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NADH- and Ferredoxin-Dependent Glutamate Synthase in the Life Span of the Second Leaf of Wheat Plant under Conditions of Senescence Induced by Nitrogen Deficiency and Natural Senescence

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Abstract. The aim of this paper was to study, in the second leaf of wheat plants with a long ontogenesis (47 d), the activity of the enzyme which catalyzes the synthesis of glutamic acid. The activity of the NADH-dependent glutamate synthase prevailed in young tissues of not yet fully expanded second leaf at the stage of incomplete autotrophy (at this stage, organic carbon and nitrogen substances are transferred into the leaf). This form was completely inhibited by azaserine (1 mmol l^{-1}). The activity of the ferredoxin-dependent GOGAT increased with increasing leaf blade area, with its peak occuring at the time of maximum expansion of the assimilation area. Thus fd-GOGAT activity was characteristic for the photosynthetic fully autotrophic phase of leaf development which is materialized in completely mature leaf tissues. In plants grown in full-strength Knop's nutrient solution containing nitrogen, fd-GOGAT was active till the early and medium senescence, whereas only to the early senescence in plants grown in a solution lacking nitrogen. No fd-GOGAT could be detected at the stage of late leaf senescence.

Both enzymes believed to be responsible for the incorporation of ammonia into organic compounds, i.e. glutamine synthetase (GS; Novosadová 1982), and glutamate dehydrogenase (GDH; Novotná and Činčerová 1985) are present in the shoots of wheat (*Triticum aestivum* L.) plants. The enzyme catalyzing the transfer of the glutamine amido-nitrogen to the amino position of glutamic acid, i.e. glutamate synthase (GOGAT), is also present in wheat leaves (Činčerová 1987). The reaction catalyzed by GOGAT, leads to formation of two molecules of glutamic acid which is the most important amino acid in the metabolism of green leaves, because it represents a very important link between the primary assimilation and recycling of ammonia and the biosynthesis of other amino acids and proteosynthesis. GOGAT exists in two forms, one of which is dependent on NADH as reductant, and the other on reduced ferredoxin. The aim of this paper was to monitor the activity of these two forms of GOGAT in order to widen the biochemical characteristic of the ontogenesis of the second leaf of winter wheat which is of great importance in the trophic (vegetative)

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phase of winter wheat plants owing to its long life span. This paper represents a logical continuation of an earlier study dealing with the ontogenesis of the second leaf of winter wheat (Činčerová 1990).

MATERIAL AND METHODS

Wheat Plant Cultivation

Winter wheat (*Triticum aestivum* L., cv. Grana) plants were grown in two nutrient solutions: (a) in a full-strength Knop's nutrient solution (+N), and (b) in a nutrient solution from which nitrogen was omitted (–N); in this variant seed protein was the sole source of nitrogen for wheat seedlings. Wheat plants were grown for 50 d under constant conditions as far as temperature, irradiance, air humidity, and photoperiod are concerned (Činčerová 1990).

The Determination of Fd-GOGAT (EC 1.4.7.1) Activity

The method described by Činčerová (1987) was employed. Ferredoxin was prepared from fresh spinach leaves. The reaction product (glutamic acid) was determined using a D-500 amino acid analyzer (Durrum Corp., USA). The procedure designed for protein hydrolysates was applied in which glutamine and asparagine interfere with serine, and thus free glutamic acid can be reliably determined. Each determination comprised blanks without ferredoxin. Another control assay mixture contained enzyme extract inactivated by boiling to estimate the amount of glutamic acid produced by the hydrolysis of the enzyme extract applied to the assay mixture. To inhibit aminotransferases, aminooxyacetic acid, a potent inhibitor of transamination reactions, was added to each assay mixture (10 mmol l^{-1}).

The Determination of NADH-GOGAT (EC 1.4.7.14) Activity

The method described by Beevers and Storey (1976) was used. GOGAT activity was estimated according to absorbance decrease at 340 nm. The specific inhibitor azaserine (1 mmol l^{-1}) which does not influence GDH activity and the activity of other NADH oxidoreductases was used to prove NADH-GOGAT activity. One enzyme unit was defined as the amount of the enzyme catalyzing the synthesis of one nmol of glutamic acid min^{-1}. All enzymic activities were expressed per leaf to maintain the continuity with previous experiments (Činčerová 1990).

