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Abscisic acid as a factor in regulation of photosynthetic carbon metabolism of pea seedlings

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Abstract

The influence of abscisic acid (ABA) on carbon metabolism and the activity of ribulosebisphosphate (RuBP) and phosphoenolpyruvate (PEP) carboxylases in 8-day-old pea seedlings was investigated. It was endeavoured to correlate the changes observed in metabolic processes with the endogenous ABA level. In plants treated with ABA incorporation of labeled carbon into sucrose, glucose, fructose and sugar phosphates was depressed, while ^{14}C incorporation into starch, ribulose and malic acid was enhanced. The activity of RuBP carboxylase was considerably lowered, whereas that of PEP carboxylase was slightly increased. It is considered that inhibition of photosynthesis due to the action of ABA is caused to a great extent by the obstruction of the C-3 pathway and reduced activity of RuBP carboxylase, whereas β-carboxylation was not blocked.

INTRODUCTION

In the search for the causes of photosynthesis inhibition by abscisic acid (ABA) (Mittelheuser and van Steveninck 1971, Poskuta et al. 1972) the influence of ABA on the light reaction in photosynthesis (Bauer et al. 1976, Zima and Sestak 1978) and the photosynthetic reaction in darkness (Sankhla and Huber 1974a, 1975, McLaren and Smith 1976) were studied. No significant influence of ABA on the photochemical stage of photosynthesis could be demonstrated, but many reactions occurring in darkness were changed. Changes were found in the activity of several photosynthetic enzymes. A considerable decrease of RuBP carboxylase activity in plants subjected to the action of ABA was observed by Wellburn et al. (1973) and Sankhla and Huber (1974a, 1975), whereas the activity of PEP

carboxylase was markedly stimulated by ABA. The changes in the activity of the basic photosynthetic enzymes evoked as consequence changes in active carbon incorporation. In C-4 plants an enhanced ^{14}C incorporation into the organic acids fraction was observed (Sankhla and Huber 1974a), whereas in wheat (C-3 plants) ^{14}C incorporation into malic acid, alanine and asparagine increased, but it was decreased in 3-phosphoglyceric acid (Sankhla and Huber 1975). Abscisic acid also affected the activity of many enzymes of amino acid metabolism (Huber and Sankhla 1974, Sankhla and Huber 1974b, Huber et al. 1977). The increase in proline content (Singh et al. 1973, Huber et al. 1977) may also be a manifestation of the influence of ABA on amino acid metabolism. An increase in soluble sugars and starch content under the influence of ABA was noted in *Lemna minor* by McLaren and Smith (1976). Bauer et al. (1976) did not find differences in ^{14}C incorporation into the particular photosynthesis products in *Lemna minor* in spite of the greatly reduced intensity of labeled carbon incorporation and enhanced PEP and RuBP carboxylases and maleate enzyme activities.

The quoted data indicate unequivocally that photosynthesis inhibition by ABA may be due to the action of the latter on photosynthetic reactions occurring in the dark, however, opinions are not uniform as regards the nature of this action.

The aim of this study was investigation of the influence of ABA on carbon metabolism and the activity of the basic photosynthetic enzymes — RuBP and PEP carboxylases — in pea, in order to establish the relation between the observed changes and the endogenous ABA level.

MATERIAL AND METHODS

The material consisted of 8-day-old pea seedlings (*Pisum sativum* L.) cv. "Bordi" grown from seeds soaked for 24 h in abscisic acid 1.0 and 10.0 ppm solution (3.8×10^{-6} M and 3.8×10^{-5} M) and in distilled water (control).

The seeds were germinated for 4 days in sand in a thermostat at 24°C. Germinated seeds were transferred into Knop's full nutrient medium where they grew under controlled conditions of fluorescent tube light 7.5 W/m² at 28°C under 16-h daylight and 21°C in the night.

For measurement of the kinetics of C-14 labeled early products of photosynthesis a method of continuous $^{14}CO_2$ supply over short time periods was used. Photosynthesis measurements were performed in an air atmosphere containing 380 ppm $^{14}CO_2$ with 50 μCi under irradiation intensity of 100 W/m². Photosynthesis in $^{14}CO_2$ was run for 1, 2, 5 and 10 min, then the photosynthesising part of the plant was cut off and thrown into boiling 80 per cent ethanol. Ion exchange column chromato-

graphy was applied for separation and identification of the radioactive compounds as well as two-dimentional paper chromatography and autoradiography (M a l e s z e w s k i and L e w a n t y 1972).

