THE EXPRESSION OF GLUTAMINE SYNTHETASE ACTIVITY IN SOYBEAN NODULES

EXPRESIA ACTIVITĂȚII GLUTAMIN SINTETAZEI DIN NODOZITĂȚILE DE SOIA

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Abstract. *The work was focused on studying the expression of glutamine synthetase (GS) activity, composition and properties of nodule cytosol GS isoenzyms in relation to plant development stages and to the ontogenetic nitrogenase (NG) activity. Ion-exchange chromatography and electrophoresis showed that the enzyme activity expression and appearance of new GS isoeinzymes in nodule cytosol was determined by the ontogenetic stage of the nodule development. It has been established that the cytosol of the inffected nodule cells contains three GS isoenzymes (GSn1, GSn2, GSn3) distinguished by electrophoretic, ion-exchange properties as well as by their activity value during the period of the highest intensity of nitrogen fixation. One of them (GSm2) is similar to the root form of the enzyme. A positive correlation has been found between the activity of nitrogenase and expression of GSn2 and GSn3 activities during ontogenetic stages. A maximum expression of the GSn1 activity is observed at the stage of nodule ageing and nitrogen fixation intensity reduction. Apparently, GSn1, and, possibly, GSn3 are adaptive enzymes, while GSn2 is a constitutive one.*

Rezumat. *Cercetările din lucrarea dată au fost focusate pe studierea expresiei activității glutaminsintetazei (GS), compoziției şi proprietăților isoenzimelor GS din citosolul nodozităților în funcție de fazele de dezvoltare a plantelor şi activității nitrogenazei (NG) în ontogeneză. Datele electroforezei şi cromatografiei pe coloană au arătat că expresia activității şi apariția isoenzimelor noi de GS în citosol a fost determinată de dezvoltarea ontogenetică a nodozităților. S-a stabilit că în citosolul celulelor infectate ale nodozităților se află trei izoforme de GS (GSn1, GSn2, GSn3), care se deosebesc prin proprietățile electroforetice, cromatografiei pe coloană, precum şi prin valoarea activității lor în perioada cu cea mai înaltă intensitate de fixare a azotului. Una din ele, GSn2, este similară enzimei din rădăcinile neinfectate. Pe parcursul ontogenezei s-a observat o corelație pozitivă între activitatea nitrogenazei şi expresiei activității GSn2 şi GSn3. O expresie maximă a activității GSn1 a fost observată în perioada senescenței nodozităților şi reducerii intensității azotfixării. Este evident că GSn1, posibil şi GSn3, sunt enzime adaptive, iar GSn2 reprezintă o enzimă constituitivă.*

Ammonia represents the firs stable product of symbiotic nitrogen fixation process in the reaction catalyzed by the oxygen-sensitive enzyme, nitrogenase (NG; EC: 1.7.99.2)(2). Glutamine synthetase (GS; EC: 6.3.1.2) is a key enzyme of ammonia assimilation (5) and it has been detected in all the compartments of root nodules - bacteroids, peribacterid space with peribacteroid membranes and cytosol fraction of

infected nodule cells (2,16, 19). These results demonstrate that ammonia assimilation take places in all the nodule compartments. But the vegetative part of infected nodule cells (cytosol) has been found to play the main role in detoxification and assimilation of symbiotically formed ammonia (19).

Several GS isoenzymes, including specific nodule form have been established in nodule cytosol (4, 3, 7, 5, 14). Specific nodule GS appear only under symbiotic conditions, they belonging to the proteins – nodulins (9, 14). However, authors did not always manage to establish a constant composition of GS isoenzymes, even for the same legume species. Probably, this may be connected with age and legume species, influence of endo- and exogenous factors and also with methodic difficulties. From this view point, we studied the expression of GS isoenzyme activities in the vegetative part of soybean root nodule (cytosol) with the aim of determining the composition of GS isoenzyme spectrum and identifying nodule specific GS isoenzyme.

MATERIALS AND METHODS

Soybean seeds (*Glycine max L) cv "Lumina"* infected by effective (nod^+ fix^+) *Bradyrhizobium japonicum* sp.9 were grown in field conditions. Plants with nodules were collected during ontogenesis. The method of Bergersen and Turner (1) was used to separate and obtain main nodule fractions. GS activity was determined by methods (18, 20). The composition of GS isoenzyme pattern was studied by 7.5% PAAG electrophoresis (6). Protein content was estimated by Lowry method (12). Method of ion exchange chromatography on DEAE-52 cellulose was used to separate GS isoenzymes from the cytosol fraction of soybean nodules and roots. The nodule capacity for N_2 fixation (nitrogenase activity) was evaluated by the method of acetylene reduction to ethylene (11).

