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Ferredoxin-Dependent Glutamate Synthase Activity during the First Developmental Stages of Wheat Plants as Affected by Calcium Deficiency

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Abstract. The delay in development of winter wheat plants grown under conditions of Ca-deficiency manifested itself also in the time curve of ferredoxin-dependent glutamate synthase activity. Up to the stage of the first leaf the activity increased. Thereafter it gradually decreased similarly as under conditions of complete nutrition, but with a shift of about 7 days as compared with plants given complete nutrition. Increased glutamate synthase activity in Ca-deficient plants may be one of the factors leading to a high glutamate level under these conditions.

The investigations of the role of the macronutrient calcium in nitrogen metabolism of plants has shown, among other things, that the glutamate level is regularly increasing under conditions of Ca-deficiency in pumpkin plants which are very sensitive to Ca-deficiency, as well in wheat (ČERNÁ 1974) which is less sensitive. Glutamate occupies a central position in nitrogen metabolism in plant leaves : it is the main amino donor for transaminations, as well as for the GS/GOGAT cycle mediating primary ammonia assimilation and its reassimilation (MIFLIN *et al.* 1981). The skeleton of glutamic acid provides the immediate material for protein amino acids such as proline, arginine, hydroxyproline. Furthermore, glutamate plays an important role as a building block (peptides, proteins) as well as in transport.

Therefore, attention was focused on the basic metabolic pathways in which glutamate is formed or consumed. Glutamate dehydrogenase-GDH (NOVOTNÁ and ČINČEROVÁ 1985) and glutamine synthetase-GS (NOVOSADOVÁ 1982) were investigated. The present report deals with the glutamate synthase-GOGAT pathway. All mentioned pathways of nitrogen incorporation into organic compounds involve glutamate as substrate or as product of the single reactions. Therefore all of them affect glutamate level in some way or other.

Glutamate synthase exists in two forms in higher plants. One form uses NAD(P)H as reducing agent (E.C.1.4.7.(13).14), while reduced ferredoxin is used by the other one (E.C.1.4.7.1.). The NAD(P)H-dependent enzyme was first isolated from higher plant tissues devoid of chlorophyll (DOUGALL

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1974). The second enzyme form dependent on ferredoxin as electron source was originally detected in pea chloroplasts (LEA and MIFLIN 1974).

Recently the presence of both forms was confirmed in green, as well as non-green tissues. However, there exists a quantitative difference — the ratio of activity of the two forms is different in different tissues. In photosynthetic tissues ferredoxin-dependent GOGAT plays a central physiological role in nitrogen assimilation, while the same is true for glutamate synthase linked with pyridine nucleotides in tissues devoid of chlorophyll.

MATERIAL AND METHODS

a) Cultivation of Plants

Seeds of winter wheat, *Triticum aestivum* L., cv. Grana were germinated for 3 days in distilled water. Thereafter, the seedlings were transferred to darkened cultivation vessels containing nutrient solutions. Two experimental variants were compared:

1. Knop's nutrient solution, NS: (LAŠTŮVKA and MINÁŘ 1967): $Ca(NO_3)_2$ — 3.487 mmol $l^{-1}H_2O$, KNO_3 — 1.414 mmol $l^{-1}H_2O$, KCl — 0.953 mmol $l^{-1}H_2O$, KH_2PO_4 — 0.821 mmol $l^{-1}H_2O$, $MgSO_4$ — 1.187 mmol $l^{-1}H_2O$, EDTA Fe — 5 p.p.m.
2. Knop's solution without calcium, -Ca: Calcium was replaced by an equivalent amount of sodium.

Each plant received 60 ml of the adequate nutrient solution for a week. The solution was filled up with water only and exchanged every week.

