0.37 M $FeCl_3$, 0.67 M HCl, 0.2 M trichloroacetic acid in 1000 ml. The γ-glutamylhydroxamate formed was determined at 540 nm.

Glutamate dehydrogenase. GDH activity determination was based on the method of Pahlich and Joy (1971). The incubation mixture contained: 0.1 ml 0.2 M sodium α-ketoglutarate, pH 7.5—8.0, 0.05 ml 3.2 M $(NH_4)_2SO_4$, 0.4 ml 2 mM NADH, 2.15 ml 0.2 M tris-HCl, pH 8.0, and 0.3 ml of enzymatic extract. The samples were incubated for 20 min at 30°C. The amount of oxidized NADH was determined at 340 nm.

Alanine aminotransferase and aspartate aminotransferase. GPT and GOT were determined by the method previously described (Kubik-Dobosz 1975).

All the results reported are means of 3 replications of the particular experiments.

RESULTS

Figs. 1 and 2 show the changes in GS activity in cucumber cotyledons growing for 6 days in light or in darkness on nitrate or ammonium medium containing 200 mg of nitrogen. GS activity increased rapidly in the cotyledons of cucumber seedlings receiving the nitrate form of nitrogen, both in light and in darkness, reaching maximal values after 4 days of growth on the medium, and then decreasing (Fig. 1). Activity in light was higher than in darkness, irrespective of the date of analysis. In plants cultured on ammonia medium the changes in GS activity were analogous (Fig. 2).

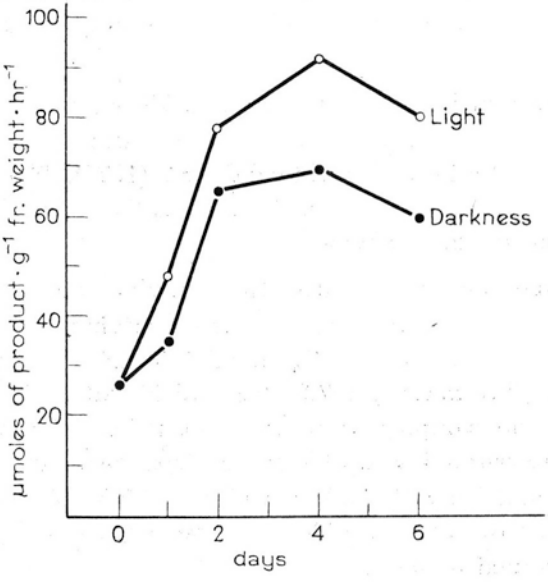

Fig. 1. Glutamine synthetase (GS) activity in cucumber cotyledons growing in light and in darkness on nitrate medium (200 mg NO_3-N)

In the successive experiment the nitrogen content in the medium was changed. Two-day cucumber seedlings were divided into 5 groups and cultivated in light or in darkness on media with basic macro- and micro-elements and nitrogen in the following amounts: no nitrogen (—N), 20 mg NO_3-N, 200 mg NO_3-N, 20 mg NH_4-N and 200 mg NH_4-N. GS, GDH, GPT and GOT activity was determined in the cotyledons. It was found that when the plants grew on media deprived of nitrogen, light after 4 days inhibited GS activity in the cotyledons (Table 1). When mineral nitrogen was present in the medium, GS activity in the cotyledons of cucumbers growing in light was always higher than

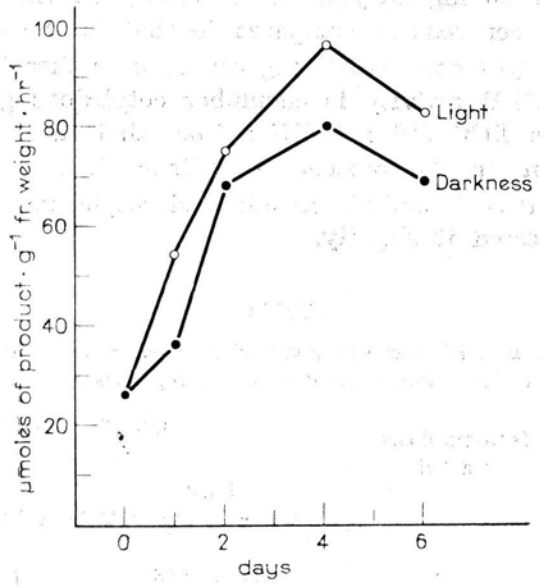

Fig. 2. Glutamine synthetase (GS) activity in cucumber cotyledons growing in light and darkness on ammonium medium (200 mg NH_4-N)

in the etiolated plants notwithstanding the form of nitrogen and the dose applied. Nitrate and ammonium ions stimulated GS when the seedlings grew in light, whereas the amount of nitrogen in the medium had no major effect on the level of GS activity. No enhancement of GS activity under the influence of nitrogen was observed when the plants grew in darkness.