Abbreviations: fd – ferredoxin, GOGAT – glutamate synthase, GDH – glutamate dehydrogenase, GLU – glutamate.

Mathematical Processing of Results

The dependence of fd-GOGAT activity on leaf blade area was determined in both experimental variants by means of correlation calculations. The dependence of the parameters under investigation on time was evaluated by means of linear correlation and regression using the method of the smallest squares according to programs available at the Department of Plant Anatomy and Physiology, Faculty of Science, Charles University, Prague. The equations employed are presented in corresponding forms.

Function L: $y = a_1 + a_2x$;
Function J: $y = 2a_1/(e^{a_3(x-a_2)} + e^{-a_4(x-a_2)})$
where: $a_1 \ldots a_3$ are parameters of the functions

Protein Determination

Protein content was determined according to Lowry et al. (1951).

Leaf Area Determination

Leaf blade area was determined using a Leaf Area Meter (LI-COR-Lincoln, Nebraska, USA).

RESULTS AND DISCUSSION

Fig. 1 shows the activity of both glutamate synthase forms in the ontogenesis of the second leaf of winter wheat plants. NADH-GOGAT activity prevailed in the phase of incomplete autotrophy in which organic carbon and organic nitrogen still were transferred to the second leaf from the first leaf. The peak in NADH-GOGAT activity occurred on d 9 leaf age in plants of both experimental variants (+N, –N); thereafter NADH-GOGAT activity decreased. This period was characterized by leaf area expansion (Fig. 2) and by a high rate of proteosynthesis (Fig. 3a). The peak in fd-GOGAT activity occurred by d 26 when the leaf completed its expansion. Fd-GOGAT activity was also detected at the stage of early and medium senescence (only in +N plants), but no fd-GOGAT activity could be detected at the stage of late senescence. In plants grown in the nutrient solution without nitrogen in which an early onset of senescence was induced, fd-GOGAT activity could be detected only at the stage of early senescence (Fig. 2). Correlation relationships between protein content and leaf age are presented in Fig. 3b, those between leaf area and fd-GOGAT activity in Fig. 4 (for +N plants), and Fig. 5 (for –N plants).

The highest protein content in the second leaf was recorded by d 19 (Fig. 3a). It appears that this term delimited the first phase of leaf ontogenesis (Činčerová 1990), in which young tissues of the second leaf were supplied with organic carbon and organic nitrogen from the first leaf. We can designate this period

as the phase of incomplete autotrophy. The transport of organic carbon into young soybean leaves was demonstrated by Thrower (1962) who used ^{14}C photosynthates. Pate (1973) showed that ^{15}N labelled organic solutes also were imported into young *Lupinus albus* leaves from older leaves. With respect to the peak in protein content on d 19, it can be presumed that the content and the activity of ribulose-1,5-bisphosphate carboxylase also culminated by this

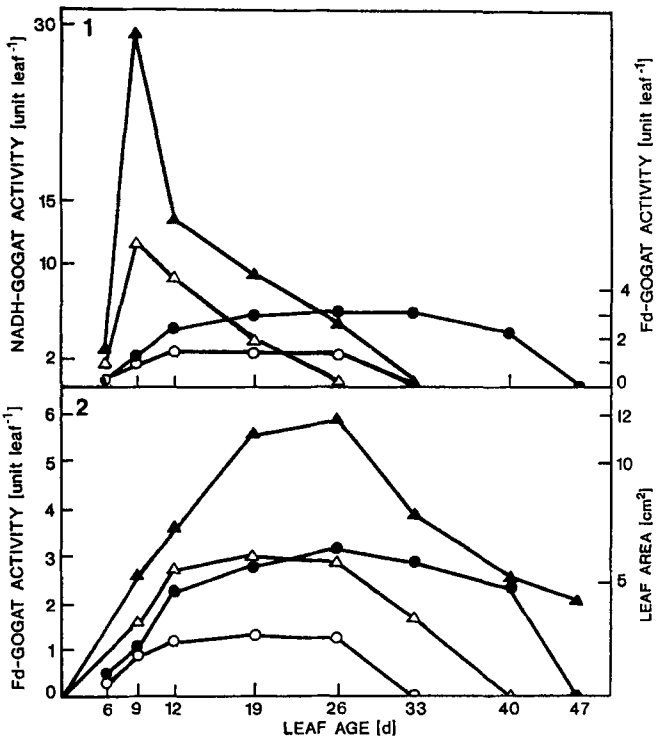

Fig. 1. The activities of NADH-dependent GOGAT (triangles) and ferredoxin-dependent GOGAT (circles) during the ontogenesis of the 2nd leaf of wheat plants. Full points (+N): plants grown in full nutrient solution, natural course of senescence; empty points (–N): plants grown in a nutrient solution lacking nitrogen, induced senescence.