RESULTS AND DISSCUSSIONS

The studies on GS isoenzymes in nodule cytosol and in uninfected root cells using 7.5% PAAG electrophoresis permitted us to establish some changes in the composition of the enzymatic spectrum. Several GS forms were observed in nodule cytosol, the activity expression of which varying during ontogenesis, while only one GS form was found out in the fraction from the uninfected root cells. Two GS isoenzyme activities were observed in the nodule cytosol, differing in their value of R_f and activity at early stage of plant development (35 days after sowing).

The comparison of the results obtained in the electrophoretic separation of GS from enzyme extract of uninfected roots, nodule cytosol and their mixture (1:1) showed that the R_f value of GS1 from nodule cytosol was similar to that of GS from uninfected roots (GSr). These data demonstrate that of the two GS isoenzymes present in nodule cytosol of 35 days old plants, one form (GS1) corresponds to GSr from uninfected roots by the R_f value, while the second one – GS2 represents a nodule specific izoenzyme, the activity of which is expressed only under symbiotic conditions.

Expression and appearance of nodule specific GS activity is connected with new metabolic needs inside nodule cells dictated by symbiotic conditions (2, 5). Since GS activity in legume nodules depends on the quantity of symbiotic ammonia (1, 2) we studied the expression of GS activity in nodule cytosol during the highest intensity of N_2 fixation. The maximum nitrogenase activity in soybean nodules corresponds to the

budding-flowering period of plants (9). With this consideration, we studied the composition of GS isoforms in nodule cytosol and uninfected roots of 60-days old plants (mass flowering period). The electrophoresis data showed the appearance of an additional GS isoenzyme in nodule cytosol with the highest R_f value. The detected GS forms were designed as GSn1, GSn2 and GSn3.

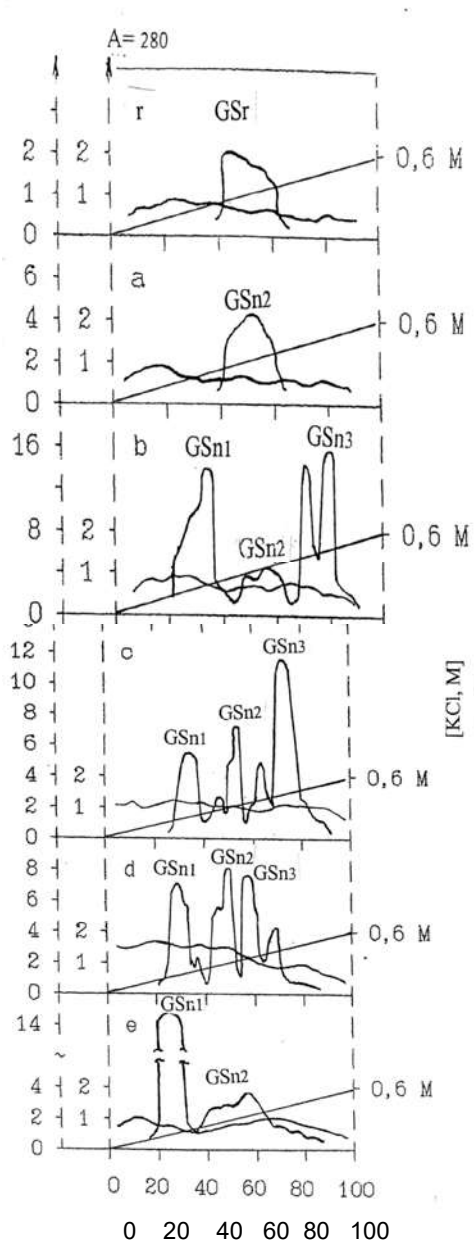

Fig. 2 - Chromatography of GS isoenzymes from cell cytosol of uninffected roots (GSr) and from cytosol of the nodule inffected cells (GSn) on DEAE-cellulose column.

It was observed that the R_f value of nodule GSn2 was similar to that of GSr from uninfected roots.

Our data have demonstrated that the GS isoenzyme composition in soybean nodules is not constant during ontogenesis. At the flowering period, nodule electrophoretic GS spectrum consists of three enzyme isoforms differed by their activity and R_f values. It is considered that the changes in the composition of isoenzimatic spectrum represent an indicator of those genetic processes in cells that occur under the influence of endo-and exogenous factors, providing viability and functioning of organisms (4, 7, 8, 10). These events have an adaptive character.