Table 1

Glutamine synthetase (GS) activity in cucumber cotyledons after 4 days of growth in light or in darkness with different mineral nitrogen sources and doses

Nitrogen source	Nitrogen doses mg N/l	Growth conditions	
		Light	Darkness
		μmoles of product · g^{-1} fr. weight · hr^{-1}	
−N	0	44.7±1.47	70.5±0.86
NO_3^-	20	85.5±1.73	72.3±1.70
	200	98.0±6.69	70.8±1.60
NH_4^+	20	92.2±2.39	68.8±1.65
	200	83.2±1.87	64.8±1.35

It results from the data presented in Table 2 that light inhibited GDH activity in cucumber cotyledons both in the -N combination and in those with various forms and doses of mineral nitrogen. GDH activity in plants cultured in light was inhibited by NO_3^- ions. Under

the influence of 20 mg NO_3-N, GDH activity in the cotyledons was depressed to 63 per cent as compared to that in the -N combination. An increased NO_3-N dose to 200 mg caused a further fall of the GDH activity level. GDH activity in cucumber cotyledons growing on ammonium salts in light did not differ from that in seedlings cultured without nitrogen. In the presence of nitrate ions no major changes were observed in GDH activity in etiolated cotyledons, whereas ammonium ions depressed it slightly.

Table 2

Glutamate dehydrogenase (GDH) activity in cucumber cotyledons after 4 days of growth in light or in darkness with different mineral nitrogen sources and doses

Nitrogen source	Nitrogen doses mg N/l	Growth conditions	
		Light	Darkness
		μmoles of oxid. NADH · g^{-1} fr. weight · hr^{-1}	
−N	0	13.50±1.68	27.48±0.81
NO_3^-	20	8.52±0.93	25.02±1.49
	200	4.98±0.82	25.50±0.96
NH_4^+	20	12.00±0.85	23.52±1.01
	200	13.50±1.06	24.00±0.62

The results of changes in GPT activity in the cotyledons of cucumber seedlings growing for 4 days in light or in darkness with various nitrogen doses are listed in Table 3.

Table 3

Alanine aminotransferase (GPT) activity in cucumber cotyledons after 4 days of growth in light or in darkness with different mineral nitrogen sources and doses

Nitrogen source	Nitrogen doses mg N/l	Growth conditions	
		Light	Darkness
		μmoles of product · g^{-1} fr. weight · hr^{-1}	
−N	0	184.8±6.7	229.8±4.3
NO_3^-	20	240.0±5.6	240.0±8.5
	200	240.0±7.1	229.8±6.7
NH_4^+	20	220.2±9.2	255.0±11.8
	200	265.2±8.6	240.0±9.6

If the medium was deprived of nitrogen, light inhibited GPT activity in the cotyledons, whereas in the presence of nitrate or ammonium nitrogen this effect was not observed. After applying to plants exposed to light 20 or 200 mg NO_3-N, GPT activity increased in the cotyledons by 30 percent. When NH_4-N was introduced into the medium

GPT activity rose by 43 per cent. The lower ammonium nitrogen dose produced a smaller effect. In etiolated cotyledons no influence of mineral nitrogen on GPT activity could be observed.

From the data in Table 4 it results that light strongly inhibited GOT activity in cucumber cotyledons. At the same time, in seedlings growing in light, a decrease of activity of this enzyme was noted after introduction into the medium of nitrate or ammonium ions. The depression of activity being more pronounced when the 10-fold increased nitrogen dose was applied. In darkness the depression of GOT activity under the influence of nitrogen occurred only in the case of the combination with ammonium.

Table 4

Aspartate aminotransferase (GOT) activity in cucumber cotyledons after 4 days of growth in light or in darkness with different mineral nitrogen sources and doses

Nitrogen source	Nitrogen doses mg N/l	Growth conditions	
		Light	Darkness
		μmoles of product · g^{-1} fr.weight · hr^{-1}	
−N	0	210.0±4.1	529.8±8.5
NO_3^-	20	195.0±2.8	529.8±6.4
	200	184.8±3.3	499.8±7.3
NH_4^+	20	195.0±2.7	469.8±3.7
	200	154.8±5.4	360.0±3.9

DISCUSSION

The maximal GS activity in cucumber seedlings in the first days of development (Figs 1 and 2) confirms the results reported by Marwaha and Juliano (1976) for rice seedlings. Maximal activity of the enzymes of nitrogen metabolism, among them of glutamine synthetase was observed by these authors between the 7th and 10th day after germination. In the present experiments it was found that in darkness the relation is identical as in light, that is maximal GS activity occurs in the cotyledons of seedlings on the 4th day of growth on the medium. Although, as demonstrated by Mecke and Holzer (1966), Mitchell and Stocking (1975), Rhodes et al. (1975), high ammonium ion doses are toxic to the enzyme, a distinct decrease of GS activity was not observed in the cotyledons growing in the presence of 200 mg NH_4-N (Table 1). Mitchell and Stocking (1975) also observed that the stimulating action of lower NH_4^+ concentrations on glutamine synthesis in isolated spinach chloroplasts is more pronounced in light than in darkness. The present results (Table 1) seem to indicate a correlation between changes in the glutamine level and GS activity.