Conditions of cultivation:	illuminance	10 000 lx
	temperature	20 ± 1 °C
	photoperiod	16 h
	moisture	80 ± 2 %

The plants were sampled 4times during their early developmental stage, *i.e.* on days 10, 17, 24 and 31 of cultivation. Further manipulations (see b) were performed at a temperature of 0—4 °C (NICKLISCH *et al.* 1976).

b) Determination of Enzyme Activity

Wheat leaves were homogenized with Na-K phosphate buffer (0.05 mol l^{-1}, pH 7.00) supplemented with Polyclar AT (1 %). The homogenate was centrifuged at 22 000 × *g*. The supernatant was desalted on a special centrifugal column containing Sephadex G-25 fine (KOHL 1971). The reaction mixture contained in 0.53 ml 0.25 ml of Na-K phosphate buffer (0.1 mol l^{-1}, pH 7.00); 0.15 ml of extract; 0.15 ml of ferredoxin solution (2.3 mg ml^{-1}); 0.05 ml of fresh solution of Na-dithionite (1 %); 0.01 ml of distilled water; 0.01 ml of glutamine (0.1 mol l^{-1}, Loba Chemie Wien, Austria). Aminooxyacetate was added in order to suppress transamination reactions (10 nmol l^{-1}). The reaction was started by adding 0.01 ml 2-oxoglutarate (0.01 mol l^{-1}, Loba Chemie, Wien, Austria) and stopped after 30 min by perchloric acid. Two controls were added to each sample: one contained the enzyme inactivated by boiling, the second devoid of ferredoxin. Activity of the reaction was evaluated according to the amount of formed glutamate. This was determined after chromatographic separation according to WALLSGROVE *et al.* (1977). Proteins were determined by the method of LOWRY *et al.* (1951).

TABLE 1
Significance of differences

Age of plants [d]	GOGAT activity [nmol mg^{-1} prot.min^{-1}] $\bar{x} \pm s_{\bar{x}}$	t_{10}	P
10	17.70 ± 0.02	13.42	**
17	15.03 ± 0.20	15.32	**
24	11.50 ± 0.11	4.89	**
31	9.97 ± 0.31		

P ** difference significant at the 1 % level

c) Isolation of Ferredoxin

Prior to experiments ferredoxin was isolated from fresh spinach leaves and purified by repeated fractionation followed by dialysis and separation on DEAE cellulose (SAN PIETRO and LANG 1958, TAGAWA and ARNON 1962, SHIN *et al.* 1963, HIEKE, personal communication). All isolation steps were carried out at a temperature of 0—4 °C.

RESULTS

Relation of the Developmental Stage of the Plant to Enzyme Activity

Ferredoxin-dependent GOGAT activity was investigated at four terms of early developmental stage of wheat plants grown in complete nutrient solution (NS):

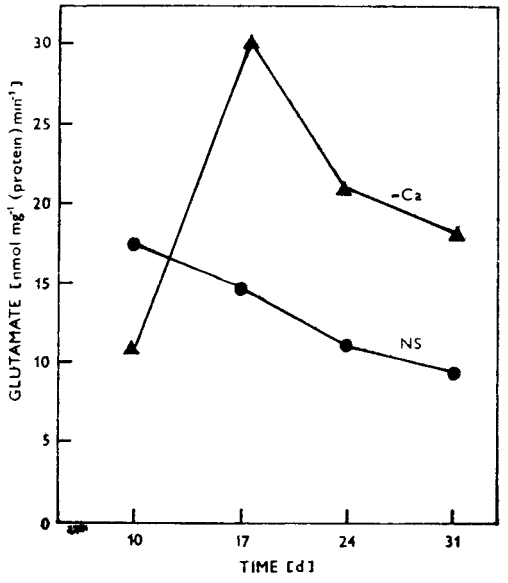

Fig. 1. Effect of Ca-deficiency on GOGAT activity during the first stages of development. — NS: variant of nutrient solution; —Ca: variant of deficient solution. Expression of the enzyme activity in nkat = 60 nmol mg^{-1} prot. min^{-1}.