Fig. 2. The activity of ferredoxin-dependent GOGAT (circles) and the expansion of leaf blade area (triangles) during the ontogenesis of the 2nd leaf of winter wheat. Full points: + N; open points: –N.

day, because it is known that this enzyme can reach at the time of maximum leaf blade expansion in *Triticum aestivum* leaves up to 40% of the total soluble protein (Evans and Seeman 1984). Chloroplast ultrastructure also is fully developed by d 19 (Kutík, personal communication). Thus this day can be considered to be the onset of the second phase, i.e. photosynthetic phase, which can be characterized as autotrophic as far as carbon and nitrogen are concerned. The activity of fd-GOGAT reached its peak by d 26 and it could be presumed that the ferredoxin activity peak also occurred by this day not only in the given

enzyme reduction step, but presumably also in photosynthetic mechanisms.

Ferredoxin-dependent glutamate synthase appears to be the sole functional GOGAT form at the stage of leaf maturity, that is from the onset of the photosynthetic phase of leaf development. Fd-GOGAT activity was also detected by d 33 and 40, that is during the presenescence period and at the beginning of actual senescence. But it must be pointed out that this situation was recorded only in plants grown in the full nutrient solution (+N). In plants

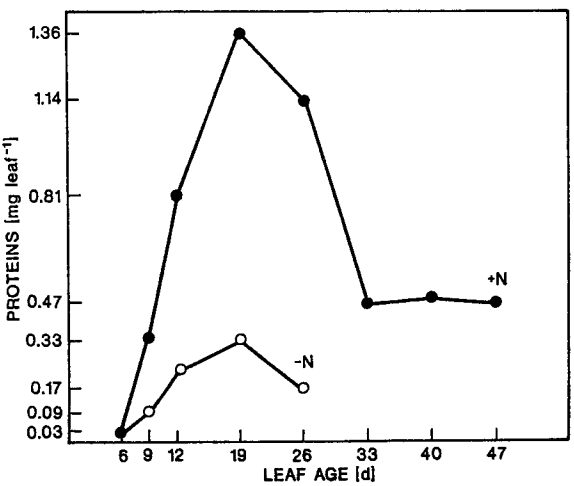

Fig. 3a. Soluble protein level during the ontogenesis of winter wheat 2nd leaf.

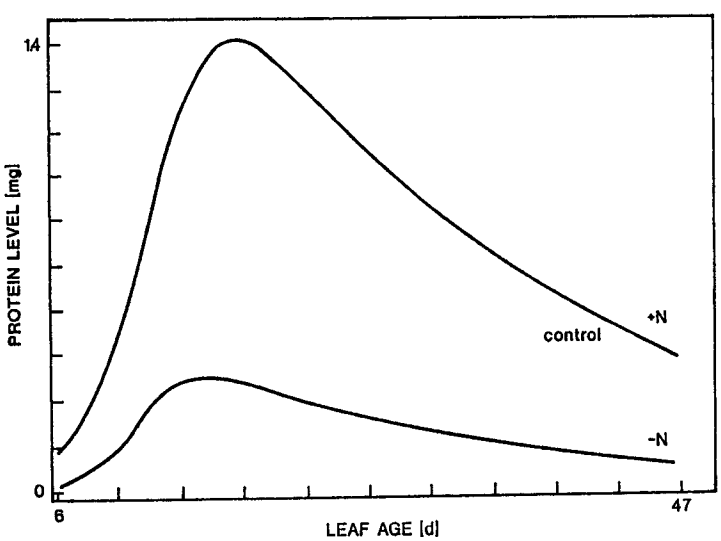

Fig. 3b. Regression between the level of soluble protein and the age of the second leaf; variants +N and –N.

in which senescence was induced by nitrogen deficiency, fd-GOGAT activity could be detected only at the beginning of senescence.