Light is known to have an important (though indirect) effect on the activity of enzymes reducing nitrates to an assimilable form that is to ammonium ions (Beevers and Hageman, 1969; Buczek, 1976). In leaves of many plant species nitrates do not induce nitrogen reductase activity as long as the plants are not exposed to light (Chen and Ries, 1969, Travis et al., 1970). It seemed strange why GS activity was so high in cotyledons of seedlings growing in darkness on nitrate medium. A satisfactory explanation was given by the results obtained in a successive experiment (Table 1). In the cotyledons of cucumbers grown on medium without nitrogen, GS activity was about 40 per cent lower in light than in those grown in darkness. Probably in etiolated cucumber cotyledons utilization of endogenous nitrogen accumulated in these storage organs is more efficient. This nitrogen may constitute the most important source of ammonium nitrogen metabolised by GS in darkness. This finds confirmation in the work of Blevins et al. (1974) who demonstrated that green barley seedlings take up more NO_3^- than do etiolated ones and in the paper of Canvin and Atkins (1974) who observed that light stimulates nitrate nitrogen and ammonium nitrogen incorporation into the amino acids occurring in barley leaves. The increase of GDH activity in cucumber cotyledons grown in darkness (Table) is in agreement with the results of Postius et al. (1976) who observed stimulation of GDH activity in darkness. These changes were correlated with the activity of the particular GDH isoenzymes and disappeared when the plants were again exposed to light. Although these authors did not find any changes in GOT activity after keeping the plants in darkness, the present investigations (Table 4) and the data of Liu and Huang (1977) prove that in cucumber cotyledons light inhibits GOT activity, this being supposedly connected with transamination of aspartic acid stored in storage protein of cucumber cotyledons. This process running more intensively in darkness than in light.

An increase of GDH activity in wheat leaves under the influence of darkness lasting 16 h was noted by Nicklisch et al. (1976). Duke et al. (1978) suggest that the probable mechanism of GDH inhibition in light may consist in an inhibitory action of sucrose produced in light. This is confirmed by the studies of Duke and Koukkari (1977) who demonstrated that sucrose added to the incubation medium markedly reduced the enzyme activity. Sahulka et al. (1975) suggest that sucrose inhibits de novo synthesis of GDH. It was found by Barash et al. (1976) that in light GDH activity in oat leaves is 12 times higher in the presence of NH_4^+ ions than in the presence of water, whereas in darkness this increase was only twofold. In the present investigations a much higher GDH activity was revealed in

the cotyledons of cucumbers growing in light in the presence of ammonium salts than in the presence of nitrate salts (Table 2).

The depression of GDH activity observed by us in green cucumber cotyledons as compared with that in etiolated ones confirms the suggestion of Nicklisch et al. (1976) hat in light biosynthesis of glutamic acid along the GS/GOGAT pathway is preferred. This pathway of amino acid synthesis would be specially favoured in the presence of exogenous nitrogen, notwithstanding its forms or doses, as indicated by the investigations of GS activity (Table 1) which showed that in light incorporation of the taken up nitrogen into the amide groups of glutamine is more intensive in light than in darkness. Thus, the results here obtained suggest that nitrogen taken up in light is built into amino acids through the GS/GOGAT system, whereas in darkness it is rather GDH that functions.

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Wpływ światła i mineralnych form azotu na aktywność niektórych enzymów w liścieniach Cucumis sativus L.

Streszczenie

W badaniach przeprowadzonych na siewkach ogórków odmiany 'Monastyrski', rosnących w pożywkach azotanowych lub amonowych na świetle oraz w ciemności, wykazano w liścieniach stopniowy wzrost aktywności syntezy glutaminowej (GS) w ciągu 6 dni uprawy. Maksymalny poziom aktywności GS zaobserwowano w 4 dniu wzrostu siewek w pożywkach. W obecności jonów azotanowych i amonowych światło stymulowało po 4 dniach aktywność GS oraz hamowało aktywność dehydrogenazy glutaminianowej (GDH) i aminotransferazy asparaginianowej (GOT) nie zmieniając jednocześnie aktywności aminotransferazy alaninowej (GPT). Jeżeli w pożywkach brak było azotu, aktywność GS, GDH, GPT i GOT w liścieniach ogórków rosnących przez 4 dni na świetle była znacznie niższa niż aktywność tych enzymów w etiolowanych liścieniach siewek. Obecny w pożywce azot azotanowy podwyższał aktywność GS i GPT oraz obniżał aktywność GDH i GOT w liścieniach siewek uprawianych na świetle, natomiast nie wpływał na aktywność enzymów w liścieniach siewek rosnących w ciemności. Jony amonowe w obecności światła stymulowały aktywność GS i GPT oraz hamowały aktywność GOT, natomiast w ciemności obniżały aktywność GDH i GOT nie wpływając równocześnie na pozostałe enzymy.