TABLE 2
Significance of differences between the variants

Variants compared	GOGAT activity [nmol mg^{-1} prot.min^{-1}] $\bar{x} \pm s_{\bar{x}}$	t	P
$NS_{10} \times -Ca_{10}$	$17.70 \pm 0.02 \times 11.16 \pm 0.04$	t_{12} 16.92	**
$NS_{17} \times -Ca_{17}$	$15.03 \pm 0.20 \times 30.76 \pm 0.41$	t_{10} 34.34	**
$NS_{24} \times -Ca_{24}$	$11.50 \pm 0.11 \times 21.31 \pm 0.38$	t_{8} 84.60	**
$NS_{31} \times -Ca_{31}$	$9.97 \pm 0.31 \times 18.07 \pm 0.53$	t_{10} 13.24	**

NS — plants from the variant of nutrient solution;
—Ca — plants from the variant of deficient solution;
P ** difference significant at the 1 % level.

Sampling 1 — 10 day-old plants at the stage of the first leaf (1.1 according to Feekes), development of the second leaf continues.
Sampling 2 — 17 day-old plants at the stage of the second leaf (1.2), development of the third leaf continues.
Sampling 3 — 24 day-old plants at the stage of initial shoot formation (2). The third leaf has developed.
Sampling 4 — 31 day-old plants at the time of full shoot formation (3). The fourth leaf begins to develop.

Only the main shoot was used for the experiments at all terms of sampling. As shown in Fig. 1 glutamate synthase activity in plants provided with complete nutrient solution steadily decreased from the stage of the first leaf, the lowest value being recorded in the last, *i.e.* the fourth sampling. All differences between the individual samplings were significant at the 1 % level (Table 1).

Effect of Ca-Deficiency on GOGAT Activity during the First Stages of Development

Similarly as in plants grown in complete nutrient solution enzyme activity was also investigated at four terms of early development. The plants were also investigated at an age of 10, 17, 24 and 31 days, however, owing to deficient nutrition their development was delayed in comparison with plants in complete nutrient solution.

Sampling 1 — 10 day-old plants, the first leaf is developing, the second one is not yet apparent.
Sampling 2 — 17 day-old plants at the stage of the first leaf (1.1), the second leaf begins to develop.
Sampling 3 — chlorotic plants at the age of 24 days at the stage of the second leaf (1.2).

Sampling 4 — 31 day-old plants, two leaves, plants in a poor condition, nevertheless initiation of shoot formation (2).

As shown in Fig. 1 the dynamics of enzyme activity under conditions of Ca-deficiency was different from that shown by plants grown in a complete nutrient solution. In the first sampling it was lower than in plants from complete nutrition. At an age of 17 days, *i.e.* at the second sampling GOGAT activity abruptly increased, followed by a steady decrease, similarly as in control plants. However, at the last three developmental stages the investigated enzyme activity was higher under conditions of Ca-deficiency. It follows from Table 2 that the difference between the variant from complete nutrition (NS) and Ca-deficient solution (-Ca) was significant at the 1 % level.

DISCUSSION

It has been reported that ferredoxin-dependent glutamate synthase activity increases during the first 10 days of growth together with maturing of the leaves, increase in the content of chlorophylls and development of the photosynthetic apparatus, as well as CO_2 assimilation proper (NICKLISCH *et al.* 1976, MATON and TAKAHASHI 1981). In the present experiments enzyme activity of photosynthetic tissues of winter wheat from complete nutrient solution was investigated in 10 day-old plants at the stage of the first leaf, at a time when the plants had already attained full development of their autotrophic functions. Therefore, increase in enzyme activity described in the papers mentioned above could not be observed. In plants from Ca-deficient nutrient solution increase in enzyme activity was observed. Maximum activity was found only on day 17 when the plants of this variant attained only the stage of the first leaf. Here the delay in development shown by plants deficient in Ca (-Ca) as compared with the control plants (NS) manifested itself and therefore it was possible also to detect the ascending part of the time curve of GOGAT activity. Thereafter, enzyme activity slowly decreased as in plants from the complete nutrient solution. With the exception of 10 day-old plants in which Ca-deficiency led to a significant decrease in GOGAT activity, enzyme activity was significantly higher at the further investigated terms of the developmental stage, *i.e.* in 17, 24 and 31 day-old plants. These results may also explain why high glutamate levels were found in overground parts of Ca-deficient wheat plants (ČERNÁ 1974, *etc.*). However, a high glutamate level has also been observed in 10 day-old plants of the -Ca variant where glutamine synthetase (NOVOSADOVÁ 1982) activity and glutamate synthase activity (see Fig. 1) as connected with so called GS/GOGAT cycle were reduced compared with control plants from complete nutrient solution. At this stage the main pathway of glutamate genesis under conditions of Ca-deficiency is obviously mediated by glutamate dehydrogenase showing a significantly higher activity (130 %) and an increase in activity at the isoenzyme level (NOVOTNÁ and ČINČEROVÁ 1985).

HARPER and PAULSEN (1969) described an increase in GDH activity in 26 day-old wheat leaves under Ca-deficiency. It seems therefore that under conditions of inadequate nutrition the plant activates mechanisms generating glutamate for metabolic needs by different pathways. Analogous data showing an increase in GDH activity under stress conditions of illuminance, nutrition,

in connection with ageing, high salt concentration, *etc.* have also been reported by other authors (POSTIUS and JACOBI 1976, NAUEN and HARTMANN 1980). It should be emphasized that this discussion is justified only with respect to glutamate synthesis, since no reduction of the level of extractable proteins under conditions of Ca-deficiency was observed in the present experiments. On the contrary, -Ca plants showed a higher content of proteins (unpublished). Proteolytic formation of glutamate is therefore excluded.

As to nitrogen incorporation in organic compounds, it may be inferred that young wheat plants are able to carry out primary nitrogen assimilation and reassimilation *via* the GS/GOGAT cycle and that they also contain a GDH system significantly active at an early stage of Ca-deficiency when the GS/GOGAT pathway is somewhat suppressed. Thus, a high glutamate level for the metabolic needs of the deficient plants is maintained.

At present there exists much information concerning the symptoms of Ca-deficiency on various structural and functional levels of the plant organism (as reviewed by SIMON 1978, *e.g.*). However, the explanation of the mechanism of Ca effect often remains an open question. That, of course, is also true of the specific role of Ca in nitrogen metabolism (BURSTRÖM 1954, HARPER and PAULSEN 1969, ČINČEROVÁ and ČERNÁ 1974, ČINČEROVÁ 1976, ČINČEROVÁ and NEČASOVÁ 1979, MERAVÝ 1979, and others). Until now even the mode of Ca penetration into the plant cell is not exactly known. There is evidence of passive as well as active Ca uptake (BENGTSSON and JENSEN 1982). The need of calcium uptake is relatively low, however a healthy plant requires an uninterrupted supply. An increasing number of papers dealing with the functions of calcium is focused on the membranes, their integrity and ability of selective absorption (EPSTEIN 1961). Here probably the qualified answers will be found to a number of questions. Recently attention has been paid to cytoplasmic calcium because in plants, too, the changes in Ca^{2+} ion availability constitute the basic control system of cytoplasmic streaming, division, elongation and cell differentiation. Calcium linked with calmodulin (MARME and DIETER 1982) mediates the activation of a number of enzymes, mainly those connected with phosphorylation processes in membranes, as for example the proteokinases (HETHERINGTON and TREWAVAS 1984). Undoubtedly investigation of the regulatory function of calcium in the plant cell on this level will promote an understanding of the function of this macronutrient in the transformations of nitrogen compounds.

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