Symbiotic nitrogen fixation, being one of the main source of endogenous ammonia in legume root nodules has a diurnal and vegetative rhythm activity (2). In order to find out whether the composition of nodule cytosol GS isoforms is related to the ontogenetic NG activity, we studied the expression of GS activities and evaluated the activity during the plant ontogenesis (Fig.1) simultaneously with activity evaluation of soybean symbiotic apparatus (fig. 2). The data on the GS activity in the vegetative part of soybean root nodules showed that the time of isoenzyme appearance and the catalytic activity level of each GS isoenzyme in this fraction are not identical and are determined by both the isoenzyme type and the ontogenetic period. At earlier stages of nodule development, we observed only one GS isoform (GSn2) which was eluted as one peak at 0.24 M KCl on DEAE – 52 cellulose (Fig. 2 a). Studies on the enzyme spectrum composition nodule cytosol during the vegetative periods from budding to senescence permit some changes in GS quantities and qualities to be observed, namely, the activity increase and appearance of new GS isoforms (Fig.2 b, c, d). Beginning with the budding period three GS isoforms (GSn1, GSn2, GSn3) were found during full flowering of the plant, differed by the ion-exchange chromatography properties and activity level.

The findings showed that the presence of two isoenzymes - GSn1 and GSn3 of three detected ones in nodule cytosol was not observed in vegetative young nodules and in uninfected root cells (fig, 1 r, a). This permitted us to suggest that both (or only one of them) represent nodule specific enzymes, activity of which is expressed only under symbiotic conditions. The studies on the nodule GSn2 and GSr from uninfected roots have demonstrated that GSn2 of nodule cytosol is similar to that from roots by electrophoretic and ion-exchange chromatography properties (Fig.1r, a).

The comparison of the dynamic activity of the GSn2 isoform with that of GSn1 and GSn3 revealed that GSn2, unlike GSn1 and GSn3, was constantly present in the soybean nodule cytosol. However, the value of its activity was not constant during the development but changed adequately with the increase of the nodule nitrogen fixing activity (3, 5, 17).

It is considered that such changes in the value of this GS form activity is the consequence of the increasing gene expression for root GS activity (gln-genes), being induced by ammonia (17).The expression of gln-genes in soybean nodules

is induced by endo - and exogenous ammonia and the transcription intensity of genes depends on the NH_4^+ concentration (23). The results demonstrated that the GSn1 activity eluting as a first peak beginning from the budding period up to the nodule senescence setting changed slightly. However, during senescence its activity increased significantly as opposed to that of GSn2 and GSn3.

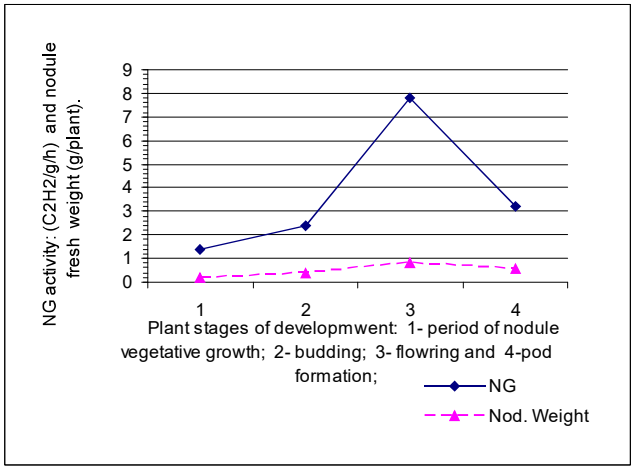

Fig. 2 - The dynamic changes in nitrogen fixation and fresh weight of soybean nodules in ontogenesis.

We suppose that the activity of the isoenzyme (GSn1) increased significantly during the comparatively low intensity of nitrogen fixation (fig.2) and the setting of destructive changes in nodules due to its degradation at the senescence period suggests that GSn1 is likely to play an important role in the assimilation of catabolically rather than symbiotically formed ammonia.

A positive correlation was found between the results on the expression of nodule cytosol GS isoenzyme activities (Fig. 1) and the changes in the activity of nitrogen fixation and nodule fresh weight (Fig. 2) during ontogenesis. It was establish that nodule capacity to fix N_2 enhanced during plant development and reached the highest activity at soybean full flowering stage.

The study of the GSn2 and GSn3 activities during ontogenesis showed that they increased during the flowering period compared with previous vegetation one by approximately 2 and 4 times, respectively.

During the senescence, when the nitrogen fixation is insignificant (fig.2) the GSn2 and GSn3 activities reduced while the GSn1 activity increased (fig.1 e), as mentioned before.

Probably, the functional activity of nodules represents a determining factor in the expression of GS activity and appearance of new enzyme isoforms in the vegetative part of nodules. Namely, this part of nodules (cytosol) represents the main compartment for symbiotically formed ammonia assimilation, and the main role in this process is attributed to the GS localized here.

Thus, it could be concluded that nitrogen fixation process catalyzed by NG influences expression of new GS isoenzyme activities in nodule cytosol, the value and patterns of which vary with ontogenetic period.

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