RuBP and PEP carboxylase activities were determined by the modified method of S l a c k and H a t c h (1967).

ABA content was determined after F a l t y n o w i c z et al. (1981). The results are means of 3-4 measurements. They were subjected to statistical analysis by Student's t test at p = 0.05 per cent.

RESULTS

As seen in Fig. 1, radioactive carbon incorporation by the control seedlings and those treated with ABA is a linear function of time. A considerable decrease in ^{14}C incorporation by the plants treated with ABA is noticeable. Inhibition of incorporation of labeled carbon into the fraction of soluble products was also observed in seedlings grown from seeds treated with ABA as compared with the control ones after all times of exposure of plants to $^{14}CO_2$ (Fig. 2). Incorporation of carbon into starch, however, was stimulated (Fig. 3) the more the higher was ABA concentration. The latter compound enhanced ^{14}C incorporation into the soluble sugars fraction, particularly after 1- and 3-minute exposure (Fig. 4 a-c). The action of ABA was more effective, when used in a 10 ppm concentration. Under the influence of ABA differences were also noted in incorporation of labeled carbon into organic acids. In the control plants the highest percentage of carbon incorporated into this fraction was observed after 1 min, and with protraction of the time of photosynthesis the share of radioactivity falling to organic acids decreased markedly in the soluble fraction. A similar course of ^{14}C incorporation into organic acids was observed in plants treated with ABA in a 1.0 ppm concentration, the percentage, however, of labeled carbon contained in this fraction was much lower than in the control. In plants treated with ABA in a 10 ppm concentration the highest percentage of ^{14}C incorporation occurred after 3 min of exposure to $^{14}CO_2$ and decreased greatly after 5 and 10 min of assimilation.

Radioactive carbon incorporation into the amino acid fraction has a similar course in the control and ABA-treated plants.

Analysis of the soluble sugars fraction in plants treated with ABA and control ones showed the greatest differences in ^{14}C incorporation into sucrose, giucose with fructose and ribulose (Fig. 5 a-c). In the control seedlings the amount of ^{14}C incorporated into sucrose was highest and remained at a constant level after all times of exposure in $^{14}CO_2$. On the other hand, in plants treated with ABA ^{14}C incorporation into sucrose was much lower. Abscisic acid inhibited also labeled carbon

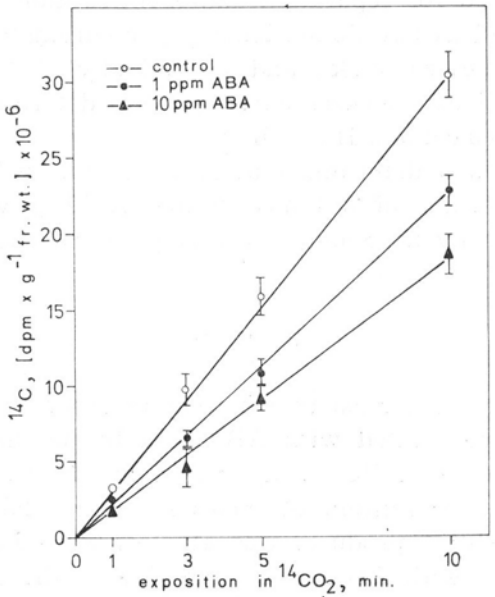

Fig. 1. ^{14}C incorporation into control plants and those treated with abscisic acid in 1.0 and 10.0 ppm concentrations

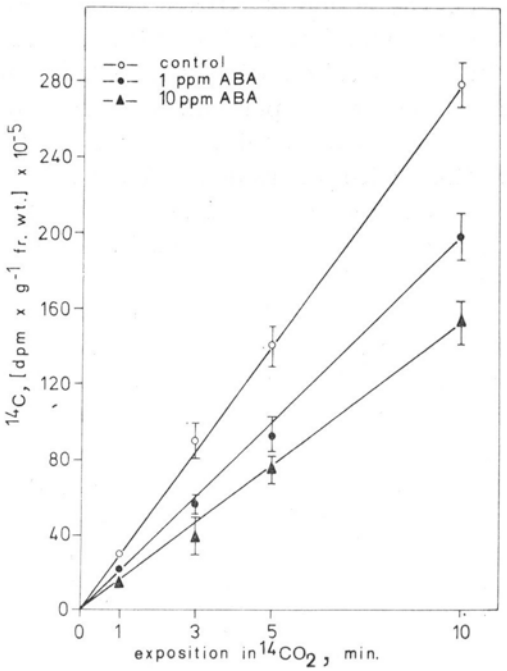

Fig. 2. ^{14}C incorporation into soluble fraction of photosynthesis products in control and ABA-treated plants at concentrations of 1.0 and 10.0 ppm

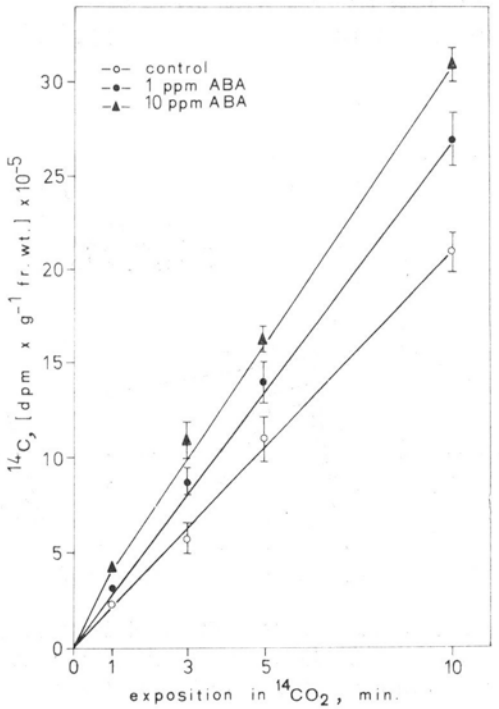

Fig. 3. ^{14}C incorporation into starch in control plants and those treated with ABA in 1.0 and 10.0 ppm concentrations

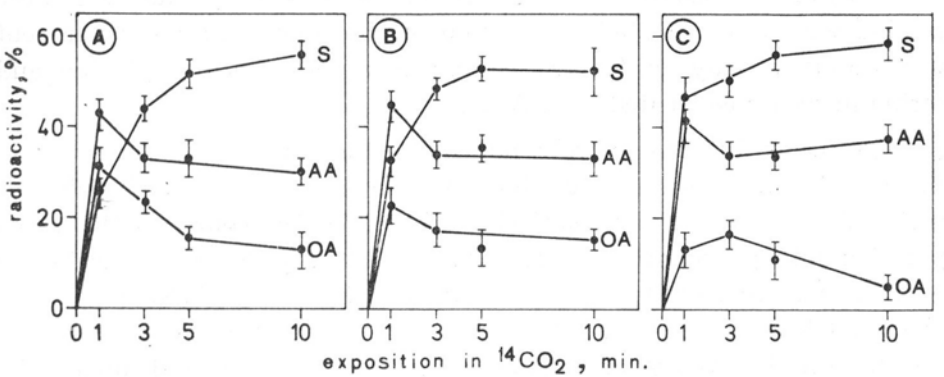

Fig. 4. Percentual share of sugars (S), amino acids (AA) and organic acids (OA) radioactivity in that of the soluble fraction of photosynthesis products in control plants (A) and those treated with ABA in 1.0 ppm (B) and 10.0 ppm (C) concentrations

incorporation into glucose and fructose. Labeled ribulose was identified in control plants as late as after 3 min of photosynthesis and the contribution of this compound to the total radioactivity of soluble sugars

was very small. In plants treated with ABA the ribulose level was much higher, particularly when the hormone was applied in higher concentrations.

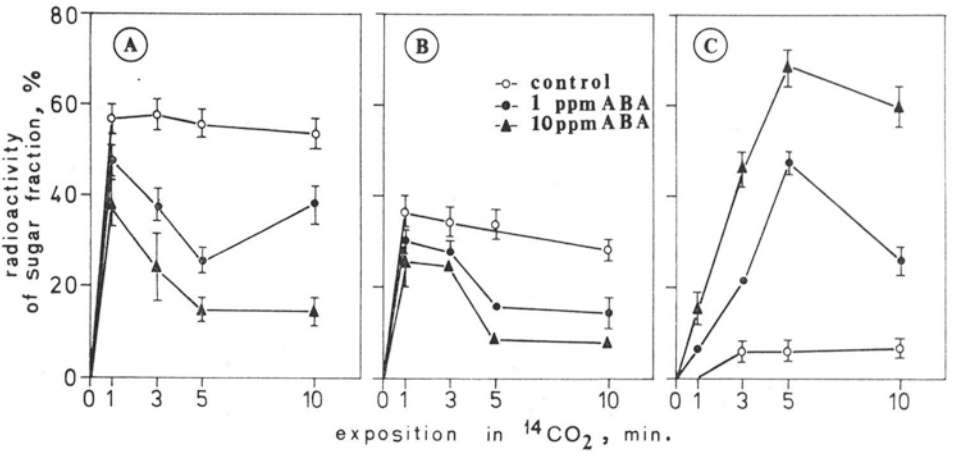

Fig. 5. Percentual share of sucrose (A), glucose + fructose (B) and ribulose (C) radioactivity in that of the soluble sugars fraction in control and ABA-treated plants in concentrations of 1.0 and 10.0 ppm

Aspartic and glutamic acid, alanine as well as glycine and serine were identified in the amino acid fraction. Labeling of these compounds with radioactive carbon was similar in the control plants and those treated with ABA. A slight inhibition of the order of several per cent was observed only in labeled carbon incorporation into glycine and serine in seedlings treated with ABA.

Significant differences were noticed in the organic acids fraction as regards ^{14}C incorporation into the phosphates of sugars, 3-phosphoglyceric acid (PGA) and malic acid (Figs. 6 and 7). The course of ^{14}C incorporation into phosphates of sugars and PGA (Fig. 6) is similar in the control and ABA-treated plants, however, there is a distinct decrease in their labeling under the influence of ABA. The share of malic acid in the total radioactivity of organic acids (Fig. 7) increased under the influence of ABA. α-Ketoglutaric acid and a nonidentified compound "y" were also determined, but the labeling of these compounds was similar in control plants and those treated with ABA.

A distinct inhibition of the activity of RuBP carboxylase was observed in plants under the influence of ABA as compared with that in control seedlings. PEP carboxylase activity, however, was slightly stimulated by ABA (Fig. 8).

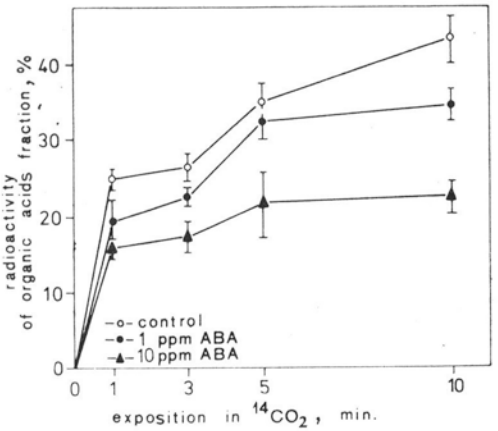

Fig. 6. Percentual share of sugar phosphates and 3-phosphoglyceric acid (PGA) radioactivity in that of the organic acids fractions in control plants and those treated with ABA in 1.0 and 10.0 ppm concentrations

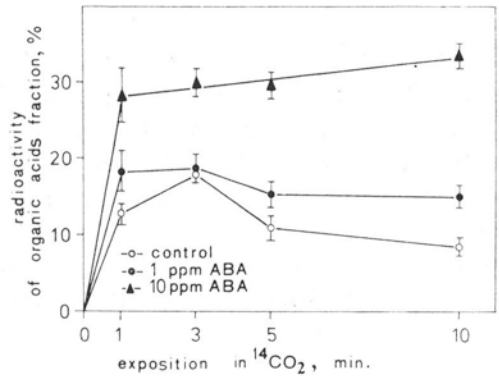

Fig. 7. Percentual share of malic acid radioactivity in that of the organic acids fraction in control plants and those treated with ABA in 1.0 and 10.0 ppm concentrations

ABA content in the leaves of plants treated with this hormone was higher than in control plants (Table 1). A greater amount of ABA was

Table 1

ABA content in leaves of 8-day-old pea seedlings grown from seeds soaked for 24 h in ABA solution of 1.0 and 10.0 ppm concentration

ABA concentration in the solution, ppm	ABA content in leaves, µg/kg fresh weight
Control	61.19±19.6
1.0	88.00±11.5
10.0	131.36±30.8

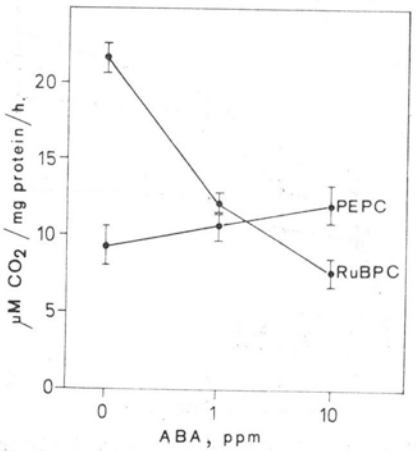

Fig. 8. Ribulosebisphosphate (RuBP) and phosphoenolpyruvate (PEP) carboxylases activity in control plants and those treated with ABA in 1.0 and 10.0 ppm concentrations

observed in plants grown from seeds treated with ABA in a 10 ppm concentration. The results obtained indicate that abscisic acid acts on carbon metabolism. Carbon incorporation was greatly reduced in the fraction of soluble products. The interrelations between the analysed three groups of compounds in this fraction were also changed, particularly between sugars and organic acids. In plants treated with ABA ^{14}C incorporation into sucrose, glucose, fructose and phosphates of sugars was decreased, while labeling of ribulose, malic acid and starch was stimulated.

DISCUSSION

In the here described experiments the photosynthetic apparatus developed under conditions of disturbed hormonal equilibrium, owing to the introduction into the seeds of exogenous ABA (Faltynowicz et al. 1981). This could lead as consequence to changes in the ultrastructure of the choloroplasts (Wellburn et al. 1973), disintegration of their thylakoids and of the membranes of the chloroplast envelope (Colquhoun et al. 1975), making in this way difficult the flow of triosophosphate beyond the chloroplast region and giving as effect a reduced production of sucrose, since it is a compound synthesized within the cytoplasm from triosophosphates translocated from chloroplasts (Kelly et al. 1976). A confirmation of the obstructed flow of tricarbon compounds beyond the chloroplasts under the action of ABA may be found in the reduced permeability of the chloroplast membranes for 3-phosphoglyceric acid, observed by McLaren and Smith (1977) in plants treated

with ABA. Sucrose is the main compound undergoing translocation and its reduced synthesis may be evidence of difficulties in the translocation of its assimilates. This in turn may be one of the causes limiting photosynthesis intensity (Hartt 1963, Starck 1976). The decreased ^{14}C incorporation into sucrose is incompatible with the accumulation of this compound under the influence of ABA, observed by McLaren and Smith (1976). These authors, however, believe that the increase in the amount of sucrose is due, not to enhanced synthesis, but rather to its limited utilisation.

It would seem that the decrease in radioactivity in glucose + fructose (Fig. 5b) is caused by an enhanced starch synthesis (Fig. 3). This result finds confirmation in many papers in which increasingly intensive synthesis and accumulation of starch was noted with higher ABA levels (Mansfield and Jones 1971, Newton 1974, McLaren and Smith 1976). This starch was consumed when the plants were transferred to a medium deprived of ABA.

A similar phenomenon was observed with a natural rise of the endogenous ABA level, for instance in resting buds in spring (van Overbeck and Mason 1968, Eagles and Wareing 1964) and during water stress (Wright and Hiron 1972, Kennedy 1977). Exogenously applied ABA thus evokes similar effects as that produced endogenously in stress conditions.

The increase in the ribulose pool observed in plants treated with ABA (Fig. 5c) seems interesting. Accumulation of ribulose, that is a sugar connected with renewal of the CO_2 acceptor (ribulose diphosphate), suggests that abscisic acid may cause splitting off of the phosphate groups, this finding confirmation in the depression of radioactivity of the sugar phosphate fraction observed in the present study. It is possible that ABA causes running off of intermediate metabolites of the Calvin cycle by stimulating splitting off of the phosphate group from ribulose--5-phosphate. The increased ribulose pool in ABA-treated plants has so far not been reported by other investigators.

Attention should also be called to the stimulation by ABA of the participation of malic acid in the organic acids pool (Fig. 7). It is a compound characteristic for the C-4 pathway and for the tendency to stimulate phosphoenolpyruvate carboxylase activity. The pea is a typical C-3 plant, and all plants of this group partly bind carbon like those of type C-4, that is as the result of carboxylation of phosphoenolpyruvate. The share of this pathway in CO_2 incorporation, however, is only several per cent (Slack and Hatch 1967, Raghavendra and Das 1977).

ABA significantly affected the ribulosebisphosphate carboxylase activity and it may be suggested that the inhibitory influence of ABA on

photosynthesis is due among other things to the limitation by this hormone of RuBP carboxylase activity.

On the basis of the present results a hypothetic mechanism may be proposed presented in Fig. 9 of the modified metabolism in plants subjected to the action of ABA. This hormone caused a rapid flow of assimilated carbon via PGA and sugar phosphates to starch, and stimulation of synthesis and accumulation of the latter compound with simultaneous inhibition of sucrose, glucose and fructose formation. Ribulose accumulation was also considerably stimulated. 3-Phosphoglyceric acid may also undergo transformation to phosphoenolpyruvate since β-carboxylation of this compound is not blocked by ABA, and there even may be a certain stimulation of CO_2 incorporation via β-carboxylation of phosphoenolpyruvate. This leads to the conclusion that transformations similar to those occurring in C-4 plants are not depressed and the limitation of CO_2 assimilation is the result of obstruction of the carbon C-3 pathway.

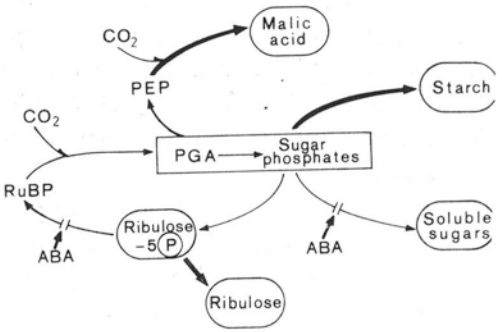

Fig. 9. Diagram of metabolic process in photosynthesis regulated by abscisic acid

It may be concluded on the basis of the results obtained that photosynthesis inhibition due to ABA was caused to a large extent by limitation of the C-3 carbon pathway and decrease of RuBP carboxylase activity, whereas β-carboxylation was not blocked by the tested hormone. It seems, however, that the depressed photosynthetic activity may be a resultant of disturbances of many processes by ABA. The latter by regulation of nucleic acids synthesis, of the activity of numerous enzymes and hormones (W a r e i n g 1978) may influence metabolic systems both in chloroplasts and in the cytoplasm and in this way regulate the photosynthesis process.

It would seem that the main role of ABA in carbon metabolism regulation consists in orienting the flow of incorporated carbon towards starch and ribulose, that is compounds stored under unfavorable conditions and readily utilised when these conditions cease to prevail. It is believed, therefore, that ABA modifies the plant metabolism so as

to increase the possibility of survival under unfavourable conditions and maintain the intrasystemic homeostasis.

On the basis of differences in the ABA content in the control plants and those treated with ABA (Faltynowicz et al. 1981) and differences in the content of this hormone in the leaves of the plants it was demonstrated that the metabolic changes observed in the seedlings were caused by an increased ABA level.

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Kwas abscynowy jako czynnik regulujący metabolizm węgla podczas fotosyntezy u siewek grochu

Streszczenie

Badano wpływ kwasu abscysynowego (ABA) na metabolizm węgla i aktywność karboksylazy rybulozodwufosforanu (RuBP) i fosfoenolopirogronianu (PEP) w 8-dniowych siewkach grochu. Starano się skorelować zaobserwowane zmiany w badanych procesach metabolicznych z poziomem endogennego ABA. W roślinach poddanych działaniu ABA było mniejsze wbudowywanie znakowanego węgla do sacharozy, glukozy, fruktozy i fosforanów cukrów oraz zachodziła znacznie wzmożona inkorporacja ^{14}C do skrobi, rybulozy i kwasu jabłkowego. Znacznemu zmniejszeniu ulegała również aktywność karboksylazy RuBP, natomiast aktywność karboksylazy PEP była tylko nieznacznie podwyższona. Sądzi się, że hamowanie fotosyntezy wywołane działaniem ABA spowodowane jest w znacznym stopniu ograniczeniem drogi C-3 i zmniejszeniem aktywności karboksylazy RuBP, natomiast β-karboksylacja nie była przez badany hormon blokowana.