Our results have shown that in addition to a high rate of proteosynthesis and expansion of leaf blade area, the first phase of leaf development can also be characterized by a high activity of NADH-dependent GOGAT and by a relatively low activity of ferredoxin-dependent GOGAT which fact can be considered as the reflection of incomplete autotrophy.

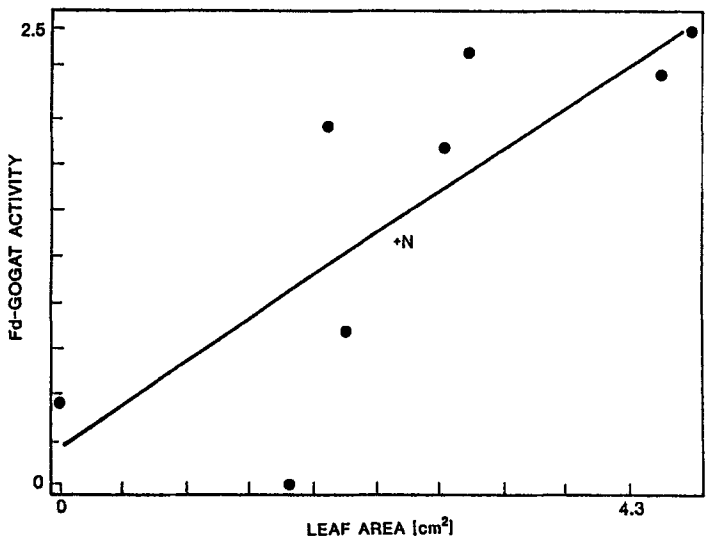

Fig. 4. Linear correlation between fd-GOGAT activity and the expansion of leaf assimilation area in dependence on time; variant +N.

Similar results were obtained by Matoh and Takahashi (1982). The ratio of NADH-GOGAT to fd-GOGAT was in shoots of 5 d-old pea seedlings 72:28, while in shoots of 17 d-old seedlings 3:97. Arima and Kumazawa (1977) experimented with roots of rice seedlings and similarly found that NADH-GOGAT was present mainly in the primary root tip and in secondary roots, whereas fd-GOGAT prevailed in mature root regions despite an apparent absence of ferredoxin in root tissues.

If we now survey the results obtained, we can draw an unequivocal conclusion: Young unripe tissues of leaves in the first phase of incomplete autotrophy are characterized by a high activity of NADH-dependent glutamate synthase. By contrast, the activity of ferredoxin-dependent glutamate synthase prevails in the second (photosynthetic) phase, i.e. in the mature leaf and during the early senescence period. This situation occurs in the second leaf of both the control

plants with a natural course of senescence and of plants in which senescence was artificially induced by nitrogen deficiency.

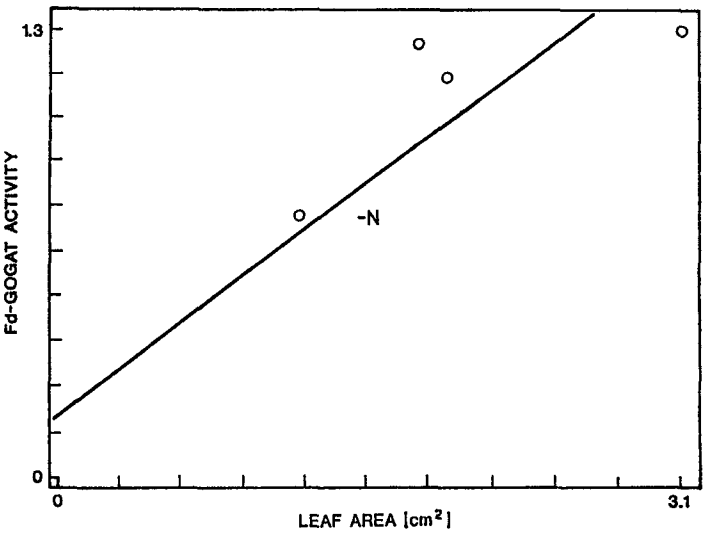

Fig. 5. Linear correlation between fd-GOGAT activity and the expansion of leaf assimilation area; variant –